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Research Report

17β-estradiol attenuates ketamine-induced neuroapoptosis and persistent cognitive deficits in the developing brain

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ABSTRACT

Previous studies have demonstrated that the commonly used anesthetic ketamine can induce widespread neuroapoptosis in the neonatal brain and can cause persistent cognitive impairments as the animal matures. Therefore, searching for adjunctive neuroprotective strategies that inhibit ketamine-induced neuroapoptosis and persistent cognitive impairments is highly warranted. The primary goal of this study was to investigate the protective effect of 17β-estradiol against ketamine-induced neuroapoptosis and persistent cognitive impairments in adult rats. Starting from postnatal day 7, Sprague-Dawley male rat pups were given a daily administration of ketamine (75 mg/kg, i.p.) or 17β-estradiol (600 μg/kg, s.c.) in combination with ketamine (75 mg/kg, i.p.). The animals were treated for three consecutive days. 24 h after the last injection, the rats were decapitated, and the prefrontal cortex (PFC) was isolated to detect neuroapoptosis by cleaved caspase-3 immunohistochemistry and by using the TUNEL assay. The neuroactive steroid 17β-estradiol was quantified using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The protein levels of BDNF and pAkt were measured by western blot analysis. At two months of age (60 days), the learning and memory abilities were tested using the Morris water maze. The results showed that ketamine triggered significant neuroapoptosis in the neonatal PFC accompanied by the downregulation of 17β-estradiol, BDNF and pAkt. The co-administration of 17β-estradiol with ketamine attenuated these changes. Moreover, 17β-estradiol significantly reversed the learning and memory deficits observed at 60 days of age. In brief, our present data demonstrate that 17β-estradiol attenuates ketamine-induced neuroapoptosis and reverses long-term cognitive deficits in developing rats and thus may be a potential therapeutic and neuroprotective method for the treatment of neurodevelopmental disorders.

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1. Introduction

Exposure to anesthetics is associated with widespread apoptotic neurodegeneration in developing brains and persistent cognitive impairments as the animal matures (Paule et al., 2011; Kong et al., 2011; Brambrink et al., 2012; Huang et al., 2012). Clinical studies also indicate that some learning disabilities and behavioral disturbances in children correlate with anesthetic exposure during surgery before 4 years of age, even in children experiencing only a single exposure to anesthesia (Flick et al., 2011; Sprung et al., 2012). Ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor ion channel blocker, is a commonly used anesthetic in pediatric patients; current studies have suggested that ketamine induces neuroapoptosis in both developing animal brains (Paule et al., 2011; Brambrink et al., 2012; Huang et al., 2012) and primary cultured neurons (Liu et al., 2013a; Li et al., 2013), and results in persistent cognitive deficits as the animal matures (Paule et al., 2011; Huang et al., 2012). To date, the mechanisms underlying the deleterious effects of ketamine exposure on the developing brain are largely elusive.

BDNF is one of the major neurotrophic factors present in the microenvironment involved in nerve regeneration and thus exerts a direct influence on the development of neurons (Xiao et al., 2010). A previous study indicated that the NMDAR antagonist MK-801 induces apoptotic neurodegeneration in the developing rat brain by depressing BDNF-mediated intracellular signaling, such as the PI3K-Akt pathway (Dzietko et al., 2004). However, whether the BDNF-mediated intracellular signaling pathway is involved in the neurotoxic effects of ketamine is still unknown.

Steroid hormones and their metabolites present within the central nervous system (CNS) are commonly defined as neuroactive steroids or neurosteroids (Melcangi et al., 2008). They can be either synthesized *de novo* in the CNS by glial cells and neurons from cholesterol or synthesized in the periphery by the adrenals and gonads. The concentration of neuroactive steroids is higher in the CNS than in the periphery. Neuroactive steroids act as important physiological regulators of nervous function with roles in mood, behavior, reproduction, and cognition and act as protective agents in models of injury and disease, including experimental models of Alzheimer's disease (AD), traumatic brain injury, stroke, autism, and mood disorders (Melcangi et al., 2008; Panzica et al., 2012; Schumacher et al., 2012). Moreover, neuroactive steroids exert key regulatory and protective roles in the fetal brain (Hirst et al., 2013). Recently, many groups have initiated research in the field of neuroactive steroid-induced neuroprotection. The neuroactive steroid estradiol has neuroprotective potential against a variety of toxic insults, including excitotoxicity, substantia nigra degeneration, ischemia, beta-amyloid toxicity, and oxidative stress (Melcangi et al., 2011; Schreihofer and Ma, 2013). It is well established that estrogens influence memory and cognition (Logan et al., 2011). A previous study suggested a possible protective role for 17β-estradiol in the NMDA receptor antagonist MK801-mediated apoptotic neurodegeneration in the developing brain by activating pro-survival proteins, which may represent one mechanism for its neuroprotective action (Asimiadou et al., 2005). Moreover, our most recent study suggested that 17β-estradiol could protect primary cultured rat cortical neurons from ketamine-induced neuroapoptosis by activating PI3K/Akt/Bcl-2 signaling (Li et al., 2013).

While the previous studies represent a broad range of actions for 17β-estradiol, the protective effects of 17β-estradiol against ketamine-induced neuroapoptosis in the developing brain and long-term behavioral deficits as animals mature have not fully been explored. Because the use of ketamine in pediatric and obstetric anesthesia is a necessity that cannot be avoided, the question becomes whether the use of 17β-estradiol as an adjuvant agent could aid in reducing the neurotoxic effects of ketamine such that ketamine could be used to its full therapeutic advantage. To address these questions, we investigated the neuroprotective effects of 17β-estradiol against the neurotoxic effects of ketamine, which has been shown to induce severe apoptotic neurodegeneration and long-term behavioral deficits in developing rats (Huang et al., 2012). We also analyzed the possible molecular mechanisms that could facilitate the neuroprotective effect of 17β-estradiol.

2. Results

2.1. 17β-estradiol exerts neuroprotective effects against ketamine-induced neuroapoptosis in the PFC

To investigate whether 17β-estradiol treatment can ameliorate apoptotic neurodegeneration induced by ketamine in the developing rat brain, we administered 17β-estradiol and/or ketamine to P7 rats and analyzed the prefrontal cortex (PFC) 24 h after the last injection. Neuroapoptosis was determined by cleaved caspase-3 immunohistochemistry and the TUNEL assay. Compared to the control group, a robust degenerative reaction was detected in the PFC of the ketamine-treated group ($P<0.01$). The administration of 17β-estradiol had no influence on the amount of ongoing physiological apoptosis ($P>0.05$). However, the co-administration of 17β-estradiol with ketamine significantly ameliorated the neuroapoptosis induced by ketamine exposure ($P<0.01$). The data are shown in Fig. 1.

2.2. 17β-estradiol treatment normalizes the endogenous 17β-estradiol levels in the PFC

Estradiol synthesized locally by neurons and astrocytes participates in the regulation of the survival of developing neurons, including those in the neocortex (Arevalo et al., 2012). To investigate whether ketamine-induced neuroapoptosis is accompanied by changes in endogenous 17β-estradiol, we analyzed the 17β-estradiol level in PFC tissue by HPLC-MS/MS analysis. As shown in Fig. 2, a significant reduction in 17β-estradiol was detected in the ketamine-treated group ($P<0.01$), indicating that endogenous 17β-estradiol is compromised following ketamine exposure in the neonatal rat brain. However, 17β-estradiol treatment restores the level of 17β-estradiol compared to ketamine treatment alone ($P<0.01$).