Neurobiology of Disease 68 (2014) 145–155

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Ketamine administered to pregnant rats in the second trimester causes long-lasting behavioral disorders in offspring

Tianyun Zhao [a], Yuantao Li [a,*], Wei Wei [b], Sinead Savage [c], Libing Zhou [d,**], Daqing Ma [c]

[a] Department of Anesthesiology, Shenzhen Maternity and Child healthcare Hospital, Southern Medical University, Shenzhen 518028, China
[b] Department of Anesthesiology, Meizhou people's hospital, Meizhou, Guangdong 514031, China
[c] Anaesthetics, Pain Medicine & Intensive Care, Department of Surgery & Cancer, Faculty of Medicine, Imperial College London, Chelsea & Westminster Hospital, London, UK
[d] Guangdong-Hongkong-Macau Institute of CNS Regeneration, Jinan University, Guangzhou, Guangdong 510632, China

ARTICLE INFO

Article history:
Received 16 November 2013
Revised 21 February 2014
Accepted 25 February 2014
Available online 26 April 2014

Keywords:
Ketamine
Brain development
Pregnancy
Cognition
Depression
NMDA receptor

ABSTRACT

Commonly used anesthetic agents, e.g. ketamine, may be neurotoxic to the developing brain but there has been little attention to the neurobehavioral consequences for offspring when used for maternal anesthesia. We hypothesize that treatment of pregnant rats with ketamine during the second trimester would affect brain development of the offspring. Pregnant rats on gestational day 14, about equal to midtrimester pregnancy in humans, received a sedative dose of ketamine intravenously for 2 h. Brain hippocampal morphology of their pups at postnatal days 0 (P0) and P30 was examined by Nissl-staining and the characteristics of dendrites were determined using the Golgi-Cox staining, while cell proliferation in subventricular zone (SVZ) and dentate gyrus (DG) was labeled with bromodeoxyuridine (BrdU). Their neurobehavioral functions were tested at P25–30 after which the NR1 and NR2 subunits of N-methyl-D-aspartate (NMDA) receptor, brain-derived neurotrophic factor (BDNF) and postsynaptic density protein 95 (PSD-95) in the hippocampus were analyzed by western blot. When pregnant rats were exposed to ketamine, there was neuronal loss, pyramidal neuronal abnormality and reduced cell proliferation in the hippocampus of offspring. These morphological abnormalities were associated with depression- and anxiety-like behaviors, and impaired memory up to young adult age. The treatment further caused NR2A receptor subunit up-regulation and NR2B receptor subunit, BDNF and PSD-95 down-regulation. These data suggest that maternal anesthesia with ketamine during the fetal brain development period can cause fetal brain damage and subsequent neurobehavioral abnormality, which is likely associated with the imbalanced expression of NMDA receptor subunits.

Introduction

There is great concern about the safety of currently used anesthetics in the young. Indeed, preclinical data suggests that commonly used anesthetics including ketamine are neurotoxic to the developing brain in rodents and even primates (Brambrink et al., 2012; Jevtovic-Todorovic et al., 2003; Satomoto et al., 2009; Slikker et al., 2007). This concept is also supported by retrospective clinical data in which there was an association between anesthesia and/or surgery early in life leading to late-onset learning disabilities (Hansen et al., 2011; Ing et al., 2012; Kalkman et al., 2009). Unfortunately, only a few previous reports looked into the effects of general anesthesia on neurodevelopmental consequences for the fetus before birth (Kong et al., 2012; Palanisamy et al., 2011; Slikker et al., 2007; Zheng et al., 2013). For example, rats exposed to isoflurane for 4 h in utero caused memory and learning deficits, as well as abnormal behaviors (Palanisamy et al., 2011). A 24-h exposure of ketamine anesthesia during late second trimester in pregnant macaques caused apoptotic neuronal death in the fetal brain (Slikker et al., 2007). However, most studies of this kind mainly concentrated on acute effect of anesthetics on neuroapoptosis without further investigation of neuronal development and neurogenesis. In particular, long-term learning and spatial memory impairments and other neurological disorders including depression, anxiety, emotional blunting and apathy were not well studied.

Ketamine is often consumed as a drug of abuse by the public, including pregnant women (Rofael et al., 2003). In addition, between 0.75% and 2% of pregnant women require surgery that is related to either the pregnancy or other medical problems (Goodman, 2002). This number is increasing, partly because of laparoscopic procedures and fetal surgery becoming more widely performed (Cheek and Baird, 2009). It is therefore important to ensure that anesthetics, such as ketamine, used in surgery or as a drug of abuse during pregnancy do not pose a risk to the unborn baby. In this study, pregnant rats on gestational day

* Correspondence to: Y Li, Department of Anesthesiology, Shenzhen Maternity and Child healthcare Hospital, Southern Medical University, Shenzhen 518028, China. Fax: +86 755 83228832.
** Correspondence to: L. Zhou. Fax: +86 20 85223563.
E-mail addresses: sylyt6788@sina.com (Y. Li), tlibingzh@jnu.edu.cn (L. Zhou).

http://dx.doi.org/10.1016/j.nbd.2014.02.009
0969-9961/

14, equivalent to the second trimester of human pregnancy (Clancy et al., 2001), were administered ketamine intravenously, and its impact on neuronal development, neurogenesis and behavior, together with underlying molecular mechanisms, were investigated in offspring.

Materials and methods

Subjects

All experimental procedures were performed according to the guidelines that have been approved by the Ethics Committees of Jinan University, Guangdong, China. All efforts were made to minimize the number of animals used. The timed-pregnant Sprague–Dawley rats were housed in polypropylene cages in a temperature and humidity regulated room with a 12 h light/dark cycle and had access to water and food ad libitum. Dams were used for experiments on gestational day 14.

Anesthesia

On the gestational day 14, 12 dams were randomly divided into control (n = 6) and ketamine (n = 6) groups. Controls were left undisturbed in their home cages while the ketamine group received a bolus dose (40 mg/kg) of ketamine (Gutian Pharmaceutical Co., Ltd. Fujian province, China) via lower back intramuscular injection, followed by continuous intravenous infusion with a pump (Sinomdt Co., Ltd, Shenzhen, China) via a tail vein at a rate of 40–60 mg/kg/h (10 mg/ml diluted with saline) for 2 h. This duration of ketamine infusion is clinically relevant while the infusion rate was varied to induce a sedative state between light anesthesia and deep sedation, evidenced by a lack of voluntary movement, decreased muscle tone, and minimal reaction to painful stimulation with the maintenance of an intact palpebral reflex but without any cardiorespiratory function being compromised (Green et al., 1981). The latter was confirmed with the blood gas analysis in a separate cohort (Nova Biomedical blood gas analyser, Massachusetts, USA) (see the Results section). The core body temperature was measured with a rectal probe and maintained between 36.5 and 37.5 °C by a servo-controlled infrared lamp and heating pad throughout experiments (RWD life science Co. Ltd., Shenzhen, China). At the end of infusion, dams were returned to their home cages after the righting reflex was recovered. Three dams per group were used for the neurogenesis study on gestational day 22 (see below) while another three in each group were allowed to give birth naturally. Two pups from each dam (total 6 pups in each group) were killed about 6 h after birth for brain histology examination. The remaining pups were allowed to grow up with their mothers until postnatal day (P) 21 (the day of birth was designated as P0) at which time pups were weaned (3–4 cages/dam) for behavioral tests. Subsequently, their ex vivo brain samples were harvested at P30 for histology, immunostaining, Golgi stain and western blot (n = 1–2/dam for each examination) (see below). A schematic representation of experimental protocols is shown in Fig. 1.

Behavioral test

Two groups (n = 10 or 11) of rats were randomly selected from each group (3–4 from each dam) at P25 to 30 for open field activity test (OFT) and forced swimming test (FST) or Morris water maze test (MWM) and sucrose preference test (SPT) for 3 consecutive days respectively. In order to avoid possible behavioral biases, each behavioral test of the two groups of animals was performed at the same time interval on the day of testing.

Open field activity test (OFT)

In order to assess general locomotor activity and anxiety-like behaviors (Prut and Belzung, 2003), the open field test was adopted. Rats (n = 10) were placed gently in a dimly lit open-field apparatus (50 cm × 50 cm × 37 cm) constructed of Plexiglas wall and black floor, and allowed to move freely for 15 min. The arena was cleaned with 70% ethanol between each trial. Data were recorded with a video camera and analyzed using EthoVision XT 7.0 (Noldus, Wageningen, Netherlands). Locomotor activity was measured with the traveling distance and speed while anxiety-like behaviors were evaluated with the duration and the frequency of the center zone visits.

Forced swimming test (FST)

The FST is widely used for assessing depressant behavior (Cryan et al., 2005). To measure this the same cohort of rats, after OFT testing, were individually placed into a transparent Plexiglas cylinder (50 cm high and 15 cm diameter) filled with 25 cm deep water at 24 ± 1 °C. Each animal was left to swim for 5 min. Data were recorded with a video camera and analyzed using EthoVision XT 7.0 (Noldus) to calculate the duration of their immobility. A rat was judged to be immobile when the velocity of center point was less than 1.7 cm/s.

Morris water maze (MWM)

MWM test was performed to assess spatial learning and memory in the animals. A water tank 120 cm in diameter and 50 cm in height was filled with water to a depth of 35 cm and maintained at a temperature of 24 ± 1 °C. It was equally divided into four quadrants. An escape platform (10 × 10 cm^2) was submerged 1.5 cm below the water surface in one quadrant. Rats were placed into the pool and allowed to search for the platform for 180 s for four trials (once from each quadrant; 15 min gap between each trial). Animals were pre-trained for 3 days before the formal test was started and guided to the platform if they could not find the platform at the end of the test, in which case the latency was defined as 180 s. Data were recorded with a video camera and analyzed using EthoVision XT 7.0 (Noldus) to determine the total distance traveled and the total latency to platform.

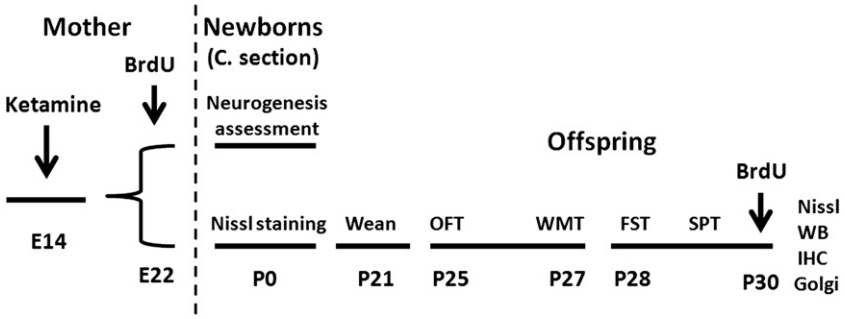

Fig. 1. The flow chart of the experimental protocols. C. section = cesarean section; E = gestation day; BrdU = Bromodeoxyuridine; Nissl staining = cresyl violet staining; P = postnatal day; OFT = open field activity test; FST = forced swimming test MWM = Morris water maze; SPT = sucrose preference test. WB = western blot; IHC = immunohistochemistry; Golgi = Golgi staining.