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Chronic Binge Ethanol Mediated Acidemia Reduces Availability of Glutamine and Related Amino Acids in Maternal Plasma of Pregnant Sheep

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Abstract

Heavy drinking during pregnancy can result in Fetal Alcohol Syndrome (FAS), of which, fetal and postnatal growth retardation and central nervous system deficits are cardinal features. While a number of mechanisms have been proposed, none fully account for these deficiencies. We have previously reported that maternal ethanol exposure (1.75 g/kg) results in transient acidemia in the mother and fetus. Alterations in pH are known to regulate glutamine homeostasis. Therefore, we hypothesized that chronic binge ethanol mediated acidosis reduces glutamine concentrations in maternal plasma that result in decreases in the circulating levels of amino acids related to glutamine metabolism. Pregnant ewes were divided into three groups: ethanol (1.75 g/kg), saline control, and acidemia (inspired fractional CO₂ was manipulated to mimic the maternal arterial pH pattern created by ethanol). The experiment was conducted on three consecutive days followed by four days without treatment beginning on day 109 of gestation, continuing to day 132. Plasma samples were analyzed for nutrients and metabolites using HPLC and spectrophotometric methods. Maternal plasma concentrations of glutamate increased (58%), while those of glutamine and related amino acids decreased (between 14 and 53%) in response to an acute challenge following the chronic exposure in ethanol treated ewes. No differences in these amino acid concentrations were noted between the ethanol and acidemic group subjects. Maternal plasma lactate increased by ~100% in response to ethanol while glucose and urea did not change in any group. We conclude that maternal chronic binge ethanol consumption results in acidosis mediated reductions in circulating levels of glutamine and related amino acids that could be responsible for neuronal deficits, altered fetal growth, development, and programming. We also speculate that the consequent increase in fetal glutamate during critical periods of brain development may contribute to the pathogenesis of FAS.

Keywords

FAS; FASD; alcohol; acidosis; amino acids; glutamate

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INTRODUCTION

The teratogenic effects of ethanol, collectively referred to as Fetal Alcohol Spectrum Disorder (FASD), include physical, mental, behavioral and/or learning deficits of which Fetal Alcohol Syndrome (FAS), a severe form of FASD, is the most well known consequence (Riley and McGee, 2005; Sokol et al., 2003). Although considerable efforts have been made to educate women to not drink during pregnancy, the incidence of prenatal ethanol exposure has not declined (Caetano et al., 2006; CDC, 2004; NIAAA, 2000). Currently, there are no known effective treatments, though some amelioration has been reported in a few individuals (Institute of Medicine, 1996).

A cardinal feature of FAS is growth deficiency (Sokol and Clarren, 1989). Prenatal ethanol exposure retards both growth and development of the fetus and progeny in rats (Lee and Leichter, 1983; Leichter and Lee, 1979). In children, prenatal ethanol exposure has been associated with growth retardation that can persist through 14 years of age (Day et al., 2002), suggesting that ethanol exposure during a sensitive or critical period of development has long-term implications for the offspring. The mechanisms by which *in utero* ethanol exposure induces fetal growth deficits are not well understood though fetal malnutrition is considered a major candidate. Ethanol could lead to fetal undernutrition by at least three possible ways: reduced maternal dietary intake (Schenker et al., 1990), impaired intestinal and/or placental transport of specific nutrients (Fisher et al., 1981; Henderson et al., 1981; Lin, 1981; Polache et al., 1996), and altered maternal and/or fetal metabolism and compartmentalization of nutrients (Schenker et al., 1990). The consequences of any of these mechanisms by which the fetus is deprived of nutrients could lead to altered development and programming (Barker, 1994; Wu et al., 2004).

Normal fetal growth and development depend on a continuous supply of amino acids from the mother to the fetus. A number of amino acids have been demonstrated to be reduced in the maternal and the fetal compartments in response to gestational ethanol exposure in rodents. Acute ethanol exposure in the pregnant mouse results in a significant reduction in the plasma concentrations of threonine, serine, glutamine, glycine, alanine, and methionine (Padmanabhan et al., 2002). Chronic ethanol exposure during the first two trimester-equivalents of human brain growth modeled in the rat has been shown to reduce maternal plasma proline concentration (Marquis et al., 1984). Utilizing a chronic paradigm during the first two trimester-equivalents of human brain growth, Karl and coworkers (Karl et al., 1995) found altered plasma glutamate in the fetal rat, but not in the mother. However, none of these studies have provided a coherent explanation for ethanol induced alterations in plasma concentrations of amino acids during pregnancy.

In this study, we report for the first time a pH-based mechanism for ethanol-induced alterations in amino acid levels during pregnancy. Perturbation in pH was hypothesized to be a mechanism underlying the teratogenic effects of ethanol even before FAS was described by Jones and colleagues (Horiguchi et al., 1971; Jones et al., 1973). Clinically, in humans, ethanol ingestion results in a mixed respiratory and metabolic acidosis and the blood ethanol concentration is directly proportional to the blood pH (Lamminpaa and Vilska, 1990, 1991; Sahn et al., 1975; Zehtabchi et al., 2005). In animal models of FASD, the mother and the fetus experience transient increases in arterial partial pressure of carbon dioxide ($P_a\text{CO}_2$), resulting in a reduction in arterial pH (pH_a) with every bout of ethanol consumption (Cudd et al., 2001b; Ramadoss et al., 2006b; Ramadoss et al., 2007; West et al., 2001). Acute pH changes are known to alter glutamine/glutamate metabolism and transport across cell membranes (Curthoys and Watford, 1995; Nissim, 1999). Glutamine is a major nutrient required by the fetus for growth (Kwon et al., 2003), and a reduction in its maternal plasma concentration might be detrimental

to the normal growth and development of the fetus. Moreover, a disturbance in glutamine homeostasis could also lead to alterations in the levels of several other amino acids like arginine and citrulline whose biosyntheses depend on glutamine. Therefore, we hypothesized that ethanol mediated acidosis is a candidate mechanism governing alterations in maternal amino acid homeostasis, and that by creating in pregnant ewes the same magnitude and pattern of acidemia, independent of ethanol exposure, as that produced by ethanol would result in similar alterations in the circulating concentrations of glutamine and the amino acids that are glutamine-dependent. The experimental paradigm was designed to study, in sheep, the response to an acute dose of ethanol, following four weekly three day binge exposures (a drinking pattern common in women who use ethanol during pregnancy) (Caetano et al., 2006; Cudd et al., 2001a; Ebrahim et al., 1999; Gladstone et al., 1996; Maier and West, 2001), lasting the third trimester-equivalent of human brain development, a period of high velocity brain growth when it is known that the brain is sensitive to ethanol mediated damage (Cudd, 2005; Dobbing and Sands, 1979). A dose of 1.75 g/kg of alcohol was used to achieve blood alcohol concentrations (BAC) of around 260 mg/dl, values that have been reported for mothers of children with FAS (Church and Gerkin, 1988) and in women who abuse alcohol (Urso et al., 1981).

MATERIALS AND METHODS

Subjects

The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University. Suffolk ewes, aged 2–6 years of age, were mated and pregnancies of known date of conception were confirmed as previously described (Ramadoss et al., 2006a). In brief, time dated pregnancies were achieved by controlling the estrous cycle through the use of progesterone impregnated vaginal implants (EAZI-BREED™, CIDR®, Pharmacia & Upjohn Ltd., Auckland New Zealand). Implants were removed 11 days after placement at which time prostaglandin F_{2α} (20 mg; LUTALYSE®, Pharmacia & Upjohn, Kalamazoo MI) was intramuscularly administered. The following day, ewes were placed with a ram fitted with a marking harness for a period of 24 hours. Marked ewes were assessed ultrasonographically on gestational days (GD) 25, 60 and 90 to confirm pregnancy. Ewes were maintained in shaded outdoor pens with herdmates from before mating until GD 100. On GD 100, the ewes were relocated to an environmentally regulated facility (22°C and a 12:12 light/dark cycle) where they remained for the duration of the experiments. Animals in all treatment groups were fed 2 kg/day of a “complete” ration (Sheep and Goat Pellet, Producers Cooperative, Bryan, TX). All animals consumed all of the feed offered.

Alcohol Dosing Protocol

Animals were divided into three experimental groups: an ethanol group (n = 5), an acidemic group (n = 6), and a pair-fed saline control (SC) group (n = 6). A week before the start of the experiment, on GD 102, the ewes underwent surgery to chronically implant femoral arterial and venous catheters (0.050” inner diameter, 0.090” outer diameter polyvinyl chloride). Details of the surgery protocol have been described earlier (Cudd et al., 2001b). The experiments were conducted on three consecutive days beginning on GD 109 followed by 4 days without treatment with the weekly pattern being repeated until GD 132. In all treatment groups, the infusion solutions were delivered intravenously over an hour by peristaltic pump (Masterflex, model 7014-20, Cole parmer, Niles IL) through a 0.2 µm bacteriostatic filter. Pumps were calibrated before infusion. Ewes in the ethanol treatment group received ethanol at a dosage of 1.75 g/kg body weight, as a 40% w/v solution diluted in 0.9% saline. Subjects in the pair-fed saline control and the acidemic groups received saline (0.9% saline) at a rate and volume equivalent to that of the ethanol group on a per kg basis.

Determination of the pattern and timing of exposure

We used a chronic weekly-weekend binge ethanol exposure paradigm, a common pattern of drinking in women who abuse ethanol during pregnancy (Caetano et al., 2006; Cudd et al., 2001a; Ebrahim et al., 1999; Gladstone et al., 1996; Maier and West, 2001). In humans, the velocity of fetal brain growth increases throughout the third trimester to peak at parturition (Dobbing and Sands, 1979). This period of third trimester high velocity brain growth is a time when it is known that there is a high sensitivity to ethanol mediated brain damage (Dobbing and Sands, 1979; West et al., 2001). In order to extrapolate this timing of exposure from sheep to humans, we differentiated the equation reported by Richardson and Herbert that predicts ovine fetal brain weight as a function of gestational age, with respect to time, and the first order velocity curve was plotted similar to that of Dobbing and Sands (Dobbing and Sands, 1979; Richardson and Hebert, 1978) (Figure 1). In the sheep, the velocity of brain growth declines significantly after GD 132. Therefore, the experiment was performed in pregnant sheep from GD 109 to GD 132 (term is 147 days) in order to best model the period of human third trimester high velocity brain growth.

Experiment Protocol

On the day of an experiment, ewes were placed in a modified metabolism cart so that the animal's head was inside a plexiglass chamber. A vinyl diaphragm attached to the open side of the chamber was drawn around the animal's neck to isolate the atmosphere in the chamber from ambient air. In the acidemic group, subjects were exposed to increased inspired fractional concentrations of carbon dioxide for 6 hours, to create a similar magnitude and pattern of reduction in the arterial pH compared to that produced by ethanol in previous studies (Cudd et al., 2001b). The rate at which CO₂ was introduced into the chamber in the acidemic group was determined by monitoring maternal arterial pH; the CO₂ inflow rate was adjusted so that maternal arterial pH in the acidemic and ethanol groups were matched over the duration of the 6 hour experimental period on all 12 experimental days. The percentage of oxygen and carbon dioxide in the chamber was measured using a gas monitor (oxygen, model S-3A; carbon-dioxide, model CD-3A, Applied Technologies, Pittsburgh, PA). Normoxemic conditions were maintained throughout the experiment in the acidemic group. Subjects in the ethanol and the saline groups had their heads inside the plexiglass chamber, but the chamber bottom was removed to allow breathing of room air.

Blood (1 ml) was drawn from the femoral artery catheter at 0, 0.5, 1, 1.5, 2, 3, 4, 5, and 6 hours for blood gas analysis on all experiment days. Samples were collected in heparinized 3 ml syringes, capped and immediately analyzed using a blood gas analyzer (ABL 5; Radiometer, Westlake, OH). Blood ethanol concentration (BEC) was measured at 0 and 1 hour. A 20 µl aliquot of blood was collected into microcapillary tubes and transferred into vials that contained 0.6 N perchloric acid and 4 mM n-propyl ethanol (internal standard) in distilled water. The vials were tightly capped with a septum sealed lid and were stored at room temperature until analysis by headspace gas chromatography (Varian Associates, model 3900, Palo Alto, CA) at least 24 hr after collection. The basic gas chromatographic parameters were similar to those reported by Penton (Penton, 1985), with the exception of the column (DB-wax, Megabore, J&W Scientific Folsom, CA) and the carrier gas (helium) used (West et al., 2001).

Assessment of plasma amino acid, lactate, urea and glucose concentrations in response to the acute challenge following chronic exposure was performed on GD 132, the last day of the study, by withdrawing blood samples (1 mL) at the beginning and at the end of the infusion (1 hour), the time at which peak BEC is achieved (Cudd et al., 2001b). Samples were immediately centrifuged for 10 minutes at 3000 × g and 4°C. Plasma was acidified with 1 mL of 1.5 mol/L HClO₄ and then neutralized with 0.5 mL of 2 mol/L K₂CO₃. The supernatant was used for amino acid analysis by HPLC, as previously described (Wu et al., 1997). Plasma levels of

glucose, lactate, and urea were analyzed by spectrophotometric enzymic methods (glucose, using hexokinase and glucose 6P dehydrogenase; urea, using urease; lactate, using lactate dehydrogenase) as previously described (Fu et al., 2005; Wu, 1995).

Statistical Analyses

The percentage change in the levels of the nutrients/metabolites at one hour (the end of ethanol/saline infusion) compared with that at 0 hour (baseline), were analyzed using a one-way ANOVA with treatment group as the sole independent factor. Arterial pH was analyzed using a mixed ANOVA with treatment as a between factor and time as a within factor. Post-hoc tests were conducted using Fishers protected LSD test. The α level was established *a priori* at $p < 0.05$ for all analyses; p values between 0.05 and 0.10 were considered trends.

RESULTS

We modeled a weekend binge drinking pattern during the brain growth spurt by intravenously infusing pregnant ewes with ethanol or saline for an hour on three consecutive days beginning on gestational day (GD) 109 followed by 4 days without treatment with the weekly pattern being repeated until GD 132. The mean $\pm$ SEM maternal blood ethanol concentration at the end of ethanol infusion (1 hr; the time point when BECs were known to peak) (Cudd et al., 2001; West et al., 2001; Ramadoss et al., 2007) was 260 ± 10 mg/dl in the ethanol treated ewes.

Maternal acid-base status

Arterial pH at the 1st hour after the beginning of the treatment was reduced significantly in the ethanol and the acidemic groups, compared to the pair-fed saline control group and these decreases persisted for 5 hours after the end of infusion (Figure 2). The magnitude of pH reduction in the acidemic group was nearly identical to that in the ethanol group at all time points. This nearly identical decrease in the maternal arterial pH in the acidemic group was created by increasing the inspired partial pressure of carbon dioxide, independent of ethanol. Maternal P_aCO_2 similarly peaked at the 1st hour in the ethanol and the acidemic groups compared to the pair-fed saline control group, while measurements at 0th hour were not different among groups (baseline P_aCO_2 , SC, 34 ± 1 mm Hg; ethanol, 33 ± 0 mm Hg; acidemia, 34 ± 0 mm Hg). P_aCO_2 values at the 1st hour were 35 ± 0 mm Hg, 39 ± 0 mm Hg, and 52 ± 1 mm Hg in the saline control, ethanol, and acidemia groups respectively. Lactate levels were elevated at the 1st hour in the ethanol group ($\sim 100\%$), but not in the acidemic or the pair-fed saline control group (Figure 3), explaining why a greater P_aCO_2 elevation was required to produce a similar magnitude of acidemia as that in the ethanol group. Consequently, ethanol mediated acidemia had both a respiratory and a metabolic component associated with it, while the acidemia group was not subjected to metabolic acidosis.

Plasma glucose and urea

No differences were detected in the maternal plasma glucose level at the beginning of the last day of treatment (SC, 45 ± 3 mg/dl; ethanol, 46 ± 5 mg/dl; acidemia, 53 ± 4 mg/dl) or at the end of the last acute challenge among treatment groups (Figure 3). Plasma urea concentrations were not different when comparing among groups.

Maternal plasma concentrations of amino acids

Maternal plasma concentrations of most amino acids at the beginning of the last day of the experiment were not different among groups (Table 1). However, some differences were noted: glutamine and glutamate were significantly reduced in the ethanol group; arginine, asparagine, and serine were significantly elevated in the acidemic group, while branched chain amino acids

(BCAAs) were significantly higher in the ethanol as well as the acidemic groups, compared with that in the pair-fed saline control group.

A significant reduction in the maternal plasma concentrations of amino acids in response to an acute exposure following the chronic exposure was observed in the ethanol and the acidemic groups compared with those in the pair-fed saline control group (Figure 4). The reductions in the levels of arginine (~ 42%), citrulline (~ 25%), asparagine (~ 44%), serine (~ 30%), threonine (~ 44%), tryptophan (~ 24%), methionine (~ 46%), leucine (~ 31%), histidine (~ 14%), tyrosine (~ 24%), valine (~ 53%), and isoleucine (~ 35%) were significant at one hour in the ethanol group. The same specific amino acids were reduced significantly in the acidemic group. Glutamine was significantly reduced (~ 40%) in the ethanol group, whereas it trended lower (~ 25%) in the acidemic group. The only amino acid that exhibited a significant increase in response to ethanol was glutamate (~ 58%). In the acidemia group, glutamate was elevated (~ 30%) but the response was not statistically significant. Post-hoc analysis did not identify differences between the ethanol and acidemia groups for any of these amino acid concentrations. In contrast, glycine, taurine, alanine, ornithine, and phenylalanine were not different among groups. The pair-fed saline control group exhibited no alterations in any of the amino acids at the 1st hour, compared with baseline values.

DISCUSSION

Ethanol alters maternal plasma amino acid levels in the sheep model system

To our knowledge, this is the first study to identify dynamic changes in maternal plasma concentrations of amino acids in response to an acute challenge of ethanol following a chronic exposure throughout the third trimester-equivalent of human brain growth in the sheep model system. The sheep was chosen because the third trimester equivalent of brain development in this species occurs *in utero*, as it does in the human and not postnatally as occurs in rats. Using a postnatal model of third trimester-equivalent of human brain development like the rat or mouse would add the potential confounds of eliminating maternal, placental and fetal interactions or a role of parturition in mediating the damage. In addition, we have previously established that the third trimester equivalent in sheep is a period of increased vulnerability to ethanol as it is in humans and in other animal models (Livy et al., 2003; West et al., 2001). Further, the sheep was utilized because of the robustness of this species in experiments requiring chronic instrumentation.

The maternal plasma amino acid concentrations in this study in the pair-fed saline control group were similar to those reported previously in normal pregnant ewes on a similar day of gestation (Kwon et al., 2003). The concentrations of most amino acids at the beginning of the last day of experiment were not different among the treatment groups though glutamine and glutamate concentration were reduced in the ethanol group and BCAA concentrations were elevated in the ethanol and the acidemic groups. A final acute challenge of ethanol or acidemia following the chronic exposure resulted in a significant reduction of glutamine, arginine, citrulline, asparagine, serine, threonine, tryptophan, methionine, leucine, histidine, tyrosine, valine, and isoleucine, compared with that at baseline, while glutamate was elevated. Glucose, in contrast, was not altered in response to ethanol, a finding that is consistent with previous reports; maternal ethanol consumption reduces fetal, but not maternal plasma glucose levels (Falconer, 1990; Marquis et al., 1984; Richardson et al., 1985). Utilizing a chronic, but not a binge ethanol exposure during the first two trimester-equivalents of human brain growth in rats, Karl and coworkers (Karl et al., 1995) found increased fetal glutamate levels, but glutamine was not altered in the mother or the fetus. Other rat studies involving a similar exposure paradigm have demonstrated a decrease in maternal plasma proline levels (Marquis et al., 1984), as well as reduced sodium dependent intestinal absorption of methionine (Polache et al., 1996), and taurine (Martin-Algarra et al., 1998). In contrast to these chronic exposure studies, an acute

ethanol study in the pregnant mouse resulted in a significant reduction in the plasma concentrations of several amino acids, including threonine, serine, glutamine, glycine, alanine, and methionine (Padmanabhan et al., 2002). The potential adverse effects of these decreases in maternal amino acid levels would be further compounded by ethanol mediated reductions in transplacental amino acid transport (Fisher et al., 1981; Henderson et al., 1981; Lin, 1981; Schenker et al., 1990), leading to further decreased availability of fetal nutrients.

Feed intake was not affected by the intravenous infusion of a modest amount of ethanol in our ovine model. The fact that maternal plasma concentrations of glucose and most amino acids were not lowered in ethanol-infused ewes at the beginning of the last infusion indicates that our model of FASD is distinctly different from the model of severe maternal malnutrition. The metabolic consequence of the changes in plasma nutrients on fetal development requires further investigation. In addition, glutamate levels in the fetus and brain are tightly regulated by uptake, glutamine synthesis and glutamate synthesis. We did not obtain fetal brain in the study and thus could not measure glutamate in this tissue.

Plasma concentrations of amino acids in ewes are regulated by many factors including dietary intake of protein, net amino acid synthesis by rumen microorganisms, as well as whole-body protein turnover and amino acid metabolism. Complex metabolic studies using stable isotopes would be required to precisely explain changes in plasma levels of amino acids, including methionine, leucine, and tyrosine. These experiments are beyond of the scope of the present work.

Acidemia is a central mechanism governing ethanol mediated alterations in amino acid levels

Acidemia was suggested as a mechanism underlying the teratogenic effects of ethanol even before FAS was described by Jones and coworkers (Horiguchi et al., 1971; Jones et al., 1973). In humans, ethanol consumption results in a mixed respiratory and metabolic acidosis and the blood pH is directly proportional to the blood ethanol concentration (Lamminpaa and Vilska, 1990, 1991; Zehtabchi et al., 2005). In this study, ethanol resulted in a mixed acidosis, a finding consistent with human clinical studies and previous reports from this laboratory where we have shown that with each bout of ethanol, the mother and the fetus were subjected to transient acidemia, irrespective of the period of gestation (Cudd et al., 2001b; Ramadoss et al., 2007). We mimicked the pH pattern produced by ethanol by manipulating maternal $P_a\text{CO}_2$ rather than by using an exclusively metabolic or a mixed respiratory/metabolic approach because 1) altering CO_2 affects pH in all fetal and maternal body fluid compartments and 2) precise manipulation of arterial pH could be maintained over 6 hours by respiratory means without the potential confounds of creating fluid volume or electrolyte changes that might occur in response to prolonged acid infusions. Further, it has been demonstrated in other animal model systems that the renal glutamine uptake in respiratory and metabolic acidosis is comparable (Gougoux et al., 1982; Windus et al., 1984).

The current study extends our previous work by demonstrating that an acute acidemic challenge (independent of ethanol), of a nearly identical magnitude to that brought about by ethanol, resulted in similar amino acid alterations as in the ethanol treatment group. The same specific amino acids were altered in both groups suggesting that acidemia is the central mechanism mediating these changes in amino acid concentrations. We propose the following model to explain the amino acid dynamics in ethanol induced acidemia.

The ethanol-glutamine model

The role of the kidney—The maternal kidney is one of two organ systems that are largely responsible for alterations in maternal amino acid levels in response to acidosis (Figure 5). In

the present study, we observed a significant decrease in glutamine levels in response to an acute challenge following the chronic exposure, a finding that is consistent with previous reports where acidosis has been shown to decrease plasma glutamine levels (Heitmann and Bergman, 1980) due to increased renal glutamine uptake, by 365% in nonpregnant sheep (Heitmann and Bergman, 1980), and by even higher values in humans and rats (Welbourne, 1987; Welbourne et al., 1986). The kidneys extract and catabolize more than one third of plasma glutamine in a single pass through the organ (Taylor and Curthoys, 2004). Several factors contribute to this increased renal absorption of plasma glutamine (Brosnan, 1987). First, acidosis stimulates glutamine uptake by renal mitochondria and its metabolism (Nissim, 1999). Second, fall in pH, to a magnitude similar to that in this study elicits increased renal expression of the system N glutamine transporter SNAT3 (Moret et al., 2007). This alteration in the renal glutamine transporter is glucocorticoid dependent (Karinch et al., 2007). Previous studies conducted in our laboratory have demonstrated that ethanol increases fetal as well as maternal cortisol levels (Cudd et al., 2001a; Ramadoss et al., 2008). Therefore, these increases in cortisol would be expected to increase the glutamine uptake by the kidneys (Karinch et al., 2007), further contributing to the observed decreases in plasma glutamine levels in acidotic ewes. On going experiments in our laboratory are investigating the role of ethanol-induced elevations in cortisol in maternal and fetal amino acid levels. Third, formation of glutamine from glutamate and ammonia is negligible in the kidneys, especially in acidosis (Nissim, 1999). Taken together, these three processes explain the decreased plasma glutamine levels. Even though the gut and liver of the sheep also play a role in utilizing plasma glutamine, the intestinal extraction does not change in response to acidosis and the hepatic uptake of glutamine decreases in chronic acidosis (Heitmann and Bergman, 1980). It is worthy of note that in humans, the kidney is the only organ that is involved in plasma glutamine extraction during acidosis (Welbourne, 1987).

A reduction in plasma glutamine concentration will directly affect the levels of several other amino acids that are synthesized from glutamine (Wu and Morris, 1998); these include ornithine, citrulline (an intermediate in arginine synthesis from glutamine and proline), and arginine (Wu and Morris, 1998). In addition, elevated levels of lactate can reduce intestinal synthesis of citrulline and arginine from proline by inhibiting proline oxidase activity (Dillon et al., 1999). The glutamate formed from glutamine in the kidney is removed by glutamate dehydrogenase, by transamination reactions, and by transport from the intracellular (ICF) to the extracellular (ECF) compartment (Nissim, 1999), probably explaining the observed increases in plasma glutamate level in this study. Finally, an acidemia mediated increase in expression of renal glutamine SNAT3 transporter would result in increased renal uptake of histidine and asparagine, probably accounting for their decreased availability in maternal plasma (Chaudhry et al., 2001; Karinch et al., 2002).

The role of the skeletal muscle—The major source of plasma glutamine in sheep and humans is skeletal muscle (Welbourne, 1987). Some evidence suggests that glutamine release from muscle is not altered in response to acidosis in sheep (Heitmann and Bergman, 1980), a finding that would explain the observed decreases in plasma glutamine concentration. In the presence of ethanol mediated increases in glucocorticoids (Cudd et al., 2001a), acidosis would up-regulate the transamination of BCAAs with α -ketoglutarate to form glutamate and branched chain α -ketoacids (BCKAs) and would also directly stimulate the oxidative catabolism of BCKAs through the activation of BCKA dehydrogenase, ultimately leading to decreased plasma levels of BCAAs (May et al., 1987). Interestingly, plasma concentrations of BCAAs at the beginning of the last ethanol or acidemia treatment were elevated (Table 1), suggesting a decrease in their intramuscular catabolism or an increase in net proteolysis.

Fetal:maternal ratios of plasma amino acids

The transport and metabolism of glutamine and glutamate during fetal development differs from that in the adult; glutamine enters the fetal circulation and is taken up by the fetal liver and the total fetal glutamate is virtually identical to the fetal hepatic conversion of glutamine to glutamate (Battaglia, 2000; Moores et al., 1994; Vaughn et al., 1995). Glutamine and glutamate are transported across the placenta primarily by the N and X_{GA} transport systems, respectively. Degradation of glutamine in placenta is limited because of the absence of glutaminase, the enzyme that is chiefly responsible enzyme for initiating glutamine hydrolysis. In contrast, glutamate is extensively metabolized by the placenta. Thus, in sheep, fetal:maternal ratios of plasma glutamate and glutamine are < 0.6 and approximately 2.0, respectively, between Days 60 and 140 of gestation (Kwon et al., 2003). The fetal liver hydrolyses glutamine to yield glutamate and releases glutamate into the systemic circulation for utilization by the placenta and other fetal tissues. We are currently investigating the role of ethanol-induced acidemia on fetal amino acid levels. We speculate that plasma concentrations of amino acids, including arginine, citrulline, and glutamine may be reduced in the fetus due to decreased maternal bioavailability. Finally, we wish to point out that the magnitude of response to ethanol-induced acidemia may be different in non-pregnant subjects. Marked changes in maternal and fetal plasma amino acid concentrations are observed with conceptus development (Kwon et al., 2003). The significance of the metabolic changes during gestation is evidenced by the amino acid levels in the allantoic fluid that is rich in alanine, citrulline, glutamine, and serine derived from fetal and maternal secretions and from placental transport mechanisms (Kwon et al., 2003). However, it should be noted that a decrease in blood pH has been reported to reduce plasma glutamine levels in non-pregnant sheep, humans, and rats (Heitmann and Bergman, 1980; Welbourne, 1987; Welbourne et al., 1986).

Conclusion and Perspectives

Results of this study support the conclusion that chronic binge ethanol induced acidosis alters amino acid homeostasis in pregnant ewes. These novel findings may help explain intra-uterine growth retardation and structural damage to the nervous system observed in FASD. Women who drink during pregnancy may exhibit decreases in plasma amino acid concentrations and thus availability of glutamine and related amino acids with each bout of ethanol consumption. Repeated perturbations in the fetal availability of specific nutrients due to altered concentrations in maternal plasma could result in impaired development and altered programming with life-long consequences for the offspring. Therefore, we conclude that maternal acidosis and glutamine dependent pathways should be taken into consideration in the development of effective therapeutic measures for FASD.

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Figure 1.

Fetal sheep brain growth velocity. The ethanol exposure paradigm was designed to occur during the period of peak brain growth velocity like that during the third trimester in humans, a period when it is known that the brain is sensitive to ethanol mediated damage. In order to reasonably extrapolate the timing of exposure from sheep to humans, we differentiated the equation reported by Richardson and Herbert that predicts ovine fetal brain weight as a function of gestational age, with respect to time, and the first order velocity curve was plotted in a manner similar to that of Dobbing and Sands (Dobbing and Sands, 1979; Richardson and Hebert, 1978).

Figure 2.

Maternal arterial pH (pH_a). The magnitude decrease in the maternal pH_a produced by ethanol was created in the acidemic subjects by increasing the inspired partial pressure of carbon dioxide independent of ethanol (baseline pH_a , SC, 7.47 ± 0.01 ; ethanol, 7.51 ± 0.01 ; acidemia, 7.46 ± 0.01). pH_a was significantly decreased (*) in the ethanol and acidemia groups compared to the pair-fed saline control group (SC). The pH_a at the end of the infusion (1 hour) in the ethanol and acidemia groups was different (#) from that at all other time points.

Figure 3.

Maternal plasma glucose, lactate, and urea responses to 1 hour ethanol, acidemia, or control manipulations following a chronic weekly weekend binge exposure. Lactate was significantly elevated (*) in the ethanol group compared to the acidemic and saline control (SC) groups. Maternal plasma glucose and urea concentrations were not different when comparing between groups.

Figure 4.

Maternal plasma amino acid responses to 1 hour ethanol, acidemia, or control manipulations following a chronic weekly weekend binge exposure. Significant reduction (*) in specific amino acids were observed in the ethanol and acidemia groups compared with that in the pair-fed saline control group (SC). Glutamine was significantly decreased (#) in the ethanol group whereas the acidemia group trended lower compared to the control group. The only amino acid that exhibited a significant increase in response to ethanol was glutamate (#). In the acidemia group, glutamate trended higher compared to the control group. No differences were detected between the ethanol and the academic groups.

Figure 5.

The ethanol-glutamine model illustrating putative maternal amino acid homeostatic responses to an acute challenge of ethanol following chronic exposure. The kidney acts as a major sink for glutamine during ethanol induced acidosis. Increased renal extraction of plasma glutamine results in decreased availability of arginine and citrulline whose syntheses are glutamine dependent. Acidemia induced elevations in renal glutamine SNAT3 transporter expression result in increased renal uptake of histidine and asparagine. In the muscle, ethanol induced acidosis up-regulates the transamination of branched chain amino acids (BCAA) with α -ketoglutarate to form glutamate and branched chain α -ketoacids (BCKA) and also directly stimulates the oxidative catabolism of BCKAs, leading to decreased plasma levels of BCAAs.

Table 1

Maternal plasma amino acid concentrations ($\mu\text{mol/L}$) at the beginning of the last day of experiment were not different among groups for most amino acids. Glutamine and glutamate were significantly (*) reduced in the ethanol group; arginine, asparagine, serine were significantly elevated in the acidemic group, while valine, methionine, and total branched chain amino acids (BCAA) were significantly higher in the ethanol and the acidemic groups compared with that in the pair-fed saline control group (SC).

Amino acids	SC	Ethanol	Acidemia
ARG	142 $\pm$ 10	148 $\pm$ 13	204 $\pm$ 24*
GLU	106 $\pm$ 14	64 $\pm$ 8*	99 $\pm$ 20
ASP	8 $\pm$ 0	5 $\pm$ 1	8 $\pm$ 1
SER	62 $\pm$ 4	60 $\pm$ 8	85 $\pm$ 8*
THR	45 $\pm$ 4	52 $\pm$ 8	62 $\pm$ 10
CIT	119 $\pm$ 17	123 $\pm$ 15	145 $\pm$ 25
ALA	97 $\pm$ 7	77 $\pm$ 12	102 $\pm$ 4
TRP	20 $\pm$ 1	22 $\pm$ 1	27 $\pm$ 4
ASN	23 $\pm$ 1	26 $\pm$ 4	37 $\pm$ 3*
GLN	179 $\pm$ 16	112 $\pm$ 15*	165 $\pm$ 12
THR	45 $\pm$ 3	52 $\pm$ 8	62 $\pm$ 10
MET	13 $\pm$ 0.5	20 $\pm$ 2*	23 $\pm$ 2*
LEU	66 $\pm$ 7	84 $\pm$ 7*	83 $\pm$ 7*
HIS	22 $\pm$ 3	23 $\pm$ 1	23 $\pm$ 1
TAU	76 $\pm$ 12	62 $\pm$ 11	75 $\pm$ 18
TYR	36 $\pm$ 2	40 $\pm$ 3	48 $\pm$ 5
VAL	79 $\pm$ 3	148 $\pm$ 24*	170 $\pm$ 19*
PHE	29 $\pm$ 1	28 $\pm$ 0	32 $\pm$ 2
ILE	59 $\pm$ 3	66 $\pm$ 6	69 $\pm$ 3
ORN	35 $\pm$ 3	35 $\pm$ 4	44 $\pm$ 4
BCAA	204 $\pm$ 11	298 $\pm$ 35*	322 $\pm$ 22*