Dexmedetomidine reduces isoflurane-induced neuroapoptosis through regulating BDNF and proBDNF

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Research

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Abstract

Background: It is well-acknowledged that Isoflurane induces neuroapoptosis in neonatal rats. Dexmedetomidine, as an α2-adrenergic agonist, was previously demonstrated to provide neuroprotection when administered during isoflurane anesthesia. Our study aims to investigate the mechanisms concerning the neuroprotective effect of dexmedetomidine from the alterations of BDNF,ERK, and JNK signals in the hippocampal region.

Methods: Neonatal Sprague-Dawley rats at postnatal day 7 were assigned into Control group, Isoflurane group, Dexmedetomidine group and Inhibitor group. After exposed to 2% isoflurane in 40% of oxygen mixed with nitrogen for 4h, the hippocampus tissues were separated and critical signal pathway proteins of BDNF, proBDNF, JNK, ERK, and caspase 3 were detected.

Results: Neuroapoptosis was triggered by Isoflurane with the increased expression of caspase 3 and TUNEL-positive cells. This effect was reversed by dexmedetomidine accompanying with up-regulation of BDNF and phospho-ERK and down-regulation of proBDNF and phospho-JNK.

Conclusions: This study revealed that dexmedetomidine pretreatment can attenuate neurotoxicity caused by isoflurane in neonatal rats by regulating BDNF, proBDcNF, ERK, and JNK, which would provide a new target for neuroprotection.

Background

With the rapid development of medical technology, growing numbers of surgical procedures can be carried out in infants under general anesthesia. It is reported that some non-obstetric surgery of pregnant women under general anesthesia resulted in later learning and memory deficits in the immature fetal brain of infants. Inhaled anesthetics have a significant advantage in general anesthesia in infants due to their characteristics of high anesthetic efficacy and strong maneuverability. Without the respiratory irritation, isoflurane is widely used in clinical anesthesia in infants due to the rapid induction and awakening properties and easy adjustment of anesthesia depth. In recent years, a multitude of experiments focus on the effects of isoflurane on the cognitive function, and some of the studies have found that prolonged exposure to isoflurane in children may cause acute cognitive dysfunction. It is well known that anesthetics may cause apoptotic neurodegeneration in the neonatal brain in rats, and results in subsequent cognitive dysfunction in adulthood [1] [2][3][4] [5] [6]. A crucial factor mediating anesthetic neurotoxicity is that the brain is more vulnerable to injury during the period of synaptogenesis than any other phase [7]. It is reported that isoflurane neurotoxicity is partially mediated by the action of proBDNF/p75NTR (p75 neurotrophin receptor), and the inhibitor of p75NTR was able to attenuate this neurotoxicity [8][9].

Dexmedetomidine is a highly selective agonist of α2-adrenergic receptors which is widely used in clinical practice with a broad spectrum of effects, including clinical sedation, anesthetic sparing effects, and analgesia [10] [11]. In addition, dexmedetomidine has gained widespread attention for its neuroprotective

properties on different experimental models of brain injury [12] [13] [14] [15] [16] [17] [18]. Recent experimental works indicate that dexmedetomidine reduced the isoflurane-induced neurotoxicity in neonatal rats [13], but the mechanism remains unclear. Several studies found that dexmedetomidine could improve cognitive function after the operation. Using in vitro approaches, it has been suggested that the neuroprotective mechanism of dexmedetomidine may be involved in the regulation of brain-derived neurotrophic factor (BDNF) expression [19]. BDNF is the product of proneurotrophin (proBDNF). BDNF activation of tropomyosin receptor kinase B (TrkB) enhances neurite growth and causes cell differentiation, and signaling by proBDNF through p75NTR induces cell apoptosis [9]. Activation of TrkB leads to extracellular signal-regulated kinase (ERK) phosphorylation, which is essential to cell survival. In contrast, the activation of proBDNF/p75NTR induces c-Jun N-terminal kinase (JNK) phosphorylation, which is critical for cellular apoptosis.

Despite the proposed mechanisms concerning the neuroprotective effect of dexmedetomidine, the possible interaction between dexmedetomidine and proBDNF has not been explored. Our experiment was designed to investigate whether dexmedetomidine pretreatment can attenuate neurotoxicity caused by isoflurane in neonatal rats by regulating the expressions of BDNF, proBDNF, phospho-ERK, and phospho-JNK in the hippocampal region.

Methods

Animal and group

Seven-day-old (P7) Sprague-Dawley rat pups (Liaoning Changsheng Biotechnology Co. Ltd., permission number: SCXK 2015-0001) weighing (15.2□3.2)g were used because this is the time when they are vulnerable to anesthesia-induced neurologic deficits. Seventy-two neonatal Sprague-Dawley rats at postnatal day 7 were assigned randomly to four groups: Control group (group Con) where rats were injected 150μl saline intraperitoneally; Isoflurane group (group Iso) where rats were injected intraperitoneally with 150μl saline; Dexmedetomidine group (group Dex) where rats were injected intraperitoneally with dexmedetomidine 50μg/kg in 150μl saline; Inhibitor group (group Inh) where rats were injected intraperitoneally with (dexmedetomidine 50μg/kg+TAT-PEP5 10 μM/kg+K252a 80μg/kg) in 150μl saline.

Anesthetic Neurotoxicity Model

Seven-day-old Sprague-Dawley rat pups were exposed 4 h to 2.0% isoflurane in 40% oxygen in a chamber (n=18 per group). Saline or dexmedetomidine (50μg/kg) or TrkB inhibitor K252a (80μg/kg) or p75NTR inhibitor TAT-PEP5 (10 μM/kg) were injected intraperitoneally 15 minutes before the exposure. Rats in group Iso received 2% isoflurane and saline (150μl); rats in group Dex received 2% isoflurane and dexmedetomidine (50μg/kg intraperitoneally); rats in group Inh received 2% isoflurane and dexmedetomidine (50μg/kg) and K252a (80μg/kg, CST) and TAT-PEP5 (10 μM/kg, millipore). Rats in group Con received 40% oxygen and saline (150μl intraperitoneally).

Some animals were sacrificed (with sodium pentobarbital 100 mg/kg intraperitoneally) 2 hours after the end of gas exposure. Their hippocampi were isolated immediately on ice and then stored at -80℃ until used for western blot study (n=8). The other rats were sacrificed 2 hours after the end of gas exposure and perfused transcardially with ice-cold saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Their brains were post-fixed for 48h, and then dehydrated and embedded in paraffin. The paraffin blocks stored at 4℃, and then used for TUNEL and immunohistochemistry study (n=10).

TUNEL fluorescent assay

The paraffin blocks were separated from tissue holders, 3.5μm sections were then cut, placed on glass slides. The paraffin sections were allowed to dry at 67℃ for 24h, and then removed paraffin and rinsed in phosphate-buffered saline (PBS). TUNEL fluorescent assay was performed using the In Situ Cell Death Detection Kit, POD (Roche, USA) according to the manufacturer's protocol. Tissue sections were incubated by proteinase K solution for 20 minutes, exposed to equilibration buffer for 10 minutes and the terminal deoxynucleotidyl transferase (TdT) and incubated in a humidified chamber at 37℃ for 1 hour, and the slides were protected from direct light during the experiment. Afterward, the reaction was stopped, and then the nuclei staining with DAPI. For negative-controls, the slides were exposed to reaction buffer without TdT. Images were acquired at 400x by NIS-Elements F 3.0 software linked to Nikon E800 microscope. TUNEL positive cells in the hippocampus were analyzed with Image-Pro Plus software. The apoptotic index was defined as the percentage of TUNEL positive cells among the total number of nuclei of the hippocampal region.

Protein extraction and Western blot Analysis

The samples were homogenized in RIPA buffer (Beyotime, China) with protease inhibitors (1 mM phenylmethanesulfonylfluoride) on ice. The homogenates were then centrifuged at 12,000 g at 4°C for 20 min to remove tissue debris. The supernatant, stored at -80°C and subsequently used to Western blot. Prepare samples in EP tubes, add 4X SDS sample buffer so the total protein amount was 50 μg per sample according to the protein amount measured by BCA protein assay (Beyotime, China), and then heated to 100℃ for 5 minutes. Load samples and protein markers onto the 10% acrylamide gels (Beyotime, China). Set electrophoresis power pack to 80V (through the stacking gel), before increasing it to 120V when the protein front reaches the separation gel. Then transferred to polyvinylidene difluoride membranes (PVDF, Millipore, USA) by electroelution at 4℃. Membranes were blocked with 1x Tween-containing Tris-buffered saline (TBST) containing 5% nonfat dry milk with constant rocking for 2 hours at room temperature. The following primary antibody was incubated with the membrane under gentle agitation at 4℃ overnight: anti-caspase 3 at 1:1000 dilution (Cell Signaling Technology, USA), anti-BDNF at 1:1000 dilution (abcam, USA), anti-proBDNF at 1:1000 dilution (alomone labs, Israel), anti-ERK1/2 at 1:1000 dilution (Cell Signaling Technology, USA), anti-phospho-ERK1/2 at 1:1000 dilution (Cell Signaling Technology, USA), anti-JNK at 1:1000 dilution (R&D, USA), anti-phospho-JNK at 1:1000 dilution (abcam, USA), and anti-β-actin at 1:5000 dilution (proteintech, China). After washing three times with 1x TBST for 10 minutes each, the membranes were incubated for 2 hours with suitable horseradish peroxidase-

conjugated secondary antibody (1:5000, ZSGB-BIO, China) with constant rocking for 2 hours at room temperature. After washing three times with 1x TBST for 10 minutes each, the membranes were incubated with sensitive chemoluminescence (ECL) substrate (Thermo, USA). Then read the membrane using a chemiluminescence imaging system (Azure C300, USA), and quantitative analysis of protein bands was measured using Image J. The protein expression of phospho-ERK1/2 or phosphor-JNK was normalized to the total ERK1/2 or JNK respectively. The band signals of other proteins were normalized to those of β-actin from the same samples.

Immunohistochemistry Staining

The paraffin blocks were separated from tissue holders, 3.5μm sections were then cut, placed on glass slides. The paraffin sections were allowed to dry at 67℃ for 24h, and then immersed slides in xylene for deparaffinization and rehydrated sections by sequentially incubating with 100%, 95%, 80% and 60% ethanol for 5 minutes each. Rinsed sections with PBS three times for 5 minutes each. Transferred slides to a microwave-proof container and covered with citrate buffer, and heated in the microwave on high power for 7 minutes. Afterward, the brain sections were blocked with 3% hydrogen peroxide (ZSGB-BIO, China) at room temperature for 30 minutes for endogenous peroxidase ablation, and then washing with PBS. Sections were then blocked with 10% normal goat serum (ZSGB-BIO, China) for 45 minutes at room temperature. Discarded the goat serum and dropped the anti-caspase 3 (1:1000, Cell Signaling Technology)、anti-BDNF (1:100, abcam)、anti-proBDNF (1:100, alomone labs)、anti-phospho-ERK1/2 (1:100, abcam) and anti-phospho-JNK (1:100, abcam) primary antibody at 4℃ overnight. Tissue sections were rinsed in PBS and dropped the goat anti-rabbit antibody (ZSGB-BIO, China) for 30 minutes at 37℃, followed by incubation with the streptavidin-horseradish peroxidase (ZSGB-BIO, China) for 20 minutes at room temperature. Tissue sections were colored with diaminobenzidine (DAB, ZSGB-BIO, China) for 0.5-5 minutes and finished coloration with the distilled water, and then counterstained with hematoxylin. The sections were then dehydrated, cleared and mounted with neutral gums and covered with a coverslip. Negative control sections were carried out with the same steps as described above, but the primary antibody was replaced by PBS. Images were acquired at 400x using NIS-Elements F3.0 software linked to Nikon E800 microscope. Quantitative analysis of protein expression was performed using Image-Pro Plus. The quantitative analysis of target positive cells in the hippocampus was calculated by the optical density (OD) of positive cells by the area of that brain region.

Statistical Analysis

All statistical analyses were performed using SPSS version 21.0 software (IBM Corporation, Somers, NY, USA). All residuals followed normal distribution as detected by Shapiro-Wilk test. All values are presented as mean SD and analyzed using one-way analysis of variance (ANOVA), followed by LSD's posttest. Significance was set at $P<0.05$.

Results

Neuroapoptosis induced by isoflurane

All rats survived throughout the experiments. The percentage of TUNEL positive cells was increased in the isoflurane-treated rats compared with control rats ($P<0.001$, Fig.1). In addition, the expression of caspase 3, which is a marker of apoptosis and cell death, detected by western blot increased in group Iso compared with control rats ($P<0.05$, Fig.2). Furthermore, the results of caspase 3 detected by immunohistochemistry staining were similar to those of the above ($P<0.05$, Fig.3).

Neuroprotection induced by dexmedetomidine

Dexmedetomidine attenuated apoptosis of neurons in the hippocampus induced by isoflurane. Additionally, compared with group Iso, in group Dex, the expression of caspase 3 detected by western blot was decreased ($P<0.001$, Fig.1). Likewise, the results of caspase 3 detected by immunohistochemistry significantly reduced by dexmedetomidine against isoflurane ($P<0.05$, Fig.3).

The change of BDNF and proBDNF expression

Western blot showed that the isoflurane reduced the expression of BDNF ($P<0.05$, Fig.2) which was significantly reversed by dexmedetomidine pretreatment ($P<0.05$, Fig.2). Furthermore, the protein expression of proBDNF in group Iso is significantly higher than group Con ($P<0.05$, Fig.2), and dexmedetomidine pretreatment reversed the increase of proBDNF ($P<0.05$, Fig.2). Immunohistochemical detection of BDNF and proBDNF had the same results consistent with the results of Western Blot ($P<0.05$, Fig.3).

The expression of phospho-ERK and phospho-JNK

The expression of phospho-ERK detected by western blot was reduced in group Iso($P<0.05$, Fig.4), and this was significantly reversed by dexmedetomidine pretreatment($P<0.05$, Fig.4). In addition, the rat's exposure to isoflurane increased the protein expression of phospho-JNK increased significantly compared with group Con($P<0.05$, Fig.4), and dexmedetomidine pretreatment reversed the change of phospho-JNK ($P<0.05$, Fig.4). Furthermore, compared with group Dex, the phosphorylation of ERK and JNK were significantly reduced by the inhibitors of TrkB and p75NTR ($P<0.05$, Fig.4). Meanwhile, immunohistochemical detection of phospho-ERK and phospho-JNK had the same results ($P<0.05$, Fig.5).

Discussion

The results of our study manifested that pretreatment with dexmedetomidine led to a significant decrease in neuroapoptosis in the hippocampus in neonatal rats in isoflurane anesthesia. These data demonstrate that pretreatment with dexmedetomidine may offer a strategy for neonatal brain protection during anesthesia of isoflurane. This protection was associated with activation of BDNF-mediated phospho-ERK, and a decrease of proBDNF-mediated phospho-JNK which are important for neuron survival. To our knowledge, this is the first time to demonstrate that the dexmedetomidine could attenuate isoflurane-induced neuroapoptosis, as evidenced by the decrease of the production of proBDNF and thereby inhibiting the activation of JNK.

Consistent with other evidence that apoptotic neurodegeneration can be induced by exposure to drugs during the brain growth spurt, neonatal exposure to isoflurane was shown to induce widespread apoptosis in several major brain regions [1] [20]. The growth and development of the brain involve various processes, including neurogenesis, neural differentiation, migration, degeneration, apoptosis, and synapse formation [2], which are closely related to the aging process. In humans, the last trimester of pregnancy to the age of 2–3 years is the rapid development period of the brain, which is equivalent to rats from the late embryonic stage to 2–3 weeks after birth, and the brain synapse appeared rapidly during this period [21] [22]. Synapse is an important structure of information transmission among neurons. It has been hypothesized that the developing nervous system is more susceptible to certain neurotoxic injury than the mature brain because of the complexity and temporal features associated with brain development [1]. Anesthesia-induced neuroapoptosis is age-dependent, and the developing brain is most vulnerable at the age of 7 days [1] [23] [24].

Isoflurane is one of the most frequently used volatile anesthetics for induction and maintenance of anesthesia during surgery. Because isoflurane has rapid induction and recovery properties with limited irritation to the airway, it is particularly beneficial for infants undergoing the surgical process. Isoflurane exerts its effects through the GABA receptor and NMDA receptor. Exposure to isoflurane leads to neuronal apoptosis of immature brain in neonatal rats, which influence the function of learning and memory in adulthood [24]. The hippocampus is closely related to the function of learning and memory. It is reported that isoflurane induced neuronal apoptosis and damage in the cognitive function in adulthood. Its mechanism of toxicity comprises a wide range of factors, such as calcium concentration, mitochondrial pathway and inflammatory factor release [25] [26]. In the preliminary experiment, we found that 7-day-old rats exposed to 2% isoflurane, which is approximately equivalent to 0.8MAC [27], for 4 hours, induced significant apoptosis in hippocampal regions, and that is consistent with previous reports [28]. Some reports showed that neurotoxicity of isoflurane was associated with down-regulation of phosphor-ERK and up-regulation of phosopho-JNK [28]. Head et al. found that isoflurane neurotoxicity in the neonates is mediated by enhanced proBDNF/p75NTR and reduced BDNF [9].

The results of our study suggest that dexmedetomidine may be a clinical accessible tool to be used in children. α2-Adrenergic receptors are functionally coupled to G proteins from early in development [29]. and have an inhibitory influence on neuronal activity [30]. As a newer α2-adrenergic agonist, dexmedetomidine is often used as a sedative and anesthetic adjuvant for patients because of its anesthetic-sparing effects and properties of anti-inflammation and cardiovascular protection [31] [32] and neuroprotection [12] [33].

Dexmedetomidine provided neuroprotection against isoflurane-induced neuroapoptosis in a dose-dependent manner. Some reports [13] [34] found that dexmedetomidine lacks neurotoxicity even at extremely high dose such as 75μg/kg. Single administration of dexmedetomidine 75μg/kg provides a similar effect to that of applying 25μg/kg for three times during a 6 hours isoflurane exposure. However, Neuroprotective effects of dexmedetomidine had ceiling-effect, more than 12.5μg□kg-1□h-1 can’t produce a larger anti-apoptotic effect. Thus, in our study dexmedetomidine of 50μg/kg induced an adequate

effect of neuroprotection during 4 hours of exposure to isoflurane. We observed that pretreatment with dexmedetomidine at a dosage of 50μg/kg significantly attenuated apoptosis in the hippocampal region of developing rats.

The neuroprotective effects of α2-adrenergic agonists have been studied for more than 20 years [35], however, the mechanisms of protection remain unknown. α2-Adrenoceptor is a subtype of norepinephrine receptors, which is widely distributed in the central nervous system and regulated the function of each system. Dahmani found that dexmedetomidine played a neuroprotective role in cerebral ischemia-reperfusion injury in rats by activation of α2-adrenoceptor and inhibition of adenylate cyclase [36]. It is known that dexmedetomidine attenuated neurotoxicity induced by isoflurane through upregulation of extracellular signal-regulated kinase (ERK1/2) and B cell lymphoma/leukemia-2 (BCL-2) by the activation of α2-adrenoceptor [18] [37]. Previous reports suggest the antiapoptotic effect of dexmedetomidine on axoneuron is mediated by α2-adrenoceptor [12] [13]. In addition, some of the neuroprotective effects of dexmedetomidine are mediated via activation of ERK pathway [16] [38] [39]. On the other hand, one current study manifested that the neuroprotection of dexmedetomidine was mediated by BDNF [19] [40]. BDNF is one neurotrophin that can regulate the survival, development, and function of neurons and has been associated with inhibition of apoptosis [41] [16] [42]. The proBDNF is a precursor peptide encoded by the BDNF gene. Intracellular proBDNF can be cleaved by fusin or proprotein convertases to mature BDNF. Binding of BDNF to its high-affinity receptor TrkB activates Ras/ERK signal transduction pathway, which is important in the formation of synaptic plasticity and the function of learning and memory. While proBDNF activated the JNK pathway mediated its high-affinity receptor p75NTR [43] [44]. Our experiment demonstrated that dexmedetomidine pretreatment resulted in an increased expression of BDNF. Furthermore, dexmedetomidine administered in neonatal rats lead to a decrease expression of proBDNF, which was the first time to show further evidence of neuroprotection. The experimental results show that the inhibitors of TrkB and p75NTR reduced the expression of phospho-ERK and phospho-JNK, which suggested that at least a part of the activation of ERK and JNK was mediated by BDNF and proBDNF.

Clinically, there is no definite conclusion about the neurodegeneration by anesthetics of infants. According to the process of neural development, the 7-day old rat is equivalent to the age of 2–3 years in the human. However, if the neurodegeneration induced by anesthetics as a clinical problem in pediatric anesthesia, the application of α2-adrenergic agonist requires further study in newborns. So, this study was reasonable and meaningful and provided theoretical support for clinical application.

A limitation of our study is that it is not possible to determine if the beneficial effects of dexmedetomidine were due to attenuation of the up-regulation of BDNF, the down-regulation of proBDNF independently, or there is an interaction underlying the action mechanism. Nonetheless, dexmedetomidine pretreatment exhibited neuroprotection against isoflurane anesthesia.

Conclusions

In summary, our study results support our hypothesis that dexmedetomidine attenuated isoflurane-induced neurotoxicity in the hippocampus of neonatal rats through enhanced BDNF signaling via TrkB and reduced proBDNF signaling via p75NTR.

Abbreviations

BDNF
Brain-derived neurotrophic factor
p75NTR
p75 neurotrophin receptor
proBDNF
Proneurotrophin
TrkB
Tropomyosin receptor kinase B
ERK
Extracellular signal-regulated kinase
JNK
c-Jun N-terminal kinase

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Use Committee at Shengjing Hospital, China Medical University (Animal Welfare Assurance) and performed in accordance with the principles of Laboratory Care, supervised by a qualified veterinarian.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that there are no conflicts of interest.

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Authors' contributions

Xudong Ding had the idea for the article and worked on the project throughout. Hui Zhang and Shiwei Sun are responsible for the animal model establishment. Morphological results of this paper was performed by Chenguang Ma. Ningning Zheng was responsible for the writing and English polishing of this paper. Hongtao Liu provided the overall theoretical guidance for the project.

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Figures

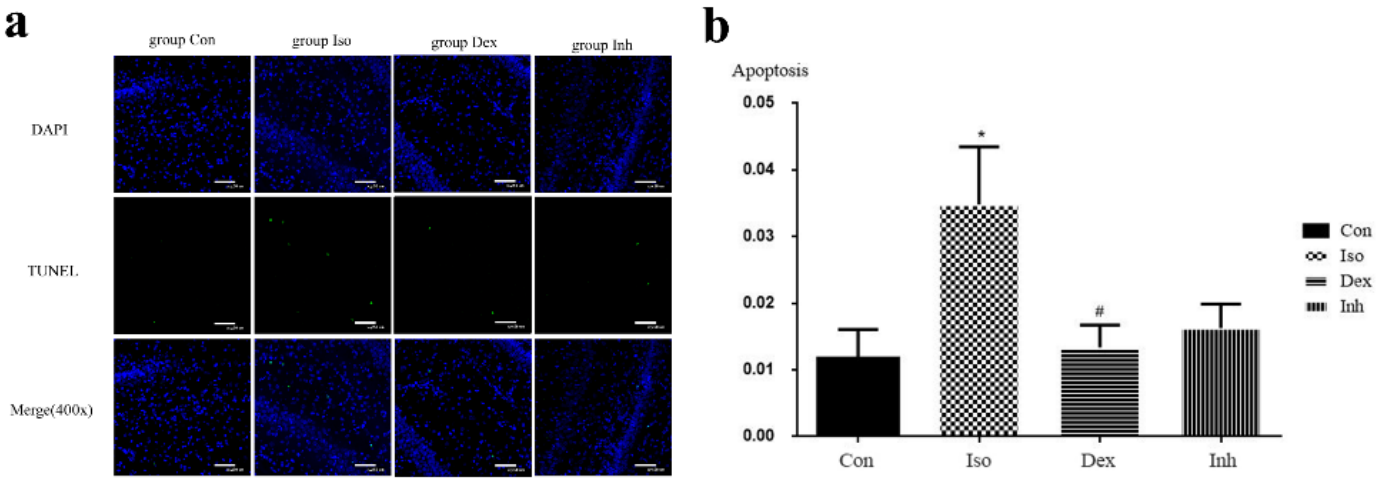

Figure 1

Dexmedetomidine attenuated the increase of isoflurane-induced neuroapoptosis in hippocampus of P7 rats (n=10 in each group, P<0.05). Representative images of TUNEL in hippocampal region (a) Green staining indicated TUNEL-positive cells and blue staining indicated nuclei. Scan bar-50 μm. Quantification of TUNEL-positive in the hippocampal region by one-way ANOVA with LSD's multiple comparisons. Results are the means□SD (b)*P<0.05 versus group Con; #P<0.05 versus group Iso.

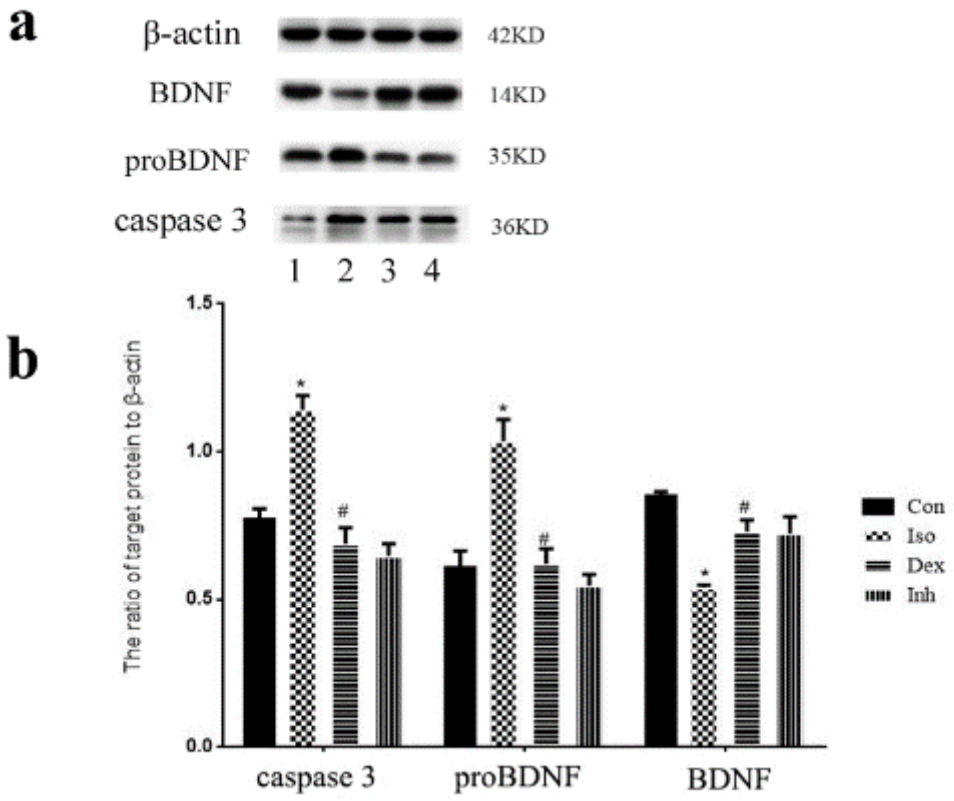

Figure 2

Dexmedetomidine reversed isoflurane-induced inhibition of BDNF and increase of proBDNF and caspase 3 in the hippocampus of P7 rats (n=8 in each group□P<0.05). (a) Representative western blot of BDNF, proBDNF, caspase 3; (b) the quantitative analysis of BDNF, proBDNF, caspase 3 by one-way ANOVA with LSD's multiple comparisons. Results are the mean SD. Lane1:group Con; Lane2:group Iso; Lane3:group Dex; Lane4:group Inh. *P<0.05 versus group Con; #P<0.05 versus group Iso.

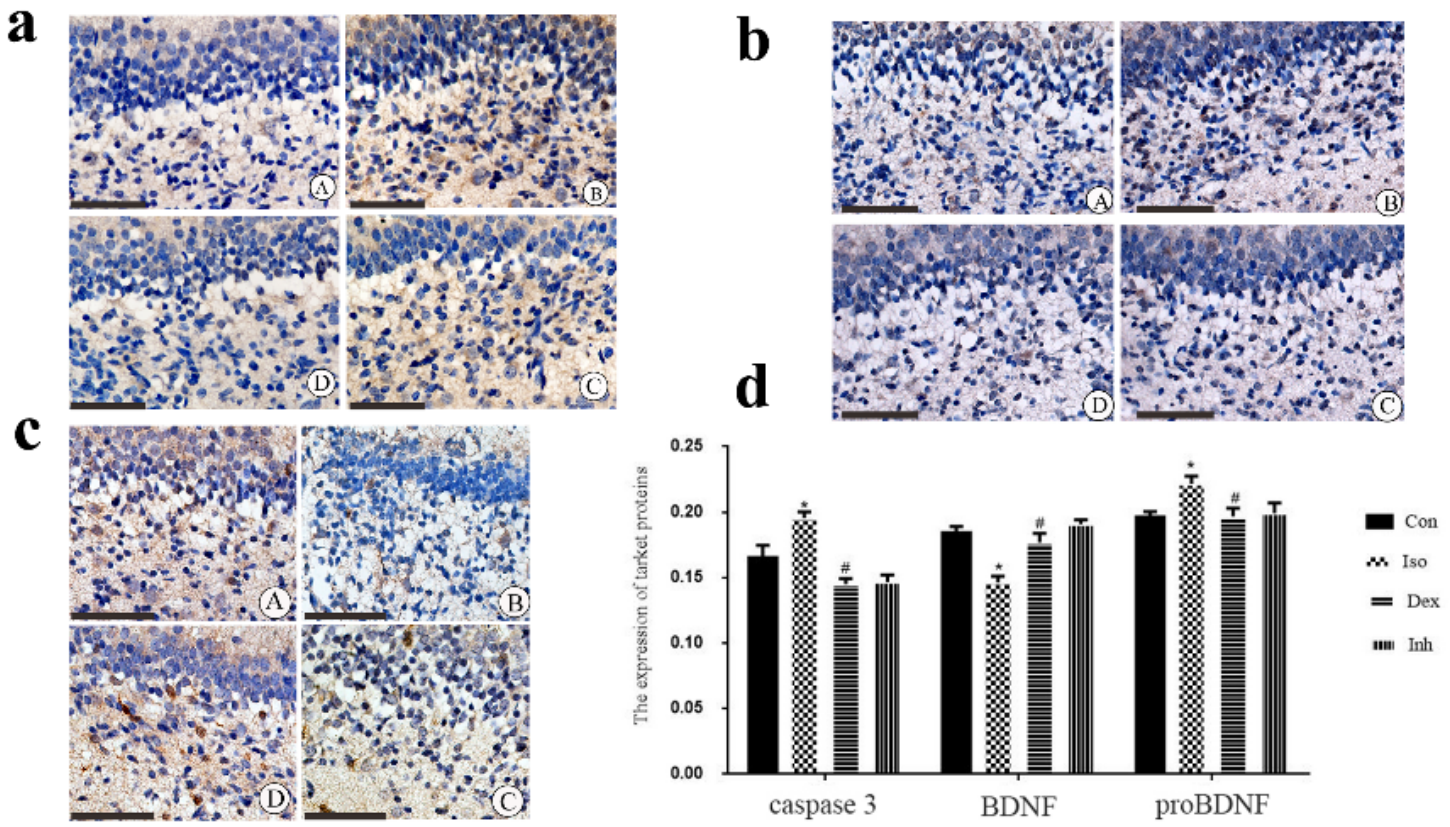

Figure 3

Dexmedetomidine reversed isoflurane-induced inhibition of BDNF and increase of proBDNF and caspase 3 in the hippocampus of P7 rats (n=10 in each group□P<0.05). (a)caspase 3; (b)proBDNF; (c)BDNF. Magnification=400x, Scan bar=50μm. A:group Con; B:group Iso; C:group Dex; D:group Inh. (d) The quantitative analysis of BDNF, proBDNF and caspase 3. *P<0.05 vs group Con; #P<0.05 vs group Iso.

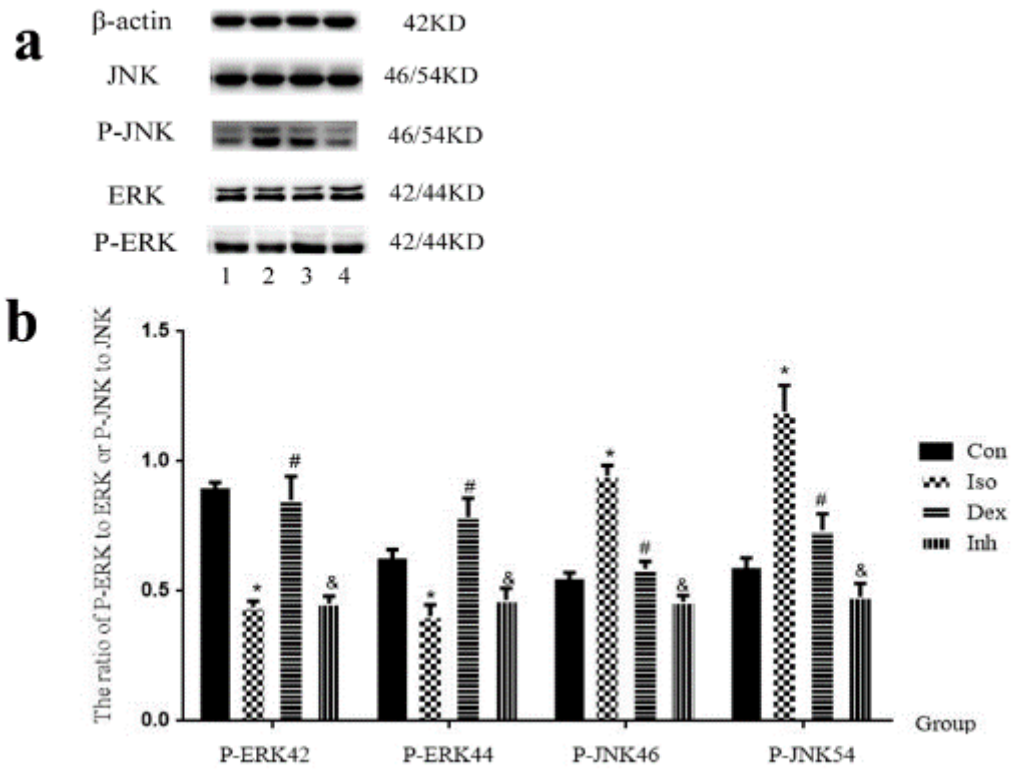

Figure 4

Dexmedetomidine reversed isoflurane-induced inhibition of P-ERK, and increase of P-JNK in the hippocampus of P7 rats (n=8 in each group, P<0.05). (a)Representative western blot of P-ERK and P-JNK; (b) The histogram represented the quantitative analysis of P-ERK and P-JNK by one-way ANOVA with LSD's multiple comparisons. Results are the mean□SD. Lane1:group Con; Lane2:group Iso; Lane3:group Dex; Lane4:group Inh. *P<0.05 versus group Con; #P<0.05 versus group Iso; & P<0.05 versus group Dex.

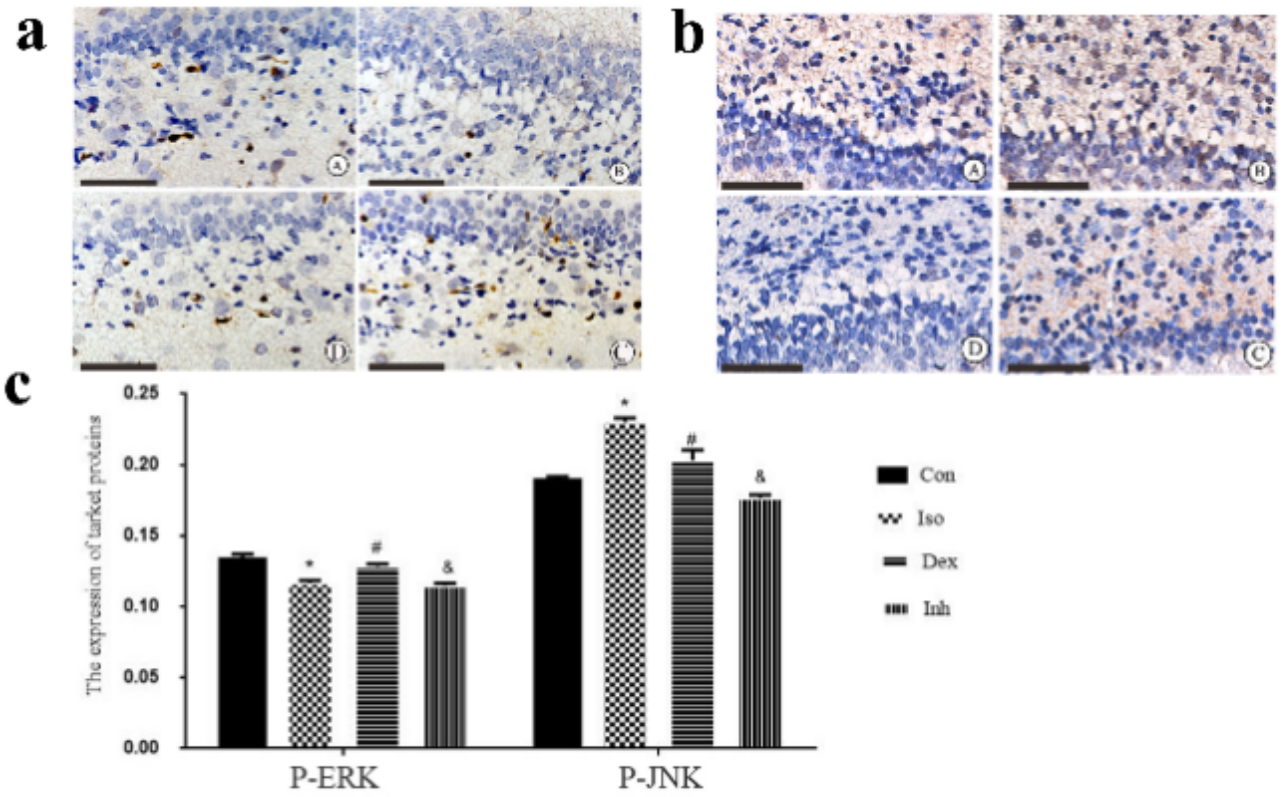

Figure 5

Dexmedetomidine reversed isoflurane-induced inhibition of P-ERK, and increase of P-JNK in the hippocampus of P7 rats (n=10 in each group, P<0.05). (b)(a),(b)Representative images of immunohistochemical (IHC) staining in the hippocampal region from various experimental groups and the sample sections were illustrated with high magnification (400x). Scan bar=50μm. a:P-ERK; b:P-JNK. A:group Con; B:group Iso; C:group Dex; D:group Inh. (c) The quantitative analysis of P-ERK and P-JNK by one-way ANOVA with LSD's multiple comparisons. Results are the mean□SD. *P<0.05 versus group Con; #P<0.05 versus group Iso; &P<0.05 versus group Dex.