Influence of prenatal ethanol exposure on vascular contractile response in rat thoracic aorta

Leigh-Anne Turcottea, Nicholas S. Aberle IIa, Faye L. Norbya, Guei-Jane Wangb, Jun Rena,*

aDepartment of Pharmacology, Physiology, and Therapeutics, University of North Dakota School of Medicine, 501 N. Columbia Road, Grand Forks, ND 58203, USA
bNational Research Institute of Chinese Medicine, Taipei 112, Taiwan

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Abstract

Fetal alcohol syndrome is associated with cardiovascular malformation. However, the impact of prenatal ethanol exposure on vascular function is not clear. The purpose of this study was to examine the influence of prenatal ethanol exposure on vascular response in adulthood. Timed-pregnancy, female rats were fed an ethanol-containing liquid diet (36% calorically or 6.36% [vol./vol.]) or control diet from gestation day 2 until labor. The pups continued to receive a standard rat chow through adulthood, and the force-generating capacity of aortic ring segments was examined. Prenatal ethanol exposure did not significantly affect postnatal growth, but it did lead to elevated blood pressure in adulthood. The contractile response to potassium chloride was similar in vessels with intact endothelium, although the median effective concentration (EC$_{50}$) was significantly reduced by prenatal ethanol exposure in rings with denuded endothelium. The response to norepinephrine was attenuated by prenatal ethanol exposure in rings with either intact or denuded endothelium. The endothelium-dependent relaxation to carbamylcholine chloride was significantly attenuated by prenatal ethanol exposure. Vasorelaxant response to the nitric oxide donor sodium nitroprusside or $\beta$-adrenergic agonist isoproterenol was similar between control and prenatal-ethanol-exposed groups with either intact or denuded endothelium. Ethanol elicited a dose-dependent endothelium-dependent vasorelaxation, which was comparable between the two animal groups. The ethanol-induced endothelium-dependent vasorelaxation was attenuated by the nitric oxide synthase inhibitor $N$-nitro-$L$-arginine methyl ester. These findings seem to indicate that prenatal ethanol exposure contributes to alterations of both endothelium-dependent and endothelium-independent vascular contractile responses. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Maternal alcohol (ethanol) consumption often leads to a series of fetal teratogenic effects, such as growth retardation, abnormal facial features, and central nervous system damage (Stratton et al., 1996). These abnormalities are collectively defined as fetal alcohol syndrome (FAS), which is characterized by organ abnormalities in the central nervous system, gastrointestinal tract, and cardiovascular system. Defects in the cardiovascular system appear in up to 50% of FAS children (Loser & Majewski, 1977). Ethanol exposure during embryogenesis contributes to abnormal cardiac and vascular development (Adickes et al., 1990; Daft et al., 1986). Reduction and delay in the development of myosin and actin, as well as alteration in Ca$^{2+}$ transport, mitochondrial function, sarcoplasmic reticulum Ca$^{2+}$ uptake/binding, and intracellular Ca$^{2+}$ homeostasis, have been reported in several animal models of FAS (Altura et al., 1996; Ren et al., 2002). Despite these important findings, the role of prenatal ethanol exposure on vascular contractile response has not been well characterized. The aim of the present study was to examine the teratogenic effects of ethanol on vascular function in offspring exposed to ethanol in utero. Pregnant Sprague–Dawley rats fed with ethanol liquid diet from gestational day 2 until delivery were used as a model for FAS, and the force-generating capacity of thoracic aorta was evaluated in FAS rats at adulthood.

2. Materials and methods

2.1. Prenatal ethanol exposure animal model

The experimental procedures described in this study were approved by the Animal Care Committee from University of North Dakota. Nulliparous female Sprague–Dawley rats from an in-house colony were mated with males overnight, and the presence of a vaginal plug the next morning indi-
cated successful mating and was designated gestational day 1. The pregnant rats were divided into two groups (weight-matched) and introduced to a liquid diet (Shake & Pour, Bioserv Inc., Frenchtown, NJ) with or without ethanol supplementation, beginning on gestational day 2 until the pups were born. This liquid diet has been used extensively and proven to be nutritionally complete (Posner et al., 1987; Ren & Brown, 2000). The ethanol-containing diet had 36% of total calories isocalorically replaced by ethanol (equivalent to 6.36% [vol/vol]). An isocaloric pair-feeding regimen was used for the control group (Ren & Brown, 2000), in which case non-ethanol-consuming animals were offered the same quantity of diet ethanol-consuming animals drank the day before. Freshly made liquid diet (80 ml per tube) was given between 1700 and 1800 daily. Both male and female offspring of the ethanol-exposed and control groups were housed in a temperature-controlled room under a 12-h/12-h light/dark illumination cycle and allowed access to standard rat chow and tap water ad libitum. Systolic blood pressure was measured weekly by using a semiautomated, amplified tail-cuff device (IITC Inc., Woodland Hills, CA). The offspring were used for study at approximately 25 weeks of age.

2.2. Plasma ethanol determination

Mid-gestational blood samples were collected between 0900 and 1000 on gestational day 12. Plasma was separated by centrifugation (4,400 rpm for 20 min at 4°C), and plasma ethanol concentrations were determined by using the alcohol dehydrogenase assay (Sigma Chemical Co., St. Louis, MO).

2.3. Aortic ring isolation procedure

At 25 weeks of age, the animals were anesthetized with a ketamine/xylazine mixture solution (3:1, 1.32 mg/kg, i.p.). Muscle relaxant ketamine and local anesthetic xylazine were selected (approved by the Animal Care Committee of University of North Dakota) to avoid potential direct cardiovascular effect associated with other anesthetics such as pentobarbital. Thoracic aorta was quickly removed and placed in an oxygenated physiologic salt solution (PSS) of the following composition (in mM): KCl, 5.4; NaCl, 136.9; NaHCO₃, 11.9; NaH₂PO₄, 0.45; MgCl₂, 0.50; CaCl₂, 2.7; and glucose, 5.6. Vessels were trimmed free of connective tissue and fat. The transverse segments (3 mm in length) were cut with special care to avoid damage to the endothelial layer. One aortic segment from each animal was left intact while the other segment was mechanically denuded of endothelium by gently rubbing the intimal surface of the vessel. Next, vessel rings were mounted vertically by using fine wires inserted through the lumen for isometric tension recording in 10-ml organ baths containing PSS gassed with 95% O₂ and 5% CO₂ at 37°C and pH ∼7.4. All rings were stretched to ∼0.7 g of resting tension and allowed to equilibrate for a period during which the bath solution was changed frequently. Isometric contractile tension was measured by using Grass FT03 force-displacement transducers coupled to a Grass Model 79 polygraph (Grass Instrument, Quincy, MA). Presence of functional endothelium was determined by greater than 50% relaxation by addition of carbamylcholine chloride (2 μM) to segments precontracted with norepinephrine (1 μM). Aortic segments relaxing less than 50% were considered to be without functional endothelium (Brown & Savage, 1996).

2.4. Experimental protocols

Aortic ring segments, once mounted, were allowed to equilibrate in PSS for 1 h before the start of each experiment. Dose-response curves of norepinephrine, potassium chloride, carbamylcholine chloride, sodium nitroprusside, isoproterenol, and ethanol were then determined. In some studies, Nα-nitro-L-arginine methyl ester (L-NAME, 100 μM) was incubated with the vessels for 30 min before addition of ethanol. Each agonist was added cumulatively, and concentrations were allowed to elicit maximal response before subsequent doses were applied.

Fig. 1. Mean body weight of pups from birth to week 25. Pups were weaned from the dam at 4 weeks of age and placed on regular laboratory chow and water ad libitum until the experimental day. The postnatal growth was not affected by prenatal ethanol exposure.
2.5. Statistical analysis

For each experimental series, data are presented as mean ± S.E.M. Statistical significance \( (P < .05) \) for each variable was estimated by two-way analysis of variance (ANOVA) or \( t \) test, where appropriate. A Dunnett test was used for post hoc analysis when required.

3. Results

3.1. General features of control and experimental animals

The average daily ethanol diet intake in pregnant dams was 51.3 ± 0.2 ml (8.50 ± 0.03 g of ethanol per kilogram of body weight). Weight-matched control dams received the same volume of diet consumed by the ethanol-exposed counterparts the previous day (pair-fed). As expected, serum ethanol levels were significantly greater among the ethanol-consuming dams (24.4 ± 6.6 mg/dl, \( n = 6 \), \( P < .05 \)) compared with findings for the control dams (0 ± 0 mg/dl, \( n = 6 \)). Pups from dams consuming either ethanol or control diets during pregnancy displayed similar birth weight, growth curves, and adulthood body and organ weight per size (Table 1 and Fig. 1). Interestingly, pups with prenatal ethanol exposure showed elevated systolic blood pressure compared with findings for control pups at 25 weeks of age (Table 1).

3.2. Vasoconstrictive response to potassium chloride and norepinephrine (both intact and denuded endothelium)

Bath application of potassium chloride (10–120 mM) produced dose-dependent vasoconstriction in aortic ring segments with either intact or denuded endothelium from both control and prenatal-ethanol-exposed pups (Fig. 2A and B).

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**Fig. 2.** Effects of prenatal ethanol exposure on potassium chloride–induced (panels A and B) or norepinephrine-induced (panels C and D) vasoconstriction in isolated rat aortic ring segments with intact or denuded endothelium. Data are mean ± S.E.M. \# \( P < .05 \) compared with findings for control group. The median effective concentration (EC\(_{50}\)) was significantly reduced by prenatal ethanol exposure in rings with denuded endothelium. The response to norepinephrine was attenuated by prenatal ethanol exposure in rings with either intact or denuded endothelium.
The maximal tension developed at 120 mM potassium chloride in endothelium-denuded rings was not significantly different between control and prenatal-ethanol-exposed groups. The potassium chloride–induced vasoconstrictive response in endothelium-intact ring segments was comparable between control and prenatal-ethanol-exposed groups, with a median effective concentration (EC50) of 18.7 ± 2.2 mM (n = 18) and 20.1 ± 2.0 mM (n = 16), respectively (Fig. 2A). However, the EC50 of potassium chloride–induced response in endothelium-denuded rings was significantly reduced in the prenatal-ethanol-exposed group (14.8 ± 2.7 mM, n = 14) compared with findings for the control group (30.1 ± 7.7 mM, n = 13, P < .05) (Fig. 2B). Similar to potassium chloride, bath application of norepinephrine (0.01–5 μM) produced dose-dependent vasoconstrictive responses in aortic ring segments with either intact or denuded endothelium from control and prenatal-ethanol-exposed groups (Fig. 2C and D). Interestingly, prenatal ethanol exposure shifted the dose-dependent response curve to the right when compared with findings for the control group in ring segments with either intact endothelium [EC50 = 0.18 ± 0.03 μM (n = 17) and 0.27 ± 0.07 μM (n = 13) for control and ethanol-exposed group, respectively] or denuded endothelium [EC50 = 0.20 ± 0.07 μM (n = 11) and 0.40 ± 0.12 μM (n = 10) for control and ethanol-exposed group, respectively].

3.3. Vasorelaxant response to carbamylcholine chloride (intact endothelium)

The endothelium-dependent vasorelaxation to the acetylcholine analog carbamylcholine chloride was significantly reduced by prenatal ethanol exposure in ring segments with intact endothelium (Fig. 3). Carbamylcholine chloride (0.25–4 μM) exhibited a dose-dependent vasorelaxation in ring segments from the control group, with a maximal relaxation of ~80%. However, carbamylcholine chloride only elicited approximately 43% maximal vasorelaxation in ring segments from the prenatal-ethanol-exposed group.

3.4. Vasorelaxant response to sodium nitroprusside and isoproterenol

Sodium nitroprusside is a nonspecific nitric oxide (NO) donor mediating vascular smooth muscle relaxation, whereas isoproterenol relaxes vessels through a β-adrenergic-cyclic adenosine monophosphate–dependent pathway. Results shown in Fig. 4 exhibit that aortic ring segments from the prenatal-ethanol-exposed group showed a similar vasorelaxant response to both sodium nitroprusside (0.1–1,000 μM) and isoproterenol (0.1–10 μM), when compared with findings for the control group, in ring segments with either intact or denuded endothelium. Sodium nitroprusside induced almost complete relaxation, whereas isoproterenol generated only a ~45% maximal relaxation. Isoproterenol-induced vasorelaxation in endothelium-denuded ring segments was not significantly different between control and prenatal-ethanol-exposed groups.

3.5. Vasorelaxant response to ethanol in absence or presence of Nω-nitro-L-arginine methyl ester

To evaluate the impact of prenatal ethanol exposure on postnatal-ethanol-induced vascular contractile response, ethanol was administered acutely into the tissue bath, and the vascular response was recorded 5 min later in vessels of both control and prenatal-ethanol-exposed groups. Results shown in Fig. 5 exhibit similar dose-dependent vasorelaxant responses of ethanol (80–640 mg/dl) in aortic ring segments with intact endothelium between the two animal groups. The vasorelaxant response of ethanol was reversible on washout (data not shown). Interestingly, when the endothelium-intact ring segments were pretreated with the NO synthase inhibitor L-NAME (100 μM), the ethanol-induced dose-dependent vasorelaxant response was significantly attenuated in both control and prenatal-ethanol-exposed groups, indicating a potential role of NO in ethanol-induced vasorelaxation.

4. Discussion

Daft et al. (1986) and Adickes et al. (1990) have shown that prenatal ethanol exposure may induce morphological alterations within the cardiovascular system. The results from the current study indicated that prenatal ethanol exposure is also capable of altering vascular function and blood pressure. Our findings also indicated that prenatal ethanol exposure–induced vascular contractile alteration is unlikely to be associated with changes in responsiveness to postnatal exposure of the vasculature to ethanol.

In this study, prenatal ethanol exposure did not affect birth weight or postnatal growth of the pups. Disparate findings have been observed in the growth retardation effect of prenatal ethanol exposure, including both unchanged (Henderson et al., 1995) and reduced (Weinberg, 1985) growth rate. This discrepancy is largely unknown, although differences in animal

![Endothelium-Intact](image)
Fig. 4. Effects of prenatal ethanol exposure on nitric oxide donor sodium nitroprusside–induced (panels A and B) or isoproterenol–induced (panels C and D) vasorelaxation in isolated rat aortic ring segments with intact or denuded endothelium. Data are mean ± S.E.M. The vasorelaxant response to sodium nitroprusside or isoproterenol was similar between control and prenatal-ethanol-exposed groups with either intact or denuded endothelium.

models and the timing of ethanol exposure may be considered to play a role. The elevated systolic blood pressure in pups at adulthood (25 weeks old) is somewhat similar to the observation made in postnatal chronic ethanol ingestion (Brown et al., 1998). However, differences in the experimental setting (e.g., pups were never fed with ethanol diet) make it difficult to apply the scenario for postnatal ethanol ingestion–induced vascular alteration to vascular response under prenatal ethanol exposure conditions. It may be speculated that ethanol-induced teratogenic effects in the formation of vasculature may play a role in the enhanced vascular resistance leading to elevated blood pressure. An increased incidence of vascular accidents, such as hematomas or subdermal hemorrhaging, has been reported in prenatal ethanol exposure (Salo et al., 1996; Weinberg, 1985). However, to the best of our knowledge, no study has directly investigated vascular function in the offspring whose dam was fed a diet enriched in ethanol during pregnancy. Results of our study revealed that prenatal ethanol exposure attenuated the vasoconstrictive response to norepinephrine and, to a lesser extent, potassium chloride in aortic segments with or without endothelium, favoring a potential change in the receptor-dependent over the voltage-dependent Ca\(^{2+}\) channel. Postnatal ethanol ingestion has been shown to attenuate the \(\alpha_1\)-adrenergic-induced contraction by phenylephrine (Sahna et al., 2000), supporting the notion of reduced responsiveness to norepinephrine observed in our study. However, other mechanisms such as postreceptor mechanisms (e.g., G-proteins) may also play a role in the altered vascular activity and cannot be excluded at this time.

Results from the present work revealed an impairment of carbamylcholine chloride–induced vasodilation after prenatal ethanol exposure, favoring the notion that prenatal ethanol exposure interferes with the production or utilization of endogenous NO by endothelium. The vasorelaxant response
to the NO donor sodium nitroprusside and to the β-adrenergic agonist isoproterenol was not affected by prenatal ethanol exposure, regardless of the existence of endothelium, suggesting minimal contributions from NO sensitivity and β-adrenergic activity to impaired relaxation. Although the origin of vascular dysfunction induced in the offspring of the alcoholic dams cannot be directly inferred from the present study, it seems that these offspring had acquired an endothelial defect similar to that observed in arteries of rats with chronic ethanol ingestion (Cricicone et al., 1989; Hatake et al., 1991). The observation that prenatal ethanol exposure did not affect relaxation responses mediated by the NO donor sodium nitroprusside is also consistent with findings obtained in the postnatal ethanol ingestion study (Hatake et al., 1991).

To date, reports on the effects of acute administration of ethanol on vascular contractile response are conflicting, depending on the vascular beds and experimental models used. Both vasoconstriction (Cricicone et al., 1989; Yang et al., 2001) and vasorelaxation (Greenberg et al., 1993) have been reported. The results of this study seem to indicate that the vasorelaxant effect of ethanol is mediated by endothelial generation and release of NO, as it was attenuated by the NO synthase blocker L-NAME. Nitric oxide is one of the most potent vasodilators, the effects of which are mediated by activation of guanylate cyclase and production of cyclic guanosine monophosphate, which in consequence leads to smooth muscle cell relaxation (Furchgott & Zawadzki, 1980). The NO involvement in ethanol-mediated vasorelaxation has been shown previously (Greenberg et al., 1993). The failure of L-NAME to completely block ethanol-induced vasorelaxation in our study may support the possible existence of an endothelium-independent component in the ethanol-induced vascular response.

In summary, the findings obtained from our study seem to indicate that prenatal ethanol exposure may play an important role in altering vascular contractile function, although it may not affect the postnatal effect of ethanol. Future work should focus on the mechanisms of action, especially intracellular Ca²⁺ mobilization, ion channel function, and protein synthesis/sensitivity, to understand better the teratogenic effect of ethanol on the vascular system.

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**References**


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**Fig. 5. Effect of prenatal ethanol exposure on ethanol-induced vasorelaxation in endothelium-intact aortic ring segments in the absence or the presence of the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 100 μM). Data are mean ± S.E.M., # P < .05 compared with findings for the non-L-NAME-exposed group. Ethanol elicited comparable vasorelaxation between the two animal groups in the absence or the presence of L-NAME. The ethanol-induced endothelium-dependent vasorelaxation was attenuated by L-NAME.**