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Effects of prenatal propofol exposure on postnatal development in rats

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ABSTRACT

Preclinical studies suggest that propofol may cause damage to immature neurons. However, the effect of maternal propofol exposure on the neuronal development of the offspring is largely unknown. In this study, pregnant rats were assigned to receive continuous infusion of saline (control) or propofol for 1 h (1HP) or 2 h (2HP) on gestational day 18. An additional group (lipid) was assigned to receive continuous infusion of intralipid fat emulsion (vehicle of propofol) for 2 h. Pups were then tested on the appearance and progression of sensory and physical motor abilities between postnatal day 1 (P1) and P28. The brain and body weights of pups from 2HP group on P10 were significantly lower than those from the saline control group, although they were the same in all four groups at birth (P0). Pups from 1HP and 2HP groups, but not lipid group, showed slower maturation of eyes (delayed opening) and several neurological reflexes (hindlimb reflex, righting reflex); they also showed delayed improvement in execution on gait reflex and inclined board tests. The forelimb reflex and negative geotaxis were also delayed in 2HP group. All parameters examined except body weight of 2HP pups recovered to normal levels by P28. We conclude that administration of propofol to pregnant rats leads to retardation in physical and neurological reflex development in their offspring.

1. Introduction

Accumulating evidence indicates that the administration of volatile anesthetics during pregnancy may cause neurotoxicity in the developing brain and subsequent impairment of cognitive function in adulthood (Palanisamy et al., 2011; Kong et al., 2012; Li et al., 2007; Yang et al., 2011). However, the effects of prenatal exposure to intravenous anesthetics on development of the offspring are largely unknown.

Postnatal development can be assessed by the rate of physical growth and maturation of neurological reflexes and motor coordination (Altman and Sudarshan, 1975). Although the emergence of certain neurological reflexes can be modulated by various factors (Hill et al., 1991; Smart and Dobbing, 1971a,b; Lubics et al., 2005), the effects of exposure to maternal anesthesia on development of these reflexes in the offspring are relatively unknown.

The intravenous general anesthetic agent, propofol, is widely used in numerous surgical procedures including non-obstetric surgery because of its rapid onset of action and short duration. Propofol readily crosses the placenta and may depress the metabolism of the fetus; the significance of this depression is unknown (Bacon and Razis, 1994; Jauniaux et al., 1998). Studies in mice indicate that a sub-anesthetic dose of propofol caused neuroapoptosis in the neonate and led to a persistent decrease in the dendritic growth in cultured GABA neurons (Cattano et al., 2008; Vutskits et al., 2005). Recently, Creeley et al. demonstrated that general anesthesia induced by propofol in pregnant rhesus macaques caused wide spread apoptosis in the fetal brain (Creeley et al., 2013). These data suggest that the immature brain during the period of rapid synaptogenesis is vulnerable to propofol-induced neurotoxicity (Dobbing and Sands, 1979). Moreover, it has been demonstrated that exposure of neonatal rats to propofol caused neurotoxic insults as well as behavioral modifications (Yu et al., 2013). In the present study, we examined the development of neurological reflexes in pups to explore the consequences of propofol administration to pregnant rats on the maturation of their offspring. On gestation day 18 (G18), we administered propofol, intralipid emulsion (the vehicle of propofol) or normal saline to the dams by continuous infusion via a tail vein catheter. This age was chosen because according to the developmental time across mammalian species (Clancy et al., 2001; Workman et al., 2013), pregnant rats on G18 approximately correlate to the later first trimester in human. The need for anesthesia and surgery during pregnancy occurs in 0.75% to 2.0% of all pregnancies. Although surgery can be required during any stage of pregnancy depending on the urgency of the procedure (Reitman and Flood, 2011), surgery occurs in 0.2% to 1% of all parturients during the first

Abbreviations: 1HP, offspring of dams prenatally exposed to 1 h infusion of propofol on gestational day 18; 2HP, offspring of dams prenatally exposed to a 2 h infusion of propofol on gestational day 18; G18, gestation day 18; IV, intravenously; N, number of dams or litters; n, number of offspring or pups; P0, postnatal day 0; Pxx, postnatal day xx.

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trimester (Mazze and Kallen, 1989; Czeizel et al., 1998). The effects of propofol exposure at this age on the fetal brain and the neurobehavioral development of the offspring remain largely unknown. In this study, we used a Fox's battery adapted for rats (Fox, 1965; Heuland et al., 2010) to quantify the physical maturation and the sensory motor development of the progeny.

2. Methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the Rutgers-New Jersey Medical School, Newark, New Jersey. Pregnant Sprague–Dawley (SD) rats (Taconic Farms, Germantown, N.Y., USA) and their offspring were used in this experiment. N refers to the number of dams or litters and n refers to the number of their offspring or pups in each experiment.

2.1. Preparation and delivery of drugs

Propofol was obtained from APP Pharmaceuticals, LLC (Schaumburg, IL). Intralipid 20% IV fat emulsion was obtained from Baxter (Deerfield, IL). Sterile normal saline, intralipid 20% IV fat emulsion or propofol was administered to pregnant rats by continuous infusion via a tail vein catheter. On G18, the pregnant rats were randomly assigned to three groups: 1) Control (which received a normal saline infusion for 2 h, N = 8); 2) propofol for 1 h (1HP, N = 6); 3) propofol for 2 h (2HP, N = 6). To test whether propofol carrier-intralipid infusion could affect the neurobehavioral development of the offspring, a separate group (lipid, N = 6) was assigned to receive continuous infusion of intralipid fat emulsion (vehicle of propofol) for 2 h. Pregnant rats were restrained gently to facilitate insertion of a 24 gauge IV catheter in the lateral tail veins. The catheter was stabilized in animals using regular tape. In the 1HP and 2HP groups, the rate of propofol infusion was adjusted to 0.3–0.6 mg/kg/min (average: 0.4 mg/kg/min) so it induced sedation (low activity but intact righting reflex) but not a surgical plane (loss of righting reflex). The final dose of propofol was 9–10 mg (0.9–1.0 ml), and 19–20 mg (1.9–2.0 ml) for the dams in the 1HP and 2HP groups, respectively. To decrease the effects of stress caused by monitoring the vital signs and metabolic state of dams on fetus, two single 24-gauge IV catheters were placed in the tail veins (one in each lateral tail vein) in a separate group of dams (N = 6). One catheter was used to administer the continuous infusion of propofol (propofol infusion rate, sedation status and total dosage of propofol were similar to those in 2HP group) and the other catheter was used for drawing venous blood at 0, 1, and 2 h during propofol infusion. The blood was analyzed for pH, $PvCO_2$, PvO_2, HCO_3^-, K^+, Na^+ and blood glucose levels (iStat analyzer, Abaxis, Union City, CA). The arterial oxygen saturation, pulse strength, heart rate, and breath rate were continuously monitored using Pulse Oximeter (Harvard Apparatus, Holliston, MA) in this group. Maternal blood pressure was monitored by non-invasive blood pressure system (Harvard Apparatus, Holliston, MA). The temperature of pregnant rats during propofol infusion was monitored with the temperature controller (Harvard Apparatus, Holliston, MA) and maintained at 37 ± 0.5 °C with a heating lamp. The control and lipid dams received an infusion of normal saline or intralipid emulsion for 2 h, respectively. The final volume for saline and intralipid emulsion was around 2.0 ml. Dams were allowed to move freely in the home cage during saline or intralipid infusion. The experimenter closely observed the activity of dams to ensure that the IV catheter was not removed. The dams wear a collar to reduce the biting of the IV lines. We observed that the pregnant rats of control and lipid groups tolerated the 2 hour infusion without any abnormal behavior. Pregnant rats were returned to their respective cages after stopping infusion and delivered pups after 3 or 4 days via normal spontaneous vaginal delivery. The pups from the group used for monitoring vital signs and blood gas were excluded from the following behavioral studies.

Progeny at birth (P0) were counted, weighed, sex typed, and culled to 10 per litter; no attempt was made to cull sick or underweight pups. Pups' body weights were measured on postnatal day 10 (P10) and 28 (P28) again. To measure brain growth, two pups from each litter were euthanized with chloral hydrate on P0, P10, and P28, and were perfused intracardially with 0.9% saline. The brain was extracted from the skull, trimmed at the obex, and weighed. For this evaluation, only two male siblings from the same litter were assigned to the same experimental group in order to minimize litter effects. The animals were selected for body weights closer to the average body weight of the litter.

2.2. Examination of neurobehavioral development

The Fox's battery itemizes the steps of cerebral development and provides benchmarks for pathological and normal development of a young rat (Altman and Sudarshan, 1975; Lubics et al., 2005; Heuland et al., 2010). According to a review on statistical issues regarding developmental neurotoxicity, one important aspect of the developmental neurotoxicity guideline is the requirement that treatment effects be assessed in both sexes (Holson et al., 2008). In order to minimize sex effects, one male and one female were randomly chosen from each litter on P1 to subsequently perform the neurobehavioral tests daily from P1 to P28 during the rats' active period—between 9:00 p.m. and 11:00 p.m.

Marked pups were monitored daily for appearance of physical characteristics: the days that their two incisors were visible or that both eyes had opened were recorded. Pups were tested for the following neurological signs and reflexes. (1) *Limb grasp*: the first day that the touched fore- and hindlimbs grasped onto the thin rod was noted. (2) *Disappearance of crossed extensor reflex*: the first day that the pinched left rear paw no longer caused the animal to extend the right leg was documented. (3) *Righting reflex*: the time in seconds that pups in a supine position take to turn over to prone position with all four paws contacting the surface was recorded on days P1 to P13. (4) *Negative geotaxis*: the day that pups that were positioned head down on an inclined board (20° incline on a 30 cm board) used their forelimbs to turn around was recorded. (5) *Inclined board test*: the maximum angle that pups could maintain position for 5 s on a board with increasing slope (5° degree increments) was logged on P10 to P14 daily and on P17, P21, and P28. (6) *Gait reflex*: the first day that pups used their two forelimbs to move outside a white paper circle (13 cm diameter) in <30 s and the time that the pups took on subsequent days (to P19) to leave the circle were recorded.

2.3. Maternal behavior

To test the effect of maternal sedation on maternal rearing behavior, pregnant rats on G18 were treated with propofol (N = 6, propofol infusion rate, sedation status and total dosage of propofol were similar to those in 2HP group) or saline (N = 6) for 2 h. Pregnant rats were allowed to undergo normal spontaneous vaginal delivery. Maternal behavior in the saline control and the 2HP group was observed during the light phase of the light/dark cycle, between 08:30 p.m. and 11:30 p.m. in the home cage where the dam and her pups were left undisturbed. A video camera was used to record maternal behavior for three periods of 10 min separated by 40 min intervals on postnatal days 2, 6, 10, and 15 (Heuland et al., 2010; Liu et al., 2000). The following behaviors were analyzed: (1) mother's suckling pups in an arched-back and in a 'blanket' postures in which the mother lays over the pups; (2) mother's behavior directed towards the pups (carrying, licking the pups, passive contact with pups), and (3) mother's behavior off the pups (self-grooming, digging the sawdust, straightening up, immobility with no contact with pups). Using time sampling observation techniques, the number of times that the mother's activities directed towards the pups or off the pups occurred during each 10 min period was recorded as frequency. Because the mother's suckling behavior occurred less frequently and continued for a period of time, it was