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Association of the Glu298Asp Polymorphism of the Endothelial Nitric Oxide Synthase Gene with Preterm Labor

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Introduction Prematurity, defined as birth before 37 weeks of gestation, has been shown to occur in about 10% of all singleton live births and is a significant public health issue. The mechanisms involved in preterm labor (PTL) are still unclear, and no biochemical markers are predictive of preterm birth. Production of nitric oxide (NO) in the uterus is increased during pregnancy and decreases during delivery (1). Genotypic alterations of endothelial nitric oxide synthase (eNOS) induce changes in plasma NO concentration (2). In the human eNOS gene, a mutation resulting in a glutamine to aspartate substitution at codon 298 (Glu298→Asp) has been associated with ischemic heart disease (3). Our hypothesis is that PTL may be preferentially associated with a particular genotype of eNOS.

Methods With IRB approval and informed consent, we obtained blood samples from 30 Hispanic and Caucasian parturients delivering with PTL and 279 controls who delivered at term (TL). The ethnic groups were combined since there was no difference in genotype distribution in the TL group. PTL was defined as spontaneous onset of labor resulting in delivery before 37 weeks of gestation, in a singleton pregnancy, with no chorioamnionitis, uterine malformation, abnormal placental implantation, fetal abnormality, nor drug abuse. Genomic DNA was isolated and the alleles of the eNOS gene were identified by established techniques. Data were analyzed using χ^2 tests.

Results The distribution of genotypes was not significantly different between the PTL and TL groups ($p=0.08$). However, when analyzed by the gene counting method (4), the presence of Asp at position 298 of eNOS was associated with preterm delivery ($p=0.02$).

Table: eNOS genotype distribution

	Glu298Glu	Glu298Asp	Asp298Asp
PTL (n=30)	47%	37%	17%
TL (n=279)	64%	29%	7%

Discussion If mutations of the eNOS gene affect plasma NO concentration, and NO is necessary to maintain uterine quiescence during pregnancy, our data suggests that alterations in the eNOS genotype might predispose to PTL. If confirmed in a larger sample, this might have implications for understanding the pathogenesis, risk factors and/or treatment of PTL.

Refs: 1. *J Reprod Fert* 1996; 107: 249-54. 2. *Arterioscl Throm Vasc Biol* 1997; 17: 3147-53. 3. *Hypertension* 1998; 32: 521-6. 4. *Pharmacogenetics* 1999; 6:511-516.

A15

Title: Blood Ionized Magnesium Concentration in Preterm Fetal Sheep Increases during Umbilical Cord Occlusion

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Introduction: This study tested the hypothesis that a pathophysiologic insult to the fetus that decreases pH (umbilical cord occlusion) produces an increase in physiologically active (i.e. ionized) magnesium concentration.

Methods: Preterm pregnant sheep (n=7) were instrumented with maternal and fetal catheters and an inflatable vascular occluder was placed around the umbilical cord. After a two-day recovery period, each ewe received a 4-g loading dose, followed by continuous IV infusion of 1-g magnesium sulfate/h. After 48 h, an episode of acute fetal distress was produced by inflation of the umbilical occluder for 10-min. Maternal and fetal arterial blood samples were collected at regular intervals to quantitate ionize magnesium concentration and monitor physiologic status.

Results: Magnesium sulfate infusion increased maternal and fetal blood ionized magnesium concentration. In vitro blood analysis demonstrated that there was a linear inverse correlation ($r^2=0.99$) between fetal sheep blood pH and ionized magnesium concentration. In vivo, 10 min of umbilical cord occlusion produced an increase in fetal blood ionized magnesium concentration in all animals ($p=0.02$) that was temporally related to the decrease in fetal blood pH.

Discussion: Whether this increase in physiologically active magnesium concentration is beneficial (via neuroprotection) or deleterious (via suppression of stress response) to the distressed fetus remains to be determined.

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Expression of Rat Myometrial Adenylyl Cyclase mRNA at the End of Gestation.

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Uterine relaxation can be effected by activation myometrial β -adrenergic receptors, which couple to G proteins that activate the enzyme adenylyl cyclase (AC), which catalyzes the conversion of intracellular ATP to cAMP. The family of AC enzymes consists of at least 9 distinct isoforms that have been identified in a variety of species and tissue types. We showed that late gestation (day 19-21 of a 22 day gestation) rat myometrium expresses mRNA for isoforms II-IX.¹ Furthermore, we showed that total AC protein increases from the nonpregnant state to pregnancy, reaching a maximum on day 20, and then decreases abruptly on day 21, the day before parturition.² Such an abrupt decrease in total AC on day 21 would diminish activity of a major uterine relaxation pathway and enable labor to begin on day 22. In the present study, we questioned whether quantities of mRNA for AC isoforms paralleled total AC protein quantities on day 20 and day 21. RNA was isolated from myometrium derived from uteri harvested from pregnant rats killed under halothane anesthesia. Probes specific for each AC isoform were made.¹ Northern blot analysis, using densitometric scanning, was used to quantify mRNA for AC isoform IV. We found that quantities of mRNA for AC isoform IV did not differ significantly between day 20 and day 21 of gestation. Quantification of mRNA for isoforms II, III, and V-IX is ongoing. We conclude that decreased total AC protein on day 21 of gestation is unlikely to result from decreased synthesis of AC isoform IV. Either changes in expression of mRNA for other AC isoforms or changes in protein degradation are more likely explanations for this phenomenon. Supported by R29 HD 34782¹ Biol Reprod 59:169, 1998; ²Biol Reprod 62:506, 2000.

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The Effect Of MgSO₄ On Bupivacaine-Induced Convulsions In Awake Rats.

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The aim of this study was to determine the effect of MgSO₄ on bupivacaine-induced convulsions in the pregnant and non-pregnant awake rat.

The experimental protocol was approved by the Columbia University Animal Care and Use Committee. Using a chronically catheterized rat model, we studied 19 non-pregnant Sprague-Dawley rats in 2 groups. Group I (n=9) received a continuous MgSO₄ iv infusion (6 mg/kg/min) and Group II (n=10) received an equal volume of saline throughout the experiment. Sixty min into the infusion of MgSO₄ or saline, a bupivacaine infusion (1 mg/kg/min) was started until the onset of convulsions. Arterial blood samples were withdrawn for the analysis of MgSO₄ and bupivacaine concentrations. Differences between all chronological variables were tested using repeated measures ANOVA, with paired t tests used for post hoc comparisons. Differences between two groups were tested with unpaired t tests. A $p < 0.05$ was considered statistically significant.

By 60 min into the MgSO₄ infusion, mean arterial blood pressure (MAP) and heart rate (HR) in Group I decreased significantly; at that time the magnesium serum concentration was 4.8 ± 0.1 mEq/L, without any other adverse effects. In Group II, MAP and HR remained unchanged during the saline infusion. In Group I, the time (17.6 ± 2.5 min) and bupivacaine dose (4.9 ± 0.8 mg) necessary to produce convulsions were significantly different ($p<0.05$) from Group II: 11.5 ± 1.1 min and 3.0 ± 0.3 mg, respectively.

The mechanism of magnesium's anticonvulsant effect is thought to be the central inhibition of the NMDA receptor (1, 2). Since CNS local anesthetic toxicity may be mediated by the NMDA receptor, which stimulates excitatory neurotransmitter systems in the CNS (3, 4), the interaction between magnesium and bupivacaine is of interest. In our conscious rats, the therapeutic level of MgSO₄ was effective in reducing the CNS toxicity of bupivacaine. In this abstract, we refer only to non-pregnant rats; however, the study of pregnant animals is in progress.

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Reference: (1) *Am J Obstet Gynecol* 168: 974-8, 1993 (2) *Am J Obstet Gynecol* 166: 1127-36, 1992 (3) *Reg Anesth* 21: 243-8, 1996 (4) *Reg Anesth Pain Med* 23: 71-6, 1998