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DOPAMINE TREATMENT DURING ACUTE HYPOXIA IS NEUROPROTECTIVE IN THE DEVELOPING SHEEP BRAIN

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Abstract—Dopamine is often used to treat hypotension in preterm infants; these infants are at risk of developing brain injury due to impaired autoregulation and cerebral hypoperfusion. However the effects of dopamine on the immature brain under conditions of cerebral hypoxia are not known. We hypothesized that pretreatment with dopamine would protect the immature brain from injury caused by cerebral hypoxia. Preterm fetal sheep were used to determine the effects of intravenous dopamine on hypoxia-induced brain injury. In 16 pregnant sheep at 90 days of gestation (0.6 of term, term = 147 days) catheters were implanted aseptically into the fetal carotid artery and jugular vein; an inflatable occluder was placed loosely around the umbilical cord for later induction of fetal hypoxemia. At 5 days after surgery, dopamine (10 μg/kg/min, *n* = 7 fetuses) or saline (*n* = 9 fetuses) was infused for 74 h. Two hours after commencing the dopamine/saline infusion, we induced umbilical cord occlusion (UCO) for up to 25 min to produce fetal asphyxia. Fetuses were allowed to recover, and brains were collected 72 h later for assessment of neuropathology. Unoperated twin fetuses were used as age-matched non-UCO controls (*n* = 8). In UCO + saline fetuses, microglial and apoptotic cell density in the subcortical and periventricular white matter, caudate nucleus and hippocampus was greater than that in age-matched controls; oxidative stress was elevated in the subcortical and periventricular white matter and caudate nucleus compared to that in age-matched controls. In UCO + dopamine fetuses microglial density and oxidative stress in the cerebral white matter and caudate nucleus were not different to that of age-matched controls. Apoptotic cell death was decreased in the cerebral white matter of UCO + dopamine brains, relative to UCO + saline brains. We conclude that pretreatment with dopamine does not exacerbate hypoxia-induced injury in the immature brain and may be neuroprotective because it led to decreased apoptosis, oxidative stress and neuroinflammation in the cerebral white matter and decreased neuroinflammation in the caudate nucleus.

Key words: preterm brain injury, white matter, dopamine, inotrope, microglia, hypotension.

INTRODUCTION

Acquired brain injury due to cerebral hypoxia–ischemia is a major risk for infants born prematurely, resulting in profound impact on the quality of life of affected individuals and their families (Saigal and Doyle, 2008). Reduced cerebral perfusion resulting from impaired autoregulation in preterm infants has been suggested as one of the major factors contributing to brain injury (Wong et al., 2012); it is likely that uncompensated hypotension can reduce cerebral blood flow, thereby increasing the risk of cerebral hypoxia. As hypotension and cerebral hypoperfusion may lead to brain injury in preterm infants, the inotrope and vasopressor drug dopamine is often administered to hypotensive preterm infants to raise mean arterial pressure (MAP) with the intention of maintaining adequate perfusion of critical organs such as the brain. Clinically, dopamine is administered to 25–53% of preterm infants born at < 28 weeks gestation (Laughon et al., 2007), but despite its relatively common use, and the evidence that it crosses the blood–brain barrier (Seri et al., 1984), little is known about its effects on the preterm brain itself.

A well-established feature of brain injury in preterm infants is diffuse injury in the cerebral white matter, often with accompanying long-term mild to severe motor impairment (Spittle et al., 2011). Reduced cerebral oxygen delivery also causes injury to the caudate nucleus and hippocampus in preterm infants and is thought to predispose infants to the development of cognitive and motor disorders later in life (Abernethy et al., 2004; Beauchamp et al., 2008; Thompson et al., 2013). In the preterm fetal sheep brain, hypoxemia induces white matter injury, neuronal necrosis in the caudate nucleus and a profound inflammatory response and atrophy in the hippocampus (Welin et al., 2005a; Jellema et al., 2013; Drury et al., 2014). Although dopamine is often administered to very preterm infants at risk of cerebral hypoperfusion and

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Abbreviations: EKG, electrocardiograph; ELISA, enzyme-linked immunosorbent assay; HR, heart rate; LPS, lipopolysaccharide; MAP, mean arterial pressure; PO_2, partial pressures of oxygen; SO_2, oxygen saturation; UCO, umbilical cord occlusion.

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hypoxia, there is an apparent lack of information on the impact of dopamine on inflammation and neuropathology caused by cerebral hypoxia, particularly within the highly vulnerable cerebral white matter, caudate nucleus and hippocampus.

At present, there is no consensus regarding the beneficial effects of dopamine treatment on neurological outcome in preterm infants at risk of cerebral hypoperfusion (Dempsey and Barrington, 2007). This is because studies of hypotensive infants treated with dopamine are limited and inconclusive due to the confounding effects of other clinical illness in these infants, highly variable treatment criteria and regimens, and the (understandable) absence of an appropriate control group, namely untreated hypotensive infants (Pellicer et al., 2009; Sassano-Higgins et al., 2011). Although there is some evidence that dopamine may worsen outcomes (e.g. Dempsey and Barrington, 2007), there is evidence that dopamine may be neuroprotective, presumably due to improvement of cerebral perfusion and oxygenation as shown for preterm infants (Pellicer et al., 2005), and in animal studies (Nachar et al., 2011).

Episodes of cerebral hypoperfusion are difficult to detect in very preterm infants and controversy exists regarding when and if hypotension requires early treatment (Dempsey and Barrington, 2007). We considered that early and effective treatment of hypotension may avoid organ hypoperfusion and subsequent hypoxic injury. Therefore we hypothesized that pretreatment with dopamine would protect the preterm brain from injury induced by cerebral hypoxia, or would not exacerbate the injury. Our study tested this hypothesis by inducing asphyxia using umbilical cord occlusion (UCO), in preterm fetal sheep receiving a continuous dopamine infusion, and assessing cardiovascular responses, neuroinflammation and neuropathology. We used preterm fetal sheep because the development of the cortical gray and white matter is comparable to that of preterm infants at 26–28 weeks of gestation (Raju, 1992), and because the pattern of hypoxia-induced brain injury closely replicates that in very preterm human infants (Mallard et al., 2003;Back et al., 2012).

EXPERIMENTAL PROCEDURES

Fetal surgery

All experimental procedures were approved by the Monash University Animal Ethics Committee. Sixteen time-mated pregnant sheep and their fetuses were prepared for experimentation at 90 ± 1 days of gestation (term is 147 days) using aseptic surgical techniques and general anesthesia. The ewes were initially anesthetized with sodium thiopentone (20 mg/kg; Pentothal), and after intubation anesthesia was maintained with 1–2% isoflurane in O_2. Antibiotics (500 mg ampicillin, 80 mg gentamicin) were administered during surgery and for 3 days afterward. For twin pregnancies, one fetus underwent instrumentation, while the other served as an age-matched, un-operated and non-UCO control. The fetus to be catheterized was exposed by uterotomy and polyvinyl catheters filled with heparinized saline (2 IU/ml) were chronically implanted non-occlusively into a carotid artery and jugular vein; another catheter was implanted into the amniotic sac. Two electrocardiograph (EKG) electrodes were attached to the fetal chest and an inflatable vascular occluder (uninflated) was positioned loosely around the umbilical cord. Post-operatively, ewes were housed in separate metabolic cages with *ad libitum* access to food and water; ewes received analgesia (transdermal fentanyl patch; 75 μg/hr, Janssen-Cilag) for 3 days.

Fetal monitoring

Five days after surgery, fetal arterial and amniotic catheters were attached to pressure transducers (DTXPlus, B-D Medical Systems, Australia) and pressure monitored by bridge amplifiers (ADInstruments, Australia), and EKG electrodes were connected to a differential amplifier (FE135 Dual BioAmp, ADInstruments, Australia). Fetal MAP was recorded after subtraction of amniotic fluid pressure; fetal heart rate (HR) was derived from the fetal EKG. Fetal MAP, HR and EKG were digitally recorded using an analog–digital interface (Powerlab 16/30), and the data displayed using LabchartPro v7.3.1 (ADInstruments) from at least 12 h before the umbilical cord was occluded until 72 h after release of the occlusion.

Fetal carotid artery blood samples (0.1–0.2 ml) were taken daily to monitor blood pH, oxygen saturation (SO_2), partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2), and acid–base balance (ABL5000, Radiometer, Denmark); samples were also taken at −2 h, −1 h, −15 min before UCO, and +5 min, +20 min, +2 h, +6 h and 24 h, 48 h and 72 h after UCO.

Experimental procedure

At 95 ± 1 days of gestation fetuses were randomly assigned to receive a continuous infusion of either dopamine or saline into a fetal jugular vein for 74 h until euthanasia (UCO + dopamine [n = 7], or UCO + saline [n = 9], see experiment timeline, Fig. 1). Dopamine was infused at 10 μg/kg/min in heparinized saline at 0.5 ml/h, using an estimated fetal weight of 1 kg (Wassink et al., 2007). UCO + saline fetuses were infused with heparinized saline at 0.5 ml/h. Two hours after commencement of the dopamine or saline infusion the umbilical cord was occluded for up to 25 min, using a volume of water known to completely inflate the occluder. The duration of occlusion was based on previous studies showing that 25 min of complete UCO causes severe hypoxicacidemia (Bennet et al., 1999). Successful occlusion was confirmed by rapid onset of fetal bradycardia, fetal hypoxemia and hypercapnia and serial measurement of arterial blood gases and pH. To prevent fetal death *in utero*, the occlusion was terminated prior to 25 min if fetuses became severely hypotensive (MAP < 10 mmHg) or developed periods of transient asystole. Twins of UCO + dopamine and UCO + saline experimental fetuses were non-operated, age-matched control fetuses (n = 8) which underwent anesthesia but not catheterization.