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NEONATAL KETAMINE EXPOSURE CAUSES IMPAIRMENT OF LONG-TERM SYNAPTIC PLASTICITY IN THE ANTERIOR CINGULATE CORTEX OF RATS

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Abstract—Ketamine, a dissociative anesthetic most commonly used in many pediatric procedures, has been reported in many animal studies to cause widespread neuroapoptosis in the neonatal brain after exposure in high doses and/or for a prolonged period. This neurodegenerative change occurs most severely in the forebrain including the anterior cingulate cortex (ACC) that is an important brain structure for mediating a variety of cognitive functions. However, it is still unknown whether such apoptotic neurodegeneration early in life would subsequently impair the synaptic plasticity of the ACC later in life. In this study, we performed whole-cell patch-clamp recordings from the ACC brain slices of young adult rats to examine any alterations in long-term synaptic plasticity caused by neonatal ketamine exposure. Ketamine was administered at postnatal day 4–7 (subcutaneous injections, 20 mg/kg given six times, once every 2 h). At 3–4 weeks of age, long-term potentiation (LTP) was induced and recorded by monitoring excitatory postsynaptic currents from ACC slices. We found that the induction of LTP in the ACC was significantly reduced when compared to the control group. The LTP impairment was accompanied by an increase in the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated excitatory synaptic transmission and a decrease in GABA inhibitory synaptic transmission in neurons of the ACC. Thus, our present findings show that neonatal ketamine exposure causes a significant LTP impairment in the ACC. We suggest that the imbalanced synaptic transmission is likely to contribute to ketamine-induced LTP impairment in the ACC.

Key words: ketamine, anterior cingulate cortex, long-term potentiation, excitatory synaptic transmission, inhibitory synaptic transmission.

INTRODUCTION

There is a growing concern that exposure to anesthetics during the neonatal period may cause neurotoxic injury in brain neurons that results in alterations of brain functions later in life. Both human and animal studies have indicated an association between early exposure to anesthetics and subsequent development of learning disabilities (Bouman et al., 1999; Wilder et al., 2009; Wang and Orser, 2011; Murphy and Baxter, 2013; Wang et al., 2013; Yu et al., 2013). Ketamine, a non-competitive *N*-methyl-D-aspartate receptor (NMDAR) antagonist, is commonly used for inducing anesthesia in a variety of pediatric procedures (Durrmeyer et al., 2010; Tsze et al., 2012; Asadi et al., 2013; Nielsen et al., 2013). Many animal studies have shown that ketamine can produce widespread neuroapoptosis in the neonatal brain after exposure in high doses and/or for a prolonged period (Olney et al., 2002; Zou et al., 2009a,b; Paule et al., 2011; Huang et al., 2012). It is suggested pathophysiologically that the development of neuroapoptosis in the neonatal brain is caused by a compensatory up-regulation of NMDARs during the time when the ketamine exposure is withdrawn, which subsequently leads to Ca^{2+} overload in turn triggering the apoptotic cascade (Shi et al., 2010; Liu et al., 2011; Sinner et al., 2011). A study of neurodevelopment indicates that exposure to ketamine in high doses affects the neurogenesis of rat cortical neural stem progenitor cells that subsequently alters normal brain development (Dong et al., 2012). Several studies by our and other groups also suggest that ketamine-induced apoptosis involves a pathological mechanism of learning and memory deficits that are associated with long-term

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Abbreviations: ACC, anterior cingulate cortex; ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; D-APV, D-(−)-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; eEPSCs, evoked excitatory postsynaptic currents; EGTA, ethylene glycol tetraacetic acid; HEPES, hydroxyethyl piperazineethanesulfonic acid; LTP, long-term potentiation; mEPSCs, miniature excitatory post-synaptic current; mIPSCs, miniature inhibitory post-synaptic currents; NMDA, *N*-methyl-D-aspartate; TBS, theta-burst stimulation; TTX, Tetrodotoxin.

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anesthetic treatment (Viberg et al., 2008; Paule et al., 2011; Huang et al., 2012; Womack et al., 2013). Thus, these findings imply that the development of apoptotic neurodegeneration induced by ketamine exposure during the neonatal period may contribute to long-lasting learning and memory deficits later in life.

According to the previous studies, several brain regions develop widespread neuroapoptosis after neonatal exposure to ketamine (Ikonomidou et al., 1999; Zou et al., 2009b). Among them, the anterior cingulate cortex (ACC), a key structure of the forebrain region, is severely affected by neuroapoptotic injury (Ikonomidou et al., 1999; Zou et al., 2009b). Substantial evidence has been accumulated to support the important role of the ACC in learning and memory (Frankland et al., 2004; Goshen et al., 2011; Einarsson and Nader, 2012). Activity-dependent synaptic plasticity is commonly thought to play a pivotal role in various kinds of behaviors ranging from development to learning and memory (Martin et al., 2000; Malenka and Bear, 2004; Zhuo, 2008). It is well-established that NMDARs are critically involved in multiple forms of synaptic plasticity in the brain, including long-term potentiation (LTP) and long-term depression (LTD) (Liu et al., 2004; Berberich et al., 2007; Unoki et al., 2012; Volianskis et al., 2013). A previous study reported that neonatal NMDARs antagonist exposure results in a lasting reduction in synaptic strength in the hippocampus (Bellinger et al., 2002). However, there have been few studies documenting the long-lasting effect of early treatment with ketamine on ACC synaptic plasticity. Therefore, using a rat model of ketamine exposure that has been validated to cause neuroapoptosis in neonatal brains (Zou et al., 2009b; Shi et al., 2010; Liu et al., 2011; Brambrink et al., 2012), the present study examined whether neuroapoptotic degeneration that develops in neonatal brains would subsequently produce long-term deleterious effects on synaptic plasticity in the ACC later in life. By performing *in vitro* whole-cell patch-clamp recordings from the pyramidal neurons of ACC slices, we found that the induction of LTP was profoundly suppressed in rats that were exposed to ketamine neonatally. To determine the mechanisms for this LTP impairment, we also addressed the changes in the intrinsic excitatory and inhibitory neurotransmission in the ACC following neonatal ketamine exposure. Our results demonstrate the imbalance of synaptic excitation and inhibition in the ACC of ketamine-treated rats.

EXPERIMENTAL PROCEDURES

Animals

Rats (Sprague–Dawley, male and female) in age groups of postnatal 4–7 days (PND 4–7) and 3–4 weeks were used in this study. Rats were housed under a 12–12-h constant light/dark cycle in a temperature (22–25 °C)- and humidity (55–60%)-controlled environment with free access to food and water. The study was carried out according the protocols approved by the Institutional Animal Care and Use Committee of the University of Texas at Arlington.

Neonatal treatment with ketamine

According to the previous studies, the window of vulnerability to the neurotoxic effect of ketamine is restricted to the period of rapid synaptogenesis, which occurs at ages of PND 0–14 and peaks between 3 and 7 days after birth (Ikonomidou et al., 1999; Wang and Slikker, 2008). Thus, neonatal ketamine treatment was performed on the same day in PND 4–7 rat pups that were randomly assigned to groups of drug and saline treatments. For the drug treatment group, they were administered subcutaneously with ketamine six times at 2-h intervals at the dose of 20 mg/kg per injection (Zou et al., 2009b). During drug treatment, animals were maintained at a light-anesthetized level as evidenced by lack of voluntary movement, and minimal reaction to the physical stimulation. Animals in the control group were administered with saline for the same number of injections at the volume of 0.02 ml. Pups were returned to their dams to help maintain body temperature and reduce stress between each treatment. During drug treatment, body temperature was maintained at 37 °C using a thermostatical heating blanket. Data obtained from arterial blood gas and glucose analysis by other groups in neonatal animals that were exposed to ketamine do not support that apoptotic degeneration was attributable to metabolic or respiratory distress (Jevtovic-Todorovic et al., 2003; Slikker et al., 2007). Further, several studies using the same protocol of ketamine administration have confirmed the apoptotic neurodegeneration in the neonatal rat brain (Ikonomidou et al., 1999; Zou et al., 2009b; Shi et al., 2010; Liu et al., 2011).

Brain slices preparation

At the age of 3–4 weeks, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and ACC slices were obtained according to previously-published procedures (He et al., 2009; Gong et al., 2010; Jin et al., 2013; Lu et al., 2013). Briefly, after decapitation, the whole brain was carefully removed and quickly transferred to an ice-cold bath of artificial cerebrospinal fluid (ACSF; composition in mM: NaCl 124, KCl 3.3, KH_2PO_4 1.2, $CaCl_2$ $2H_2O$ 2.5, $MgSO_4$ 2.4, $NaHCO_3$ 26, and glucose 10). The bath was continuously bubbled with a 95% O_2/5% CO_2 gas mixture (pH 7.3–7.4). After cooling for about 1 min, the anterior half of the brain was dissected, and the brain block was glued onto the stage of a vibrating tissue slicer (DTK-1000, Dosaka EM. Co., Ltd., Japan), where four to five coronal brain slices (350–400 μm), including the region of the ACC were obtained and immediately transferred to a holding chamber with oxygenated (95% O_2 and 5% CO_2) ACSF.

Whole-cell patch-clamp recordings

Patch electrodes (2–4 MΩ) were prepared using borosilicate glass (1.2 mm outside diameter, 0.69 mm inside) and made by a horizontal electrode puller (P-87, Sutter, USA). A single brain slice was then held down in the recording chamber with an anchor and was kept