**Dexmedetomidine alleviates acute postoperative anxiety in rats by suppressing the NF-κB pathway**

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**Abstract**: Evidence suggests that surgical procedures can effect the central nervous system and lead to changes in mood and behavior, rarely understood about the role of acute inflammation in promoting acute anxiety postoperatively. This study was designed to explore the possible mechanism of dexmedetomidine (DEX, a2- adrenergic receptor agonist) for reducing acute postoperative anxiety, which may be related to the activation of nuclear factor kappa B (NF-κB) and downstream signal pathway in the hippocampus. Experiments were conducted with rat, the elevated plus-maze and open field test were performed to evaluate anxiety-like behavior. Inhibit DEX with Atipamezole (AT, a2-adrenergic receptor antagonist) and inhibit NF-κB with Pyrrolidinedithiocarbamate (PDTC, inhibit phosphorylation of IkB, prevent the activation of NF-κB), the level of interleukin-6 (IL-6), IL-1β, IL-10 and Tumor necrosis factor-α (TNF-α); the nuclear translocation of NF-κB in the hippocampus and anxiety-like behavior were measured. Rats exhibited anxiety-like behavior at 6h and 12h after surgery. Preoperative administration of DEX significantly alleviated postoperative anxiety like behavior. DEX premedication inhibited the nuclear translocation of NF-κB alleviated acute postoperative anxiety. These findings are the first to show that acute postoperative anxiety may be related to NF-κB nuclear translocation in the hippocampus in rats, which can be alleviated by DEX premedication.

**Keywords**: NF-κB nuclear translocation, acute postoperative anxiety, inflammation, rats, dexmedetomidine.

**INTRODUCTION**

About 63% of children will experience perioperative anxiety (Lamiani et al., 2021). Surgery leads to local tissue damage, physical barrier damage and potential environmental and symbiotic microbial exposure, all of which may lead to local inflammation and then to a systemic inflammatory response and immunosuppression, while peripheral cytokine signals move to the brain and regulate neuronal activity in specific brain regions, particularly in the hippocampus (Alazawi et al., 2016; Ramlawi et al., 2006). However, little research appears on the role of acute inflammation in promoting acute anxiety postoperatively.

Inflammation is a key factor in acute and chronic central nervous system (CNS) disorders (Hou et al., 2022). It is believed that inflammation mediates important biochemical changes in specific areas of the brain, leading to the anxiety-like behavior (Salim et al., 2012). Studies *in vivo* also revealed that the immune system dysregulation had effect on the occurrence of anxiety (Robson et al., 2017; Maldonado-Bouchard et al., 2016). Hippocampus is implicated structurally and functionally in mood disorders (Michopoulos et al., 2015; Aguilera et al., 2003). NF-κB, a transcription factor, is expressed ubiquitously and can be activated by inflammatory and stress responses (Rhen et al., 2005). A large number of evidence indicates that anxiety behavior can be regulated by NF-κB (Salim et al., 2011; Maier et al., 1998).

Together, these data indicate preliminarily that acute postoperative anxiety is associated with inflammation mediated by NF-κB in the hippocampus.

DEX is a highly specific a2- adrenergic receptor agonist that has numerous applications. Previously, we showed that DEX premedication decreased postoperative anxiety effectively in children (Du et al., 2019). Some clinical settings and studies also demonstrated the anti-inflammatory effect of DEX is mediated by the inhibition of NF-κB activation (Zi et al., 2019; Kang SH et al., 2013). Inhibition of the NF-κB pathway may be the underlying mechanism of the neuroprotective action of DEX (Wang et al., 2017). The present study was aimed at investigating whether DEX decrease acute postoperative anxiety through suppressing NF-κB activation in the hippocampus in rats after hernia repair surgery.

**MATERIALS AND METHODS**

*Reagents and antibodies*

DEX (Hengrui Co., Ltd. Lianyungang, China); rat IL-1β ELISA kit (ke20005, Huaimei Bioengineering Co., Ltd. Wuhan, China); rat IL-6 ELISA kit (ke00007, Huaimei Bioengineering Co., Ltd.); rat IL-10 ELISA kit (ke00012, Huaimei Bioengineering Co., Ltd.); rat TNF-α ELISA kit (ke00068, Huaimei Bioengineering Co., Ltd.); p65 (ab16502, Abcam, England); p105 (ab16500, Abcam); proliferating cell nuclear antigen (PCNA, 10205-2-AP, Proteintech, USA); β-actin (60088-1-ig, Proteintech); Pyrrolidinedithiocarbamate (PDTC, S3633, Selleck, USA); Atipamezole (AT, S4650, Selleck, USA).
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**Animals**
Young Sprague-Dawley rats (equal number of males and females, 23–25 days, 60–80 g, Hunan Silaite Jingda Experimental Animal Co., Ltd., China, batch no. 4300470061675) were given food and water. In the first part, rats were randomly divided into seven groups (16 rats per group): 1) Group C: Only saline was intraperitoneally (i.p.) injected without surgery; 2) Group D 6h: DEX (50 μg/kg) was i.p. injected without surgery, behavioral and biochemical changes were measured postoperative 6h; 3) Group D 12h: DEX (50 μg/kg) was i.p. injected without surgery, behavioral and biochemical changes were measured postoperative 12h; 4) Group S 6h: Saline was i.p. injected; surgery was performed 30 mins later, behavioral and biochemical changes were measured postoperative 6h; 5) Group S 12h: Saline was i.p. injected; surgery was performed 30 mins later, behavioral and biochemical changes were measured postoperative 12h; 6) Group D+S 6h: DEX (50 μg/kg) was i.p. injected; surgery was performed 30 mins later, behavioral and biochemical changes were measured postoperative 6h; 7) Group D+S 12h: DEX (50 μg/kg) was i.p. injected; surgery was performed 30 mins later, behavioral and biochemical changes were measured postoperative 12h. In the second part, rats were also randomly divided into five groups (16 rats per group): 1) Group C: Only saline was i.p. injected without surgery; 2) Group S: Saline was i.p. injected, surgery was performed 30 mins later; 3) Group D+S: DEX (50 μg/kg) was i.p. injected, surgery was performed 30 mins later; 4) Group D+S+AT: DEX (50 μg/kg) and AT (250 μg/kg) were i.p. injected, surgery was performed 30 mins later; 5) Group S+PDTC: PDTC (100 mg/kg) was i.p. injected, surgery was performed 30 mins later. In this part, behavioral and biochemical changes were measured 6h postoperatively.

**Hernia repair surgery**
Hernia repair surgery was performed 30 min after the i.p. injections. The rats were anesthetized with sevoflurane (5% induction, 2–2.5% maintenance, oxygen flow 3 L/min). The right groin was shaved and disinfected with iodophor and a 1.5-cm incision through the skin, fascia and muscle were made with a surgical blade. The incision was enlarged with blunt scissors and the inguinal tissue was dissociated from the spermatic cord. A thin rubber tube was used to help the spermatic cord separate from the surrounding tissues. All tissues in the spermatic cord except the blood vessels and vas deferens were ligated twice with 2-0 silk thread. The skin was sutured with 4-0 antibacterial suture, pressure was applied to stop the bleeding and the wound was cleaned (Bree et al., 2015).

**Behavioral analysis**
All behavioral tests were conducted in the morning in a quiet environment. The researchers who administered the drug did not conduct the test. Thirty minutes before the test, the rats were familiarized with the surrounding environment and testers. To eliminate inter-observer variability, one observer scored each parameter for all rats.

**Elevated plus-maze**
The elevated plus-maze test is a classic experiment for measuring anxiety behavior (Komada et al., 2008). The experimental device is made of wood and include four arms. Two open arms (30 cm × 6 cm) are surrounded by a small wall made from 2 cm high plastic plate to prevent the rats from falling; two closed arms of the same size perpendicular to the open arm are surrounded by a 15 cm high wall. All arms are 40 cm above the ground. Same arms are opposite each other and there is an square (6 cm × 6 cm) in the middle of the maze. At the beginning of the experiment, each rat was placed in the square opposite a closed arm and was allowed to explore the maze freely for 5 min. The rats’ movements were recorded with a digital camera placed directly above the intersection and were scored manually when viewing the video tape. The open arm entries, the time spent in the open arms and the total number of arm entries (i.e., all four arm entries) were analyzed. Anxiety-like behavior was characterized by a decrease in entering and staying in the open arm. The total number of arm entries was a measure of general activity. After each individual test, the device was cleaned and dry completely to prevent subsequent rats from being affected by the odor deposited by the previous rat.

**Open field test**
The open field test was performed in the same room under the same conditions as the elevated plus-maze test (Dunn et al., 2023). The experimental device was a large wooden box (100 cm × 100 cm) surrounded by a 45 cm high wall and in dim light. The square of the wooden space is divided into 16 same squares with white pinstripes. Each rat was placed in the center, facing the same direction all the time. The rats were move freely for 5 min. The digital camera was placed in a high location to record the rats’ behavior. The measurements included the time spent in the central area, the frequency of central area entry (four squares in the center) and the total distance (represented by the number of crossed lines). The time spent in the central area and the frequency of central area entry are measurements of anxiety behavior, movement distance reflects motor function.

**Collection of serum and tissues**
Following the elevated plus-maze and open field tests, the rats were anesthetized with sevoflurane and decapitated. The blood was collected into 1.5-mL test tubes and allowed to coagulate at 4°C for 1 h. The sample was centrifuged at 4°C for 15 min at 3000 × g, then the supernatant was divided into equal parts and stored at -80°C. Hippocampal tissue was isolated from the brain at 4°C, frozen in liquid nitrogen then stored at -80°C.
Enzyme-linked immunosorbent assay
We measured the rat IL-1β, IL-6, IL-10 and TNF-α levels with sandwich ELISA according to the kit instructions.

Western blot analysis
Hippocampal tissue (0.03 g) was minced. Lysis buffer (200 U/L) was added to extract the total protein from the biological samples and then cytoplasmic protein and nuclear protein were extracted. We prepared 10% separation glue and added TEMED (N, N′, N″, N‴-tetramethylthelylenediamine). Then, loading buffer was added to the cytoplasmic protein and nuclear protein. The foramen primum was injected with 3.5 μL marker and 10μL denatured protein was loaded into each blank for electrophoresis. The electrophoresis was run at a constant current of 300 mA for about 84 min for p65, 90 min for p105, 58 min for PCNA and 62 min for β-actin. After the transfer of the blots to PVDF membranes, the membrane was incubated with the primary antibody and secondary antibody. Finally, color development was performed.
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Fig. 3. Effect of dexmedetomidine on NF-κB nuclear translocation in hippocampal. P65 (a) and p105 (c) levels in the hippocampal cytoplasm, p65 (b) and p105 (d) levels in the hippocampal nuclei 6 h postoperative; Data were presented as the means ± SD; (n=8). Analysis was performed using the univariate analysis of variance (ANOVA) plus Tukey multiple comparison. *P < 0.05, **P < 0.01 VS group C; #P < 0.05, ##P < 0.01 VS group S; †P < 0.05, ††P < 0.01 VS group D+S.

Fig. 4. Effect of NF-κB on acute postoperative anxiety in rats. Anxiety-like behavior was tested with the elevated plus-maze and open field test. Open arm entries (%) (a), time spent in the open arm (sec) (b), The total number of arm entries (c), time spent in the central area (%) (d), and frequency of central area entry (e), the total distance travelled (f). Data were presented as the means ± SD; (n=8). Analysis was performed using the univariate analysis of variance (ANOVA) plus Tukey multiple comparison. *P < 0.05, **P < 0.01 VS group C; #P < 0.05, ##P < 0.01 VS group S; †P < 0.05, ††P < 0.01 VS group D+S.
Ethical approval
The study was carried out after obtaining permission from the Hunan Children's Hospital medical ethics committee (HCHLL-2016-002).

STATISTICAL ANALYSIS
All studies were conducted by an investigator blinded to the treatment. All data are expressed as the mean ± standard deviation (SD). Univariate analysis of variance (ANOVA) plus Tukey multiple comparisons were used for comparison between groups. P<0.05 was considered significant. Statistical analysis was performed with SPSS software (version 20.0.0; IBM Corp, Armonk, NY).

RESULTS

Effect of DEX premedication on acute anxiety-like behavior after hernia repair surgery in rats
Anxiety-like behavior was measured by elevated plus-maze and open field test. In the elevated plus-maze, group S6h and S12h spent a shorter time in the open arms (p<0.01) and lower percentages of open arm entries (p<0.01) compared to the group C; Group D+S 6h and D+S 12h spent a longer time in the open arms (p<0.01) and higher percentages of open arm entries (p<0.01) than group S6h and S12h (fig. 1a,1b), no significant differences in the total number of arm entries (fig. 1c). In the open field test, group S6h and S12h had lower frequency of central area entry (p<0.01) and shorter time spent in the central area (p<0.01) compared to group C; Group D+S 6h and D+S 12h had a higher frequency of central area entry (p<0.01) and longer time spent in the central area (p<0.01) than group S6h and S12h (fig. 1d,1e), no significant differences in the total distance (fig. 1f). And no significant differences in elevated plus-maze and open field test between group C and group D6h and group D12h.

These results suggest that, in rats, inguinal hernia repair surgery results in acute anxiety-like behavior. DEX premedication can significantly alleviate their anxiety-like behavior after surgery. And there were no obvious anxiolytic effects by DEX itself.

Effect of DEX on inflammatory response and NF-κB nuclear translocation in hippocampus
Serum pro-inflammatory and anti-inflammatory cytokines were detected by ELISA. Compared with group C, serum levels of IL-1β (p<0.01), IL-6 (p<0.01), TNF-α (p<0.01) and IL-10 (p<0.01) increased 6h postoperative; Compared with group S, group D+S and group S+PDTC had decreased serum IL-1β (p<0.01), IL-6 (p<0.01) and TNF-α (p<0.01) and increased IL-10 (p<0.01); Compared with group D+S, group D+S+AT had decreased serum IL-1β (p=0.0435), IL-6 (p=0.0394) and TNF-α (p<0.01) and increased IL-10 (p<0.01) (fig. 2).

The changes in NF-κB subunits p65 and p105 in the hippocampal cytoplasm and nucleus were analyzed using western blotting. Compared with group C, p65 (p<0.01) and p105 (p<0.01) levels in the hippocampal cytoplasm were significantly decreased and significantly increased p65 (p<0.01) and p105 (p<0.01) levels in the hippocampal nuclei 6h postoperative; Compared with group S, group D+S and group S+PDTC had decreased p65 (p<0.01) and p105 (p<0.01) levels in the hippocampal cytoplasm and increased p65 (p<0.01) and p105 (p<0.01) in the hippocampal nuclei; Compared with group D+S, group D+S+AT had decreased p65 (p=0.027) and p105 (p=0.0315) levels in the hippocampal cytoplasm and increased p65 (p<0.01) and p105 (p=0.0211) in the hippocampal nuclei (fig. 3).

These results suggest that surgery can induce inflammatory responses in rats. And DEX premedication can significantly reduce the inflammatory responses induced by surgery through NF-κB pathway.

Effect of NF-κB on acute postoperative anxiety in rats
In the elevated plus-maze test, group S and group D+S+AT spent a shorter time in the open arms (p<0.01) and lower percentages of open arm entries (p<0.01) compared to the group C; group D+S spent a longer time in the open arms (p<0.01) and higher percentages of open arm entries (p<0.01) than group D+S+AT; group D+S and group S+PDTC spent a longer time in the open arms (p<0.01) and higher percentages of open arm entries (p<0.01) than group S (fig. 4a,4b), no significant differences in the total number of arm entries (fig. 4c). In the open field test, group S and group D+S+AT had lower frequency of central area entry (p<0.01) and shorter time spent in the central area (p<0.01) compared to group C; group D+S had higher frequency of central area entry (p<0.01) and longer time spent in the central area (p<0.01) than group D+S+AT; group D+S and group S+PDTC had higher frequency of central area entry (p<0.01) and longer time spent in the central zone (p<0.01) than group S (fig. 4d,4e), no significant differences in the total distance (fig. 4f).

These results suggest that DEX premedication can significantly alleviate acute postoperative anxiety-like behavior by suppressing the NF-κB pathway.

DISCUSSION
The present study, focusing on acute anxiety induced by surgery in an animal model, proves that inflammation induced by NF-κB activation in the hippocampus may promote the development of acute postoperative anxiety, which can be inhibited by DEX premedication.
Anxiety can inhibit the exploratory behavior of rats (Crawley et al., 1985). In the present study, rats in the surgery groups showed increased anxiety-like behavior and decreased interest in 6h and 12h postoperatively. DEX premedication significantly reduced the acute postoperative anxiety in rats.

The pathophysiology underlying anxiety is not fully understood. Regulation of the inflammatory response can promote the recovery of neurological function and decreasing anxiety-like behavior, as the IL-1β, IL-6 and TNF-α may play a key role in negative affects such as anxiety (Wüst et al., 2004; Sallinen et al., 1997; Pyndop et al., 2014; Khandaker et al., 2014; Zhang et al., 2017). Consistent with these findings, our results showed that the significantly increase of IL-1β, IL-6 and TNF-α, while IL-10 was decreased at 6h postoperatively in the rats. The rats that had undergone surgery showed obvious corresponding anxiety-like behavior. DEX premedication reduced the postoperative inflammatory reaction and alleviated acute postoperative anxiety. These results suggest that acute postoperative anxiety correlates positively with inflammation, at least to a certain extent.

NF-κB as a key role in regulating emotional disorders in the CNS (Cohen et al., 2011). Our results demonstrate that surgery induces NF-κB activation in the hippocampus. The accumulation of p105 and p65 promotes the activation of NF-κB (Meffert et al., 2005; Mincheva-Tasheva et al., 2013). NF-κB activation in the hippocampus can lead to the induction of pro-inflammatory cytokine genes, further increasing the level of pro-inflammatory cytokines in the brain, finally activating the inflammatory response, resulting in inflammation-related acute anxiety (Haftcheshmeh et al., 2022; Cheng et al., 2017; Rump et al., 2022; Koo et al., 2010; Mattson et al., 2001).

At present, our understanding of postoperative mental and behavioral disorders, including anxiety, is limited and postoperative management is still a challenge. Our results indicate that acute postoperative anxiety is associated with neuroinflammation. In a variety of postoperative neurodegenerative diseases, the increases of inflammatory cytokines in the CNS were induced by immune activation and leads to symptoms, such as anxiety (Fidalgo et al., 2023; Price et al., 2014). These findings indicate that targeting inflammatory cytokines and their signaling pathways may be a new strategy for treating postoperative anxiety and other harmful neurological sequelae.

In this study, we found that DEX inhibited the activation of NF-κB in the hippocampus of young Sprague-Dawley rats, thereby decreasing anxiety-like behavior. It is unclear how DEX inhibits NF-κB activation in the hippocampus, although its anti-inflammatory effect may be related to its central sympathetic action and/or cholinergic anti-inflammatory pathway (Zi SF et al., 2019; Iovino et al., 2020). DEX reduced the initial and subsequent inflammatory cytokine peaks, protecting the host from the excessive systemic consequences of primary injury, which may be an important reason for alleviating acute postoperative anxiety. This explanation may also help us understand why different anesthetics lead to different emotional responses in animal research or clinical practice (Luo et al., 2015).

In animal models of systemic inflammatory response syndrome, DEX reduces the severity of neuroinflammation and neuronal apoptosis (Kho et al., 2022). After DEX infusion, the incidence of postoperative delirium was reduced by 60% and the risk of POCD was reduced by 40% (Su et al., 2016; Zhou et al., 2016). The results in this study also indicate that the premedication of DEX reversed the effect of the surgery on acute anxiety-like behavior. Our study may expand the existing knowledge of the protective mechanism and clinical application of DEX. Although the application of DEX shows good results, its use in various clinical environments requires further optimization to improve its efficacy and safety.

There are some deficiencies in our research. First, the DEX dosage is 50μg/kg, which has a good anti-inflammatory effect in young Sprague-Dawley rats, while the clinical dosage is 0.5–10μg/kg (Yang et al., 2011). Although the application of high-dose DEX (5–15μg/kg) is feasible in the clinic, some risks remain (Linden et al., 2005). Studies are needed to determine the optimal concentration of DEX in animals and humans in the future. Second, we only explored the relationship between acute postoperative anxiety and peripheral inflammation and neuroinflammatory reaction 6h and 12h postoperatively. The expression of NF-κB nuclear translocation, inflammatory cytokines and postoperative acute anxiety may change with time. Therefore, it is necessary to extend the observation time in future research to further understand the effect of the inflammatory reaction on postoperative anxiety. Third, although pain is one of the important factors influencing postoperative anxiety, we did not observe acute postoperative pain in the rats. Finally, although the hippocampus is one of the key areas of CNS emotion management, the study of biochemical changes in other brain regions may aid the comprehensive assessment of the potential relationship between inflammation and anxiety.

**CONCLUSION**

In conclusion, our results provide novel evidence that the positive correlation between acute postoperative anxiety and postoperative inflammation which may be related to the activation of NF-κB in the hippocampus. A decrease
in neuroinflammation may be accompanied by a reduction in acute postoperative anxiety.

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REFERENCES


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