Maternal alcohol exposure during mid-pregnancy dilates fetal cerebral arteries via endocannabinoid receptors

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ARTICLE INFO

ABSTRACT

Prenatal alcohol exposure often results in fetal alcohol syndrome and fetal alcohol spectrum disorders. Mechanisms of fetal brain damage by alcohol remain unclear. We used baboons (*Papio* spp.) to study alcohol-driven changes in the fetal cerebral artery endocannabinoid system. Pregnant baboons were subjected to binge alcohol exposure via gastric infusion three times during a period equivalent to the second trimester of human pregnancy. A control group was infused with orange-flavored drink that was isocaloric to the alcohol-containing solution. Cesarean sections were performed at a time equivalent to the end of the second trimester of human pregnancy. Fetal cerebral arteries were harvested and subjected to *in vitro* pressurization followed by pharmacological profiling. During each alcohol-infusion episode, maternal blood alcohol concentrations (BAC) reached 80 mg/dL, that is, equivalent to the BAC considered legal intoxication in humans. Circulating anandamide (AEA) and 2-arachidonoylglycerol (2-AG) remained unchanged. Ultrasound studies on pregnant mothers revealed that fetal alcohol exposure decreased peak systolic blood velocity in middle cerebral arteries when compared to pre-alcohol levels. Moreover, ethanol-induced dilation was observed in fetal cerebral arteries pressurized *in vitro*. This dilation was abolished by the mixture of AM251 and AM630, which block cannabinoid receptors 1 and 2, respectively. In the presence of AM251, the cannabinoid receptor agonist AEA evoked a higher, concentration-dependent dilation of cerebral arteries from alcohol-exposed fetuses. The difference in AEA-induced cerebral artery dilation vanished in the presence of AM630. CB1 and CB2 receptor mRNA and protein levels were similar in cerebral arteries from alcohol-exposed and control-exposed fetuses. In summary, alcohol exposure dilates fetal cerebral arteries via endocannabinoid receptors and results in an increased function of CB2.

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Introduction

Alcohol consumption during pregnancy is a serious public health problem and a leading preventable cause of birth defects and developmental disabilities (Basavarajappa, 2015). The adverse effects of alcohol on the developing fetus result in a spectrum of structural abnormalities, behavioral defects, and neurocognitive disabilities termed fetal alcohol spectrum disorders (FASD). The most severe cases are diagnosed as fetal alcohol syndrome (FAS). FASD is estimated to affect 2–5% of children in the U.S. and Western European countries (www.cdc.gov; May & Gossage, 2001, 2011; May et al., 2009). In some regions of the world, however, FAS/FASD affects up to 29% of live births (Olivier, Curfs, & Viljoen, 2016).

It is widely documented that prenatal alcohol (ethanol) exposure causes abnormalities in several organs and systems of the body, such as the heart, kidney, liver, gastrointestinal tract, and the

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endocrine and immune systems (Caputo, Wood, & Jabbour, 2016; Gauthier, 2015). However, the brain constitutes the most severely affected organ, exhibiting both structural and functional abnormalities in response to prenatal alcohol exposure (Caputo et al., 2016; Mattson, Schoenfeld, & Riley, 2001). Unfortunately, the mechanisms of cerebral damage due to prenatal alcohol exposure are poorly understood and thus, treatment is limited (Murawski, Moore, Thomas, & Riley, 2015).

The developing brain is critically dependent on a sufficient blood supply, which ensures delivery of oxygen and other nutrients. Remarkably, the prevailing neurological symptoms in FAS and FASD, such as memory deficit, low processing speed, lack of cognitive flexibility, and many others are similar to those observed in individuals with progressive neurodegenerative disorders, in which cerebrovascular dysfunction is being increasingly recognized as one of the major pathophysiological mechanisms (Gorelick et al., 2011; Mattson & Riley, 1998; Olson, Feldman, Streissguth, Sampson, & Bookstein, 1998; Zlokovic, 2011).

Endocannabinoid (eCB) signaling is critical for regulating arterial contractility in response to physiological and pathological stimuli (Engeli et al., 2012; López-Miranda, Herradón, & Martín, 2008). The endocannabinoid system (eCBS) consists of eCB-producing enzymes, eCB ligands, eCB receptors (mostly CB1 and CB2 types), and the eCB degradation machinery (Lu & Mackie, 2016). Endocannabinoid production, functional activity of CB1 and CB2 receptors, and eCB breakdown within the vascular smooth muscle have all been reported (Czikora et al., 2012). It remains unknown whether alcohol in general, and prenatal alcohol exposure in particular, affects the components of the vascular eCB system and its regulation of cerebrovascular contractility. Thus, we hypothesized that fetal alcohol exposure *in utero* targeted key components of the eCB system within the fetal cerebral artery.

In the current work, we use a model of binge alcohol administration in pregnant baboons via gastric infusion of alcohol-containing fluid during mid-pregnancy. Non-human primate models of alcohol exposure are widely used in alcohol-related studies (Baker, Farro, Gonzales, Helms, & Grant, 2014; Clarren & Bowden, 1982; Weerts, Goodwin, Kaminski, & Hienz, 2006). A substantial number of studies use several forms of alcohol self-administration (Grant & Bennett, 2003; Grant et al., 2008). The use of anesthesia to monitor fetal cerebral artery function by an ultrasound exam during an alcohol exposure episode, however, is required and is utilized in our current work, following previously accepted models of ethanol administration (Kochunov et al., 2010). Furthermore, the model we chose offers several advantages. First, the effect of alcohol during mid-pregnancy can be estimated without exposing the ovary or fetus to alcohol at earlier intervals, as would be necessary during development of alcohol preference in a self-administration protocol. Lack of alcohol exposure prior to conception is of particular advantage, as transgenerational effects of binge drinking have been reported in a primate model of alcohol self-administration. Second, the only data available on alcohol's effects on fetal cerebral artery function in non-human primates is reported in an identical protocol (Kochunov et al., 2010). Thus, our findings can be easily compared with previous observations.

To our knowledge, the possibility that the fetal cerebral artery endocannabinoid system is targeted by maternal alcohol drinking has not been previously investigated. While largely exploratory in nature, our current work reveals that alcohol-induced dilation of fetal cerebral arteries is mediated by endocannabinoid receptors, with CB2 receptors representing the novel targets of fetal alcohol exposure in cerebral arteries.

Materials and methods

Study approval

The care of animals and experimental protocols were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center, which is an Association for Assessment and Accreditation of Laboratory Animal Care-accredited institution.

Animal subjects

A total of seven alcohol-naïve *Papio hamadryas anubis* dams (ages 7–15 years) were enrolled into the study. Dams were received from the University of Oklahoma Primate Research Center before reaching 80 days of gestation. Then, dams were given 10 days to acclimate to the new environment at UTHSC. Baboons were singly housed in standard baboon cages, with visual and auditory access to each other. A maximum of four baboons (cages) were housed per room, on a 12-h light/dark cycle (lights on at 6:00 a.m.) without access to natural light. Baboons were fed twice a day, with each feeding consisting of the High Protein Monkey Diet (~15 biscuits per meal, 21 kcal/biscuit) to sustain the baboons' weight gain as expected throughout the pregnancy. Each feeding was also supplemented by two pieces of fresh fruits or vegetables and two tablespoons of peanut butter. Drinking water was available *ad libitum*. The facilities were maintained in accordance with the USDA and AAALAC standards.

Alcohol infusion procedure

Dams were randomly assigned to the experimental groups. Dams were subjected to either alcohol or control infusion procedures at 90, 100, and 110 days of gestation as confirmed by an ultrasound exam. Prior to each procedure, the animals were fasted for 12 h. On the day of the experiment, the animal sedation was induced by a single shot of ketamine hydrochloride (Ketaset®, 10 mg/kg of body weight). Anesthesia was maintained with isoflurane (1.5–2.0%) throughout the infusion, followed by maternal blood sampling and Doppler evaluation of fetal cerebral artery function. A rectal suppository of indomethacin (25 mg) was used to prevent labor during the alcohol/control infusion. An intravenous (IV) catheter was placed into the saphenous vein for blood collection and another catheter was placed into the cephalic vein for IV sodium chloride infusion. Animal monitoring of vital signs and depth of anesthesia consisted of electrocardiography, pulse oximetry, capnography, non-invasive blood pressure, and temperature measurements. A gastric catheter was introduced into the stomach and the infusion was administered over 10 min. For the experimental group, the infusion contained 1.8 g/kg ethanol (ultrapure, 200 proof; American Bioanalytical) diluted in reverse osmosis-purified drinking water. The control group of animals received an isocaloric solution containing orange-flavored Tang® powder (Kraft Foods). In both cases, the total volume of fluid infused was equal to 200 mL. Experimental blood collection occurred at 10 time points (see below) over 180 min, and Doppler evaluation of fetal middle cerebral artery (MCA) function was performed immediately before and at 120 min post-infusion. Before awakening from anesthesia, both groups of animals received a single intramuscular injection of carprofen (Rimadyl, 4.4 mg/kg of body weight), aimed to alleviate symptoms of hangover following alcohol drinking. At 120 days of gestation, Cesarean sections (C-sections) were performed using standard methodology that included maternal anesthesia. During C-section, fetuses were euthanized by exsanguination. In the