Isoflurane Neurotoxicity Is Mediated by p75NTR-RhoA Activation and Actin Depolymerization

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ABSTRACT

Background: The mechanisms by which isoflurane injured the developing brain are not clear. Recent work has demonstrated that it is mediated in part by activation of p75 neurotrophin receptor. This receptor activates RhoA, a small guanosine triphosphatase that can depolymerize actin. It is therefore conceivable that inhibition of RhoA or prevention of cytoskeletal depolymerization might attenuate isoflurane neurotoxicity. This study was conducted to test these hypotheses using primary cultured neurons and hippocampal slice cultures from neonatal mouse pups.

Methods: Primary neuron cultures (days in vitro, 4–7) and hippocampal slice cultures from postnatal day 4–7 mice were exposed to 1.4% isoflurane (4 h). Neurons were pretreated with TAT-Pep5, an intracellular inhibitor of p75 neurotrophin receptor, the cytoskeletal stabilizer jasplakinolide, or their corresponding vehicles. Hippocampal slice cultures were pretreated with TAT-Pep5 before isoflurane exposure. RhoA activation was evaluated by immunoblot. Cytoskeletal depolymerization and apoptosis were evaluated with immunofluorescence microscopy using drebrin and cleaved caspase-3 staining, respectively.

Results: RhoA activation was increased after 30 and 120 min of isoflurane exposure in neurons; TAT-Pep5 (10 μM) decreased isoflurane-mediated RhoA activation at both time intervals. Isoflurane decreased drebrin immunofluorescence and enhanced cleaved caspase-3 in neurons, effects that were attenuated by pretreatment with either jasplakinolide (1 μM) or TAT-Pep5. TAT-Pep5 attenuated the isoflurane-mediated decrease in phalloidin immunofluorescence. TAT-Pep5 significantly attenuated isoflurane-mediated loss of drebrin immunofluorescence in hippocampal slices.

Conclusions: Isoflurane results in RhoA activation, cytoskeletal depolymerization, and apoptosis. Inhibition of RhoA activation or prevention of downstream actin depolymerization significantly attenuated isoflurane-mediated neurotoxicity in developing neurons.

What We Already Know about This Topic
• Anesthetics such as isoflurane can cause apoptotic death in developing neural tissue in vitro, in part by affecting synaptogenesis.
• Synaptogenesis depends in part on polymerization of actin, which can be blocked by RhoA.

What This Article Tells Us That Is New
• RhoA is activated by exposure to isoflurane in neuronal and brain tissue cultures.
• Inhibition of RhoA or actin depolymerization attenuates isoflurane neurotoxicity, confirming RhoA activation as a mechanism of isoflurane neurotoxicity.
pending on proteolytic cleavage, BDNF can serve either a prosurvival or a proapoptotic function.\textsuperscript{10,12,14} Signaling by mature BDNF through tropomyosin receptor kinase B enhances neurite growth, stimulates maturation and stabilization of nascent synapses, and causes cell differentiation. In contrast, proBDNF activation of p75 neurotrophin receptor (p75\textsuperscript{NTR}) induces apoptosis and actin cytoskeletal depolymerization.\textsuperscript{11,15}

In developing neurons, the actin cytoskeleton has a key role in neurite formation.\textsuperscript{16} Actin is the most prominent cytoskeletal protein at both pre- and postsynaptic terminals. Activation of RhoA, a small guanosine triphosphatase that regulates the state of actin cytoskeletal polymerization, can inhibit axonal elongation and cause growth cone collapse.\textsuperscript{17–19} Interestingly, p75\textsuperscript{NTR}-mediated signaling results in activation of RhoA and subsequent actin depolymerization.\textsuperscript{20} Recently, we demonstrated that isoflurane neurotoxicity is partly mediated by shifting the balance of BDNF signaling toward proBDNF/p75\textsuperscript{NTR} signaling toward proBDNF activation of p75NTR and causes cell differentiation. In contrast, neurite growth, stimulates maturation and stabilization of nascent synapses. Synaptic loss would subsequently lead to neuronal apoptosis. The current study was conducted to test that hypothesis.

Materials and Methods

All studies performed on animals were approved by Veteran Affairs San Diego Institutional Animal Care and Use Committee (San Diego, California) and conformed to the guidelines of Public Health Service Policy on Human Care and Use of Laboratory Animals.

Preparation of Neuronal Cell Cultures

Neonatal mouse neurons (The Jackson Laboratory, Bar Harbor, ME) were isolated using a papain dissociation kit (Worthington Biochemical, Lakewood, NJ) as previously described.\textsuperscript{15} At each session, neurons were isolated from 12–20 neonatal pups, 1–3 days old (PND1–3), and grown in vitro for 4–7 days (DIV4–7). Experiments performed on a single neuronal isolation (12–20 pups) constitute a sample size of one (n = 1). Hippocampal slices were prepared from PND4–7 mouse pups. Briefly, both left and right hippocampi were dissected at 4°C and were sectioned at 400 \( \mu \)m using a Vibratome 1000 Plus (Vibratome, Bannockburn, IL). Slices were then placed in culture plate inserts (Millipore, Billerica, MA) above 1 ml neuronal media. Neurons and slices were cultured in Neurobasal-A media supplemented with B27 (2%), 250 mM GLUTMax1, and penicillin/streptomycin (1%) as previously described.\textsuperscript{15,21} Neurons were cultured on 2 \( \mu \)g/cm\(^2\) poly-d-lysine/laminin–coated plates or coverslips at 37°C in 5% CO\(_2\) for 4–7 days before experiments. Cleaved caspase-3 (Cl-Csp3) (Cell Signaling, Danvers, MA) and drebrin (Abcam, Cambridge, MA) were used to detect apoptosis and to delineate the F-actin cytoskeleton, respectively, via immunofluorescence deconvolution microscopy. Cl-Csp3 and drebrin staining intensities were normalized to the nuclear stain 4',6-diamidino-2-phenylindole (DAPI) (Molecular Probes/Invitrogen, Carlsbad, CA). Antibodies to activated RhoA were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). RhoA activation was quantified by densitometry and normalized to glyceraldehyde 3-phosphate dehydrogenase (Imgenex, San Diego, CA). The p75\textsuperscript{NTR} inhibitor, TAT-Pep5 (H-YGRKRRQRRR-CFFRGGFNNPRY-OH), and jasplakinolide, a marine sponge cyclopdepsipeptide that stabilizes the actin cytoskeleton, were obtained from CalBiochem (Gibbstown, NJ).

Anesthetic Neurotoxicity Model

Primary neuronal cultures and acute hippocampal slice cultures were placed in a Plexiglas chamber within an incubator and exposed to 1.4% isoflurane, delivered from a calibrated vaporizer from 15 min to 4 h, in a gas mixture of 5% CO\(_2\) balanced with air, at a flow rate of 2 l/min. The concentration of isoflurane was monitored continuously by a Datex Capnomac (DRE Medical, Inc., Louisville, KY). The temperature in the incubator was maintained at 37°C.

Protein Extraction and Western Blot Analysis

Proteins in cell lysates were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using 10% acrylamide gels (Invitrogen) and transferred to polyvinylidene difluoride membranes (Millipore) by electroelution. Membranes were blocked in 20 mM phosphate-buffered saline (PBS) Tween (1%) containing 4% bovine serum albumin and incubated with primary antibody overnight at 4°C as previously described.\textsuperscript{15} Primary antibodies were visualized using secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology) and enhanced chemiluminescence (ECL) reagent (Amersham Pharmacia Biotech, Piscataway, NJ). All displayed bands were expected to migrate to the appropriate size and were determined by comparison to molecular-weight standards (Santa Cruz Biotechnology). ImageJ** was used for densitometric analysis of immunoblots with normalization of RhoA to glyceraldehyde 3-phosphate dehydrogenase.

Immunofluorescence and Deconvolution Microscopy

Neurons were prepared for immunofluorescence microscopy as previously described.\textsuperscript{15,21} Antibodies used for immunofluorescence were CI-Csp3 and drebrin; caspase-3 and drebrin were normalized to the nuclear stain DAPI; phallolidin-594 was normalized to DAPI. Hippocampal slices or cells were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature, incubated with 100 mM glycine (pH 7.4) for 10 min to quench aldehyde groups, permeabilized in buffered Triton X-100.
for 10 min, blocked with 1% bovine serum albumin/PBS/Tween (0.05%) for 20 min, and then incubated with primary antibodies (1:100) in 1% bovine serum albumin/PBS/Tween (0.05%) for 24 h at 4°C. Excess antibody was removed by incubation with PBS/Tween (0.01%) for 15 min, and the samples were incubated with fluorescein isothiocyanate or Alexa-conjugated secondary antibody (1:250) for 1 h. To remove excess secondary antibody, cells were washed six times at 5-min intervals with PBS/Tween (0.01%) and incubated for 20 min with the nuclear stain DAPI (1:5000) diluted in PBS. Cells were then washed for 10 min with PBS and mounted in gelvatol for microscopic imaging. Deconvolution images were obtained as described elsewhere (Applied Precision, Inc., Issaquah, WA). The system includes a Photometrics CCD (Photometrics, Tucson, AZ) mounted on a Nikon TE-200 (Nikon, Melville, NY) inverted epifluorescence microscope. Between 30 and 80 optical sections spaced by approximately 0.1–0.3 μm were taken. Exposure times were set such that the camera response was in the linear range for each fluorophore. Lenses included 100× (NA 1.4), 60× (NA 1.4), and 40× (NA 1.3) magnifications. The data sets were deconvolved and analyzed using SoftWorx software (Applied Precision, Inc.) on a Silicon Graphics Octane workstation (SGI, Fremont, CA). Image analysis was performed with the Data Inspector program in SoftWorx. Maximal projection volume views or single optical sections were visualized. Pixels were assessed quantitatively by CoLocalizer Pro 1.0 software (Colocalization Research Software, Japan and Switzerland). Statistical analysis was performed using Prism 4 (GraphPad Software, La Jolla, CA).

**Cytoskeletal Depolymerization Quantification**

The drebrin pixels (green) were normalized to nuclear-stained pixels (blue). Drebrin is a filamentous F-actin binding protein that stabilizes the actin cytoskeleton within neuritic processes. A reduction in neuritic processes is indicated by decreased drebrin protein expression. Twenty neurons were counted per preparation.

**Statistical Analysis**

All parametric data were analyzed by one-way analysis of variance or two-tailed unpaired t tests with Bonferroni correction as indicated. Significance was set at \( P \) less than 0.05. Statistical analysis was performed using Prism 4 (GraphPad Software). Sample size (n) represents the amount of times the experiments were repeated on separate neuronal cell culture preparations derived from 12–20 PND1–3 pups.

**Results**

**TAT-Pep5 Attenuates Isoflurane-mediated RhoA Activation in DIV4–7 Primary Mouse Neurons**

Mixed cortical and hippocampal neurons were isolated from PND1–2 mouse pups and were grown in culture 4–7 days (DIV4–7) and RhoA activation was assessed with or without TAT-Pep5 (fig. 1). Isoflurane exposure (1.4%; 15, 30, and 120 min) resulted in significantly increased (n = 3; \( P = 0.0052 \) vs. basal, \( \# \), \( P = 0.005 \) vs. basal) compared with control (Ctrl). Pretreatment with TAT-Pep5 (15 min; 10 μM) significantly decreased isoflurane-mediated RhoA activation at both 30 and 120 min (n = 3; \( * P = 0.0088 \) vs. isoflurane 30, \( ** P = 0.0304 \) vs. isoflurane 120). (B) Quantitation of the data. An immunoblot showing that the RhoA band (~23–25 kDa) corresponds to the His-Tag RhoA-loaded control (C). RhoA values were normalized to glyceraldehyde 3-phosphate dehydrogenase. Error bars, SEM.

![Fig. 1. Isoflurane exposure increases RhoA activation in DIV5 neurons in vitro](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931104/ on 06/19/2017)
that the RhoA band (both 30 and 120 min (figs. 1A and B). An immunoblot shows neurons and causes growth cone collapse17–19; we hypothesized Previous work has shown that RhoA regulates actin dynamics in Neuritic Processes and Enhanced Neuronal Apoptosis

TAT-Pep5 Attenuates Isoflurane-mediated Decrease in RhoA–loaded control (fig. 1C). 

isoflurane exposure significantly attenuated RhoA activation (n = 5–7; # P = 0.005 vs. basal) in dendritic filopodial spines as indicated by decreased drebrin immunofluorescence along dendritic shafts (E) compared with control (Ctrl) (A); isoflurane significantly enhanced Ci-Csp3 within the cell body (n = 5–7; # P = 0.04 vs. basal) (F) compared with Ctrl (E). Pretreatment with TAT-Pep5 significantly (n = 4) blocked the isoflurane-mediated decrease in drebrin (C) (* P = 0.0348 vs. isoflurane) and the increase in Ci-Csp3 (n = 5–7; * P = 0.031 vs. isoflurane) (G). Drebrin (green pixels) along dendrites is normalized to DAPI (blue pixels) or Ci-Csp3 (red pixels) is normalized to DAPI. Scale bar, 10 µm. Error bars, SEM.

Jasplakinolide Attenuates Isoflurane-mediated Loss of Neuritic Processes and Apoptosis in DIV4–7 Neurons

Our results from figures 1 and 2 demonstrate that isoflurane causes p75NTR/RhoA activation resulting in cytoskeletal depolymerization and apoptosis; thus, we tested whether actin cytoskeletal depolymerization contributes directly to apoptosis (fig. 3). Primary neuronal cultures were pretreated (1 h) with jasplakinolide (1 µM), a marine sponge cyclodepsipeptide that stabilizes the actin cytoskeleton and prevents depolymerization.22–26 Basally neurons displayed prominent drebrin immunofluorescence (fig. 3A). Isoflurane (fig. 3B) decreased (n = 4–6; P = 0.0337 vs. basal) drebrin immunofluorescence, an effect significantly attenuated with jasplakinolide pretreatment (fig. 3C, n = 4–6; P = 0.0144 vs. isoflurane). Quantitation is shown in figure 3D. Basally neurons expressed minimal Ci-Csp3 (fig. 3E). Isoflurane significantly increased Ci-Csp3 (fig. 3F, n = 4–6; P = 0.003 vs. basal), an effect attenuated with jasplakinolide (fig. 3G, n = 4–6; P = 0.001 vs. basal). Quantitation is shown in figure 3H.

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Basal, P = 0.0050 vs. basal) concentrations of activated RhoA at both 30 and 120 min compared with basal (figs. 1A and B). Pretreatment with TAT-Pep5 (15 min; 10 µM) before isoflurane exposure significantly attenuated RhoA activation (n = 3; P = 0.0088 vs. isoflurane 30, P = 0.0304 vs. isoflurane 120) at both 30 and 120 min (figs. 1A and B). An immunoblot shows that the RhoA band (~23–25 kDa) corresponds to the His-Tag RhoA–loaded control (fig. 1C).

TAT-Pep5 Attenuates Isoflurane-mediated Decrease in Neuritic Processes and Enhanced Neuronal Apoptosis

Previous work has shown that RhoA regulates actin dynamics in neurons and causes growth cone collapse17–19; we hypothesized that isoflurane would disrupt neuritic processes through p75NTR activation of RhoA. DIV4–7 primary mouse neurons were treated with or without TAT-Pep5 (10 µM, 15 min) before isoflurane exposure (1.4%, 2 h) and stained with drebrin, an F-actin binding protein, the apoptotic marker Cl-Csp3, and the nuclear marker DAPI (fig. 2). Basally neurons displayed prominent drebrin immunofluorescence (fig. 2A). Exposure to isoflurane resulted in a significant (n = 4; P = 0.005 vs. basal) reduction in neuritic processes as indicated by reduced drebrin immunofluorescence (fig. 2B), an effect attenuated by TAT-Pep5 (fig. 2C, P = 0.0348 vs. isoflurane). Quantitation is shown in figure 2D. Basally neurons expressed minimal Cl-Csp3 (fig. 2E). Isoflurane induced a significant increase (n = 5–7; P = 0.04 vs. basal) in Cl-Csp3 expression (fig. 2F), an effect significantly attenuated by TAT-Pep5 (fig. 2G, P = 0.031 vs. isoflurane), a pharmacologic agent that prevents p75NTR activation of RhoA. Quantitation is shown in figure 2H. These findings extend the notion that p75NTR activation has a central role in isoflurane-mediated neurotoxicity.

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Fig. 2. Isoflurane exposure decreases neuritic processes and enhances neuronal apoptosis in DIV4–7 (4–7 days in vitro) primary neurons. Primary neurons (DIV4–7) were exposed to 1.4% isoflurane (Iso) for 2 h with and without pretreatment with TAT-Pep5 (15 min, 10 µM) and incubated with antibodies for drebrin (neuronal F-actin binding protein) (A–C; quantitation shown in D), the apoptotic marker, cleaved caspase-3 (Cl-Csp3) (E–G; quantitation shown in H), and the nuclear marker DAPI (4′,6-diamidino-2-phenylindole). DIV4–7 neurons exposed to isoflurane exhibited a significant reduction (n = 4; # P = 0.005 vs. basal) in dendritic filopodial spines as indicated by decreased drebrin immunofluorescence along dendritic shafts (B) compared with control (Ctrl) (A); isoflurane significantly enhanced Ci-Csp3 within the cell body (n = 5–7; # P = 0.04 vs. basal) (F) compared with Ctrl (E). Pretreatment with TAT-Pep5 significantly (n = 4) blocked the isoflurane-mediated decrease in drebrin (C) (* P = 0.0348 vs. isoflurane) and the increase in Ci-Csp3 (n = 5–7; * P = 0.031 vs. isoflurane) (G). Drebrin (green pixels) along dendrites is normalized to DAPI (blue pixels) or Cl-Csp3 (red pixels) is normalized to DAPI. Scale bar, 10 µm. Error bars, SEM.
These findings extend the notion that actin cytoskeletal depolymerization has a central role in isoflurane-mediated neurotoxicity.

**TAT-Pep5 Decreases Isoflurane-mediated Reduction in Drebrin Immunofluorescence in Hippocampal Slices**

We tested whether the neurotoxic effect of isoflurane on the actin cytoskeleton seen in primary neuronal cultures also occurs in intact hippocampal slices isolated from PND4–7 pups (fig. 4). Hippocampal slices at 4x magnification with DAPI stain are shown in figure 4A (basal, CTRL), figure 4B (isoflurane, ISO), figure 4C (Pep5, CTRL), and figure 4D (Pep5, ISO). Basally hippocampal slices at 60x magnification displayed normal drebrin immunofluorescence (fig. 4E). Isoflurane exposure (1.4%, 4 h) significantly reduced drebrin immunofluorescence (fig. 4F). Pep5 treatment (10 μM, 15 min) on CTRL slices showed no change in drebrin expression (fig. 4G). TAT-Pep5 significantly attenuated isoflurane-mediated reduction in drebrin expression (fig. 4H, n = 4, P = 0.0007 vs. isoflurane). Quantitation of the data is represented in figure 4I. The results demonstrate that the cytoskeletal destabilizing effects of isoflurane occur not only in isolated primary neurons in culture but also in intact hippocampal slices from developing pups.

**Discussion**

We have previously demonstrated that isoflurane exposure during the critical period of synaptogenesis leads to neuronal...
apoptosis that is mediated in part by preferential signaling of proBDNF-p75NTR.\textsuperscript{15} The mechanism by which p75NTR activation leads to neurotoxicity after exposure to anesthetic is not clear. What is known is that RhoA, a small guanosine triphosphatase and key regulator of the actin cytoskeleton,\textsuperscript{27,28} associates with and is activated by the p75NTR.\textsuperscript{20,29,30} Accumulating evidence has also linked RhoA and apoptosis.\textsuperscript{31–34} The current data clearly show that isoflurane exposure leads to increased RhoA activation, actin depolymerization, and neuronal apoptosis. Inhibition of RhoA activation by TAT-Pep5 or downstream stabilization of the actin cytoskeleton with jasplakinolide significantly attenuated neuronal death.

Previous work has shown that RhoA initiates cytoskeletal rearrangement through activation of RhoA-associated kinase, a downstream serine/threonine kinase.\textsuperscript{35,36} To support our premise that RhoA has a central role in isoflurane-mediated apoptosis, we used a specific inhibitor of p75NTR-mediated RhoA activation, TAT-Pep5.\textsuperscript{20} Pretreatment with TAT-Pep5 before isoflurane exposure significantly attenuated RhoA activation, cytoskeletal destabilization, and apoptosis. The link between RhoA and apoptosis previously established by other investigators\textsuperscript{31–34} together with the data presented above, strongly support the premise that RhoA activation has a central role in isoflurane-mediated apoptosis. The attenuation of cytoskeletal depolymerization along with attenuation of apoptosis after RhoA inhibition suggested that cytoskeletal destabilization may have a significant role in isoflurane-mediated apoptosis.

Regulation of the actin cytoskeleton is critical for normal neuronal function, including synaptic spine morphogenesis, stability, and function. The actin cytoskeleton is directly involved in neurite arborization and has complex roles at both pre- and postsynaptic terminals.\textsuperscript{37} For example, at the presynaptic terminal, actin has been implicated in maintaining and regulating synaptic vesicle pools within the bouton as well as replenishing pools through endocytosis.\textsuperscript{38–40} Recently, two groups have shown that activity-driven induction of presynaptic boutons requires actin polymerization to convert immature nonfunctional boutons to active mature boutons capable of neurotransmitter release.\textsuperscript{41,42} At the postsynaptic neuron, actin is critical in anchoring, regulation of lateral trafficking, and assisting with exo-endocytosis of postsynaptic receptors.\textsuperscript{43,44} Actin is also highly concentrated within dendritic spines,\textsuperscript{45–47} and is critical for maintenance of spine morphology and plasticity.\textsuperscript{16,48} Furthermore, ab-

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**Fig. 4.** TAT-Pep5 decreases isoflurane-mediated reduction in dendritic spines in hippocampal slices. Hippocampi were dissected from postnatal day (PND)4–7 mouse pups, and 400-μm slices were exposed to isoflurane with or without pretreatment with TAT-Pep5 (15 min; 10 μM). Images represented in 4× magnification are as follows: basal (control (CTRL), A), isoflurane (ISO, B), Pep5 CTRL (C), Pep5 ISO (D). Images in 60× magnification are as follows: CTRL (E), ISO (F), Pep5 CTRL (G), and Pep5 ISO (H). Isoflurane exposure significantly (n = 4, *P = 0.0041 vs. basal) reduced drebrin immunofluorescence in hippocampal slice cultures; TAT-Pep5 significantly (n = 4, *P = 0.0007 vs. isoflurane) attenuated the isoflurane-mediated reduction in drebrin expression. (I) Quantitation of the data. Drebrin expression (green pixels) was normalized to DAPI (4',6-diamidino-2-phenylindole) (blue pixels). Error bars, SEM.
normal dendritic spine morphology caused by alterations in actin assembly has been linked to cognitive and behavioral changes.\textsuperscript{49–52} Given the central role of the actin cytoskeleton within neuronal synapses, any disruption in actin dynamics during the key period of synaptogenesis could potentially result in significant neuronal dysfunction. Whereas our previous data suggested that RhoA activation had a prominent role in isoflurane-mediated apoptosis, the role of actin depolymerization remained undetermined. To examine actin’s role, we used jasplakinolide, a cyclodepsipeptide isolated from a marine sponge that stabilizes the actin cytoskeleton and thus prevents depolymerization.\textsuperscript{22,53} Our data demonstrate that neurons pretreated with jasplakinolide had significantly reduced isoflurane-mediated apoptosis. The data support the premise that actin depolymerization directly contributes to loss of neuritic processes and to neuronal apoptosis.

The means by which RhoA activation leads to actin depolymerization in the setting of isoflurane exposure is not known. However, a possible mediator of the actin depolymerization initiated by RhoA is RhoA-associated kinase, a serine/threonine kinase.\textsuperscript{35,36} If RhoA-associated kinase activation is, in fact, critical to isoflurane-mediated actin depolymerization, then it might be possible to reduce isoflurane-induced neurotoxicity by specifically targeting RhoA-associated kinase. This possibility will be evaluated in future studies.

**Conclusion**

These results demonstrate that isoflurane exposure leads to RhoA activation, cytoskeletal depolymerization, and neuronal apoptosis. Inhibition of RhoA or stabilization of the actin cytoskeleton prevents these neurotoxic effects of isoflurane exposure during the critical period of synaptogenesis. These findings are consistent with our hypothesis that isoflurane-mediated apoptosis in developing neurons results from the cytoskeletal destabilizing effects of RhoA activation that is
attendant with proBDNF activation of p75NTR. As such, the results provide a mechanistic framework upon which novel therapeutic approaches for the prevention of anesthetic neurotoxicity might be developed.

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