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Brief communication

Supplemental choline does not attenuate the effects of neonatal ethanol administration on habituation of the heart rate orienting response in rats

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ABSTRACT

Several studies using rodent subjects have now shown that extra dietary choline may prevent or even reverse the deleterious effects of pre- and early post-natal ethanol administration. Choline supplementation has been shown to attenuate many, although not all, of ethanol's effects on brain development and behavior. Our laboratory has consistently reported impaired habituation of the heart rate orienting response to a novel olfactory stimulus in animals exposed to ethanol on postnatal days (PD) 4–9. Here we examine whether supplemental choline given both during and after ethanol administration could alleviate these ethanol-induced deficits. Subjects were given 5 g/kg/day ethanol or sham intubations on PD 4–9. Half of the subjects in each group were given a single daily s.c. injection of choline chloride on PD 4–20, while the other half were injected daily with saline. Pups were tested for heart rate orienting and response habituation in a single test session on PD 23. Results replicated the ethanol-induced impairment in response habituation. However, choline supplementation had no effect on orienting or habituation in either neonatal treatment group. These findings indicate that habituation deficits induced by ethanol are not alleviated by extra dietary choline using these parameters. Choline holds great promise as a treatment for some fetal alcohol effects, but is not an effective treatment for all ethanol-related deficits.

1. Introduction

Prenatal exposure to ethanol in humans can result in notable impairments in attention, memory, and other cognitive processes (Riley et al., 2011). Some of these deficits are measurable shortly after birth (Streissguth et al., 1983) and many are known to persist well into adulthood (Coles et al., 2010; Streissguth, 2007). Efforts at preventing maternal drinking during pregnancy have largely been unsuccessful; the incidence of fetal alcohol syndrome (FAS) has not decreased since the identification of the syndrome more than 35 years ago (Jones and Smith, 1973). It is estimated that nearly 1% of the population exhibits neurobehavioral problems associated with prenatal exposure to ethanol (Sampson et al., 1997). Because incidence rates seem to be relatively unchanged (Goodlett, 2010), more effective measures for prevention of maternal ethanol use are sorely needed. However, until that time, efforts to treat the afflicted individuals continue to be a major focus of research and practice.

Work with animal models has investigated approaches to treating neural and behavioral alterations caused by ethanol exposure. Neonatal handling (Lee and Rabe, 1999), rearing in an enriched environment (Hannigan et al., 2007), and therapeutic motor training (Klintsova et al., 2000) all appear to mitigate some of the negative consequences of pre- or early post-natal ethanol exposure. Antioxidants, such as vitamin E and ß-carotene, also offer promising avenues for treatment and perhaps prevention of alcohol-induced neural and behavioral deficits (Cohen-Kerem and Koren, 2003; Marino et al., 2004). Choline supplementation has also been found to attenuate many of the negative effects of neonatal alcohol exposure. In an extensive series of experiments, Thomas et al. have reported that supplemental choline given during and/or after ethanol exposure can improve measures of physical and behavioral development (Thomas et al., 2009) and reverse hyperactivity (Monk et al., 2012; Thomas et al., 2004a). Choline has also been shown to rescue impairments in spatial memory (Ryan et al., 2008; Thomas et al., 2007), visual discrimination learning (Thomas et al., 2000), reversal learning (Thomas et al., 2004a), and trace eyeblink conditioning (Thomas and Tran, 2012). Wagner and Hunt (2006) also demonstrated a beneficial effect of supplemental choline on ethanol-induced deficits in trace fear conditioning. Collectively, these findings lend support for the treatment potential of choline.

Another robust behavioral consequence of prenatal ethanol exposure is a lack of response habituation (Hepper et al., 2012; Streissguth

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et al., 1983), and we have previously observed that habituation to discrete cues is impaired in young rats exposed to ethanol during the neonatal period (Hunt and Morasch, 2004; Hunt and Phillips, 2004; Morasch and Hunt, 2009). The assessment of habituation may be particularly useful as an early indicator of cognitive dysfunction [e.g., (Guiraud et al., 2011; Sansavini et al., 2011)]. A stimulus-elicited decrease in heart rate is one component of the orienting response (Lang et al., 1997), which undergoes rapid habituation with repeated stimulus presentations. Animals exposed to ethanol on PD 4–9 show virtually no response decrement to a novel olfactory cue throughout a 10-trial test session, whereas control subjects exhibit complete habituation within 4 or 5 trials (Hunt and Morasch, 2004; Morasch and Hunt, 2009). The present experiment was designed to assess whether choline supplementation would rescue these habituation deficits. The choline administration procedure was identical to that used by Wagner and Hunt (2006), which was based on that described by Thomas et al. (2004a).

2. Methods

2.1. Animals

The subjects were offspring of 9 litters of Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), born and reared at the College of William and Mary. Pups from an additional 10 litters served as unhandled controls and underwent no treatment until the day of heart rate testing. Animal facilities were temperature- and humidity-controlled and maintained on a 14:10 h light:dark cycle (light onset 0600 h). Male and female breeders were maintained together in 50.8 × 40.6 × 21.6 cm clear polycarbonate cages with pine shavings and stainless steel lids. Animals had free access to high-protein food (LabDiet Formula 5008) and water. Cages were checked daily for pups, and the day of birth was designated as postnatal day (PD) 0. Litters were culled to 8–10 pups on PD 2. Pups were weaned from the home cage on PD 21, at which time they were group housed with all siblings in identical polycarbonate cages. Procedures were approved by the Institutional Animal Care and Use Committee of the College of William & Mary.

2.2. Apparatus

Heart rate was recorded as previously described (Morasch and Hunt, 2009) using two transcutaneous electrodes made from 27 ga stainless steel wire and shaped to resemble safety pins. Cardiac potentials were amplified with a Grass Instruments (Quincy, MA) Model P15 preamplifier. The R-spike activated a Schmitt trigger (Coulbourn Instruments, Allentown, PA). A computer stored each inter-beat interval (IBI) measured to the nearest millisecond, and controlled all timing sequences and data collection.

Subjects were tested individually in a 25-cm long cylindrical Plexiglas chamber (14 cm diameter) mounted horizontally inside a sound attenuating shell. The olfactory stimulus (0.5 ml amyl acetate + 40 ml water) was introduced into the chamber by means of a custom-made olfactometer system. The temperature inside the chamber was maintained at 28 °C by a heated airstream. The olfactory stimulus was evacuated from the chamber by negative pressure generated by an exhaust fan.

2.3. Ethanol administration

On PD 4, all pups from the litter were removed from the home cage and assigned to one of the four groups, designated by ethanol treatment [ethanol (EtOH) or sham intubated (Sham)] and choline supplementation [choline or saline injections]. Data from like-treated pups within a litter were averaged so that each of the 9 litters contributed one data point per treatment group. Subjects in the Unhandled group (1 pup per litter) were not treated during this time.

Animals assigned to group EtOH were administered a total of 5.0 g/kg/day ethanol via intragastric intubation. The total daily dose was divided into two doses of 2.5 g/kg each that were administered 2 h apart (at 0900 and 1100 h). The ethanol solution was 11.9% *v/v*, dissolved in Similac™. A third feeding, given 2 h after the second ethanol dose (1300 h), consisted of the Similac™ vehicle alone (Goodlett and Johnson, 1997). Sham controls received the tube-insertion procedure three times per day, but were not given any fluid (Goodlett and Johnson, 1997; Marino et al., 2004). Intubations were achieved using a 10 cm length of PE-10 tubing (Clay Adams, Parsippany, NJ). Animals were returned to the home cage after each feeding session. This procedure occurred daily from PD 4 through PD 9.

2.4. Choline administration

Supplemental choline injections began on the first day of ethanol administration, PD 4, and continued daily through PD 20. A single subcutaneous injection of choline chloride or saline was given to subjects immediately after the last feeding session on PD 4–9 (1300 h), and at the same time of day on PD 10–20. A constant volume of 0.10 ml of an 18.8 mg/ml solution of choline chloride (Sigma, St. Louis, MO) or saline vehicle was injected on each day of treatment (Thomas et al., 2004a; Wagner and Hunt, 2006).

2.5. Behavioral testing

All animals were tested for heart rate orienting responses and response habituation on PD 23 (+/− 1 day). Heart rate electrodes were acutely implanted on the dorsal surface, one at the nape of the neck and the other approximately 1 cm from the base of the tail. Animals were placed into the test chamber for a 10 min period of adaptation. Next, subjects were given 10 odor presentations. Each odor was 10 s in duration, and trials were separated by 100–200 s intervals. Heart rate was recorded for 1 s prior to each odor trial to provide a measure of baseline heart rate, during the 10 s odor presentation, and for 5 s after stimulus offset.

2.6. Treatment of heart rate data

Inter-beat intervals were converted into a beats-per-minute (BPM) measure for analysis. Heart rate recorded during the 1 s baseline period was subtracted from that recorded during each second of the stimulus and post-stimulus periods to obtain difference scores. Negative difference scores reflected a decrease in heart rate (bradycardia) that defines the heart rate orienting response (Lang et al., 1997), whereas positive difference scores reflected an increase in heart rate (tachycardia).

2.7. Statistical analyses

Heart rate data were analyzed using mixed-factor analysis of variance (ANOVA), with ethanol treatment and choline supplementation as between-groups variables, and seconds (orienting response) or trial block (habituation) as the within-subjects variable. In all cases involving a repeated measure, the Greenhouse–Geisser correction procedure was used to control for possible inflation of probability values. Post hoc comparisons were made using Newman–Keuls tests ($p < .05$). The second-by-second changes from baseline heart rate obtained from the first trial were analyzed separately to assess the integrity of the orienting response. Habituation was assessed by analysis of the peak change in heart rate averaged across blocks of 2 trials. The peak change was defined as the largest change observed, either positive or negative (Hunt and Morasch, 2004; Hunt and Phillips, 2004). Data from the Unhandled control group were compared separately to ethanol- and sham-treated groups using ANOVAs.