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Prenatal programming of hypernatremia and hypertension in neonatal lambs

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Ross, Michael G., Mina Desai, Catalina Guerra, and Shengbiao Wang. Prenatal programming of hypernatremia and hypertension in neonatal lambs. Am J Physiol Regul Integr Comp Physiol 288: R97–R103, 2005. First published September 16, 2004; doi:10.1152/ajpregu.00315.2004.—Maternal water restriction and the accompanying dehydration-induced anorexia may induce long-term physiological changes in offspring. We determined the impact of prenatal hypertonicity (Pre-Dehy) on offspring cardiovascular and osmoregulatory function. Pre-Dehy lambs were exposed to in utero hypernatremia (8- to 10-meq increase; 110–150 days of gestation) induced by maternal water restriction. Control lambs were born to ewes provided ad libitum water and food throughout gestation. After delivery, all ewes were provided ad libitum water and all newborns were allowed ad libitum nursing. Lambs were prepared with vascular and bladder catheters at 15 ± 2 days of age and studied at 21 ± 2 days. After a 2-h basal period, lambs received an infusion of hypotonic (0.075 M) NaCl (0.15 mol kg−1 h−1 iv) for 3 h. Lamb arterial blood pressure was monitored, and blood samples were obtained before, during, and after infusion. During the neonatal basal period, Pre-Dehy lambs had significantly increased plasma osmolality (302 ± 1 vs. 294 ± 1 mosmol/kgH2O, P < 0.01), sodium levels (144 ± 1 vs. 140 ± 1 meq/L, P < 0.01), hematocrit (28 ± 1% vs. 25 ± 1%, P < 0.05), and mean arterial blood pressure (79 ± 2 vs. 68 ± 1 mmHg, P < 0.001) compared with control lambs. Despite the infusion of hypotonic saline, Pre-Dehy lambs maintained relative hypertonicity, hypernatremia, and hypertension. However, plasma arginine vasopressin, glomerular filtration rate, and maternal osmolar and sodium excretion and clearance (per kg body wt) were similar in the groups. Offspring of prenatally water-restricted ewes exhibited hypernatremia, hypertonicity, and hypertension, which persist despite hypotonic saline infusion. In utero hypertonicity and perhaps maternal nutrient stress may program offspring osmoregulation and systemic arterial hypertension.

Recent human studies have provided evidence that the in utero environment has an impact on fetal development and may alter homeostatic regulatory mechanisms, resulting in chronic diseases of adulthood [i.e., the Barker hypothesis (5)]. Prenatal programming may provide a species survival benefit, these mechanisms may produce offspring with maladaptive phenotypes in modern society.

In view of the frequency of climatic drought, endocrine systems regulating plasma tonicity and/or sodium (i.e., AVP and thirst) (12) may be influenced by the perinatal environment. Prenatal (pregnancy and newborn) sodium depletion of rats reduces plasma and urine sodium concentration and increases hematocrit among offspring. As adults, the offspring demonstrate elevated fluid turnover (24) and a high NaCl intake (10). Conversely, extracellular dehydration during pregnancy increases the salt appetite (25) and blood pressure (3) of offspring rats. In humans, programming of osmoregulatory-salt appetite systems is suggested by evidence that infants of mothers who experienced moderate to severe emesis during pregnancy have enhanced salt preference at 16 wk of age (8, 20).

Similar to that in humans, ovine fetal development is precocial and thus provides an opportunity to examine mechanisms of in utero osmoregulatory programming. Among water-restricted pregnant ewes in which maternal plasma hypertonicity was maintained during the terminal 25% of pregnancy, 1-day-old newborns demonstrated increased pituitary AVP content and decreased hypothalamic AVP gene expression (27). At 1 mo of age, offspring of prenatally water-restricted ewes demonstrated higher plasma sodium and pituitary AVP content compared with control lambs (36). Although these results indicated biochemical evidence for programming of osmoregulatory mechanisms, we sought to determine whether there were discernible physiological alterations in offspring of water-restricted ewes compared with control singleton offspring.

MATERIALS AND METHODS

Animals and surgery. Twelve time-dated pregnant Western mixed-breed sheep with singleton pregnancies were obtained from a local source (Nebeker Ranch, Palmdale, CA). Study (prenatal dehydration, the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Pre-Dehy (prenatally dehydrated) animals were housed indoors in individual steel study cages and acclimated to a 12:12-h light-dark cycle. Food (alfalfa pellets) was provided ad libitum, and water was provided as described in Pre-natal dehydration. The care and use of the animals were approved by the Animal Research Committee of Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center and were in accordance with American Association for Accreditation of Laboratory Animal Care and National Institutes of Health guidelines.

Pre-natal dehydration. The animal model for the preparation of chronic prenatal dehydration was described previously (27, 36). Briefly, at 105 ± 1 days of gestation (term = 145–150 days), ewes (n = 6) were surgically prepared with femoral vein catheters. Maternal blood samples were drawn daily to monitor plasma tonicity and electrolytes. After the baseline plasma osmolality was established, water was removed from the ewes for 2 days, followed by water restriction of ~1 l/day during the remainder of pregnancy. The water intake was titrated to achieve an 8–10 meq/l increase in plasma sodium concentration from 110 days of gestation until spontaneous delivery at term. Matched groups of prenatally euedydrated lambs (control; n = 6) were born to ewes provided ad libitum water and food throughout gestation. Control ewes were not prepared with vascular catheters and were allowed ad libitum food and water intake throughout the pregnancy. In both Pre-Dehy and control groups, ewes were allowed to deliver naturally. Immediately after delivery, ewes were provided ad libitum water and food and newborns were allowed ad libitum nursing.

Experimental protocol. At 15 ± 2 days of age, lambs were surgically prepared with bladder and femoral arterial and venous catheters. All experiments were performed on conscious lambs maintained in a specially designed support sling. At 21 ± 2 days, Pre-Dehy and control lambs were studied. Sixty minutes before the basal period began, the lamb bladder catheter was drained to gravity and 3H-labeled inulin (2 μCi·kg⁻¹·min⁻¹) was intravenously infused (0.01 ml·kg⁻¹·h⁻¹) for measurement of glomerular filtration rate (GFR) and continued through the basal and experimental periods. During a subsequent 2-h basal period, arterial blood pressure and urine volume were continuously monitored. Neonatal arterial blood samples (4 ml) were obtained at 30-min intervals for determination of arterial pH, blood gases, plasma osmolality, electrolytes, inulin, and hematocrit, and urine samples were assayed for volume, osmolality, inulin, and electrolyte concentrations. After the basal period, lambs received an intravenous infusion of hypertonic NaCl (0.075 M; 0.15 ml·kg⁻¹·h⁻¹) for an additional 2 h. Neonatal arterial blood pressure was continuously monitored, and arterial blood and urine samples were obtained at timed intervals during and after the infusion. After the hypertonic saline study, lambs underwent additional studies on subsequent days (9).

Analytical methods. Throughout the measurement periods, neonatal arterial blood pressure was monitored continuously by means of a Beckman R-612 recorder (Beckman Instruments, Fullerton CA) and Statham P23 pressure transducers (Garret, Oxnard CA). All signals were digitized at 50 Hz and acquired on an IBM-compatible computer. Heart rate and systolic, diastolic, and mean arterial pressures were calculated from the pressure tracings.

Plasma and urinary electrolyte levels were determined with a Nova 5 electrolyte analyzer (Nova Biomedical, Waltham, MA). Osmolality was measured by freezing point depression on an Advanced Diagamic Osmometer (model MO, Advanced Instruments, Needham Heights, MA). Blood pH, arterial CO₂ tension (PaCO₂), and arterial O₂ tension (PaO₂) values were measured at 39°C with a Radiometer BM 33 MK2-PHM 72 MKS acid-base analyzer system (Radiometer, Copenhagen, Denmark). Inulin concentrations were assessed by counting 100-μl aliquots diluted to 10 ml with Hydroflour (National Diagnostics, Somerville, NJ) in a Beckman LS-355 liquid scintillation counter (Beckman, Irvine, CA). Plasma AVP levels were measured by radioimmunoassay (33).

Calculations and statistics. All values are expressed as means ± SE, with SE representing the variance between animals. All urinary values of excretion or clearance were adjusted for actual body weight and expressed per kilogram. Basal values represent the mean of measurements obtained during the basal period (~2 to 0 h). Differences over time were assessed with repeated-measures analysis of variance with Dunnett’s post hoc test. Statistical significance was accepted at P ≤ 0.05.

RESULTS

Maternal dehydration. After water deprivation for 2 days, maternal plasma osmolality increased significantly from 313 ± 1 to 325 ± 3 mosmol/kgH₂O (P < 0.01), in accord with significant increases in plasma sodium (149 ± 1 to 155 ± 1 meq/l, P < 0.001) and chloride (114 ± 1 to 117 ± 1 meq/l, P < 0.01) concentrations. With water intake restricted to ~1 l/day, plasma osmolality and sodium levels of the ewes were maintained at significantly elevated levels throughout the remaining gestation. There were no significant changes in maternal plasma potassium (4.4 ± 0.3 to 4.9 ± 0.1 meq/l) or hematocrit (31.6 ± 1.7% to 33.3 ± 2.8%) in response to water restriction.

Lamb basal values. At birth, significant differences were noted in weight between the groups. Control lambs weighed significantly more than Pre-Dehy lambs (5.0 ± 0.2 vs. 4.1 ± 0.3 kg, P < 0.05). However, Pre-Dehy singleton and control lambs were of similar weight at 21 days of age (Pre-Dehy 10.0 ± 0.8, control 10.8 ± 1.2 kg).

There were marked differences between Pre-Dehy and control neonatal lambs in arterial blood parameters (Table 1).

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<th>Table 1. Basal arterial blood and urinary values in control and Pre-Dehy offspring</th>
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Values are means ± SE for n animals. Pre-Dehy, prenatally dehydrated; P<sub>CO₂</sub>, arterial CO₂ tension; P<sub>CO₂</sub>, arterial O₂ tension; GFR, glomerular filtration rate. Control singleton vs. Pre-Dehy singleton: *P < 0.01, †P < 0.001.
Plasma osmolality (302 ± 1 vs. 294 ± 1 mosmol/kgH₂O, \( P < 0.01 \)) and sodium concentration (144 ± 1 vs. 140 ± 1 meq/l, \( P < 0.01 \)) and hematocrit (28 ± 1 vs. 25 ± 1%, \( P < 0.05 \)) were significantly greater in Pre-Dehy than control lambs, although there were no differences in basal plasma potassium, chloride, or AVP levels. Systolic (96 ± 2 vs. 87 ± 2 mmHg, \( P < 0.001 \)), diastolic (66 ± 2 vs. 53 ± 1 mmHg, \( P < 0.001 \)), and mean (79 ± 2 vs. 68 ± 1 mmHg, \( P < 0.001 \)) arterial pressures were significantly elevated in Pre-Dehy compared with control lambs. There were no differences between the groups in basal heart rate, pH, or PaCO₂. There was a small but statistically significant reduction in PaO₂ among Pre-Dehy lambs. Basal GFR, urine volume and osmolality, urinary osmolar excretion and clearance values, and fractional sodium excretion were similar between the groups (Table 1).

**Lamb responses to hypotonic saline infusion.** In response to the infusion of hypotonic (0.75 M) saline, there was no significant change from basal plasma osmolality in control lambs during or after the hypotonic saline infusion (Fig. 1). Pre-Dehy lambs continued to evidence relatively increased plasma osmolality compared with controls throughout the study and demonstrated a further trend toward increased plasma osmolality at the conclusion of the study. The relative plasma hypernatremia in Pre-Dehy lambs was maintained throughout the hypotonic saline infusion. Although hematocrit decreased slightly in both groups in response to the intravenous saline solution, Pre-Dehy lambs maintained a relatively elevated hematocrit. Plasma AVP levels and heart rate did not change in any of the groups. Pre-Dehy lambs maintained elevated mean, systolic, and diastolic arterial blood pressures compared with controls throughout the hypotonic saline infusion (Fig. 2).

Both Pre-Dehy and control lambs demonstrated significant changes in urinary values in response to the infusion (Fig. 3). Urinary osmolality and potassium and chloride concentrations demonstrated a slight decrease and a subsequent increase after the infusion. Conversely, urinary volume and GFR increased slightly during the infusion and decreased during the recovery period. Urine sodium concentration did not change during the infusion in either group but demonstrated a marked increase during the recovery period in Pre-Dehy lambs. No differences were evident in GFR, urine osmolality, or sodium between Pre-Dehy and control lambs.
DISCUSSION

Perinatal programming of osmoregulation has been suggested in the fetal and the neonatal rat. Perinatal sodium depletion (i.e., hyponatremia) of rats resulted in reduced plasma and urine sodium concentration and increased hematocrit in the offspring. As adults, these offspring demonstrated elevated fluid turnover and a high sodium intake, potentially due to activation of the angiotensin-aldosterone system (10, 24). Similarly, acute extracellular dehydration in rats treated with polyethylene glycol during pregnancy increased salt appetite (25) and blood pressure (3) in the offspring. Thus there is an early period in intrauterine development during which fluid regulation in the offspring may be influenced permanently by maternal plasma sodium levels. It is interesting to note that both hyponatremia as a result of chronic sodium depletion and hypernatremia due to acute extracellular dehydration resulted in increased salt intake of the offspring. Renal responsiveness to AVP also may be programmed, as neonatal rat exposure to subcutaneous injection of AVP during the first 7 days after birth permanently decreases renal AVP responsiveness because of a reduction in AVP binding sites in the adult kidney (11, 12). Moreover, studies in humans demonstrate a similar phenomenon of programming of neonatal osmoregulatory-salt appetite systems as a result of the maternal pregnancy osmotic environment (8). Therefore, in utero alterations in fluid and electrolyte endocrine systems may result in permanent effects on offspring.

In the present study, maternal water restriction during pregnancy significantly increased maternal plasma osmolality and sodium concentration, and these increases were maintained for the duration of pregnancy. Maternal dehydration induces fetus-mother water transfer, resulting in fetal plasma hyperosmolality in direct proportion to maternal hypertonicity and increased fetal plasma AVP (1, 29, 31). Although chronic hypertonicity maintains increased fetal plasma AVP for at least 96 h (1), it is unknown whether fetal plasma AVP remained continually elevated throughout the ≥35-day period of plasma hypertonicity. Thus Pre-Dehy fetuses were exposed to chronically elevated plasma osmolality and potentially elevated plasma AVP levels throughout the last month of pregnancy. Although maternal food intake and weight gain were not quantified in this study, subjective assessment indicated a significant reduction, consistent with prior studies of dehydration (30).

After birth, ewes and lambs were provided food and water and suckling ad libitum, respectively. Mother-infant pairs were expected to return to euhydration levels. In response to prenatal maternal water restriction, singleton offspring at 21 days demonstrated significantly increased plasma osmolality and sodium concentration, hematocrit, and systolic, diastolic, and mean arterial pressures vs. control lambs. In response to hypotonic saline, both Pre-Dehy and control lambs demonstrated an initial decrease in urine osmolality and a urinary diuresis, although Pre-Dehy lambs maintained relatively elevated plasma osmolality and sodium levels, hematocrit, and arterial blood pressures, compared with controls. Thus these results demonstrate a programmed syndrome of hypernatremic hypertension among 3-wk-old offspring of water-restricted ewes.

Fig. 3. Glomerular filtration rate (GFR, top left), urine volume (top right), urine osmolality (bottom left), and urine sodium levels (bottom right) in control (○) and Pre-Dehy (□) singleton lambs during hypotonic saline infusion. Time 0 h represents basal period before infusion, followed by intravenous infusion of hypotonic (0.075 M) NaCl over 2 h and subsequent 2-h recovery period. Values are means ± SE of n = 6 at each time point. *Significant difference from basal value among control lambs; †significant difference from basal values among Pre-Dehy lambs.
At 21 days of life, Pre-Dehy offspring demonstrated marked osmolar and cardiovascular differences compared with singleton controls. The elevated plasma sodium levels and osmolality in Pre-Dehy compared with control lambs were not due to variations in basal hydration. All animals had ad libitum access to maternal nursing, and all ewes were provided ad libitum food and water. Furthermore, similar basal hydration conditions are supported by the similar values of urinary volume, urine osmolar excretion, and fractional sodium excretion among the Pre-Dehy and control offspring. The relative plasma hypertonicity in the Pre-Dehy lambs persisted throughout the intravenous hypotonic saline infusion. The elevated plasma osmolality during both basal and infusion conditions occurred in the presence of normal plasma AVP levels. Compared with control lambs, the 7 mosmol/kgH2O increase in plasma osmolality in the Pre-Dehy lambs is above the normal threshold (~2% increase) for stimulation of AVP secretion (21), indicating a resetting of plasma osmolality threshold set points and differential regulation of plasma osmolality and plasma sodium values.

In the present study, Pre-Dehy singleton offspring demonstrated an increased hematocrit, both during the basal period and in response to the hypotonic saline infusion. Although this may be a result of increased red blood cell mass, the minor difference in Pao2 was unlikely to have stimulated erythropoiesis. Alternatively, hemoconcentration resulting from reduced vascular plasma water may correlate with findings of plasma hyperosmolality. Increased systemic arterial blood pressures may have resulted from increased peripheral resistance and/or increased cardiac output, although these values were not quantified in the present study. Prior studies of low-birth-weight infants indicate reduced renal glomerular number and an increased risk of hypertension as adults (13). Interestingly, studies of ovine twins similarly demonstrate reduced nephron number compared with control singleton lambs or late-gestation growth-restricted lambs. Hence, the authors suggested that reduced nephron endowment may be dependent on the timing of the growth restriction (23). Furthermore, growth-restricted rat pups are hypertensive but demonstrate normal GFR at 12 wk of age (2). In the present study, although GFR values were similar in Pre-Dehy and control lambs, it is possible that the elevated blood pressure among Pre-Dehy lambs may mask reduced glomerular number. Alternatively, increased salt intake and/or plasma sodium may alter intrarenal or circulating renin-angiotensin homeostasis or renal sympathetic nerve activity, contributing to hypertension.

Programming of offspring cardiovascular and renal function has been examined with models of nutrient restriction or fetal-maternal glucocorticoid administration. Despite varying models, results suggest that fetal hypocaloric-pituitary-adrenal axis activation, either directly or indirectly, is responsible, in part, for programming of offspring cardiovascular homeostasis. Emerging data further suggest that antenatal exposure to glucocorticoids modifies fetal cardiovascular development, resulting in offspring hypertension (16, 34). Among spontaneous models of intrauterine growth restriction (IUGR), adult offspring of human IUGR infants have increased blood pressure of only 1.7 mmHg (19) for each 1 kg of reduced birth weight. Conversely, newborn piglets (6) with spontaneous IUGR demonstrated decreased mean arterial blood pressure and decreased GFR, suggesting a species difference. Offspring of protein-restricted rat dams (14, 18, 26, 32) or dams treated with dexamethasone (7, 16, 17) have demonstrated increased blood pressure. Although plasma osmolality or sodium rarely has been measured, there was no change in maternal plasma sodium of nutritionally deprived dams. Although fetal and/or newborn glucocorticoids were not measured in the present study, dehydration may increase ovine cortisol responsiveness to endogenous corticotropin-releasing hormone (22).

In the present study, maternal hypertonicity was maintained during the final 25% of gestation. The sensitivity of programming likely depends on 1) developmental patterns of expression, 2) access of the programming agent to the tissue, and, perhaps most importantly, 3) the state of differentiation of that tissue. The last quarter of ovine gestation is a likely critical period in the maturation of osmoregulatory mechanisms, as ovine fetal dipsogenic systems mature (“switch on”) acutely during the last third of gestation (15). AVP secretory mechanisms and neural AVP binding sites also may mature acutely (35). At 90% of gestation, neuroendocrine responses to cellular dehydration are functional, although they evidence a relatively reduced sensitivity for AVP secretion compared with the adult (37). One-day-old newborn lambs that had been subjected to in utero hypertonicity from 119 days gestation to term had increased plasma sodium and total pituitary AVP content but lower hypothalamic AVP mRNA level compared with control newborns (27). At 2 mo of postnatal age, prenatally dehydrated lambs had higher plasma sodium levels but similar hypothalamic AVP mRNA levels and pituitary AVP content compared with controls (36). Thus increased plasma sodium levels in the presence of normal plasma AVP levels indicate that altered central sodium receptor set points and/or renal AVP responsiveness may account for the mechanism of prenatal osmoregulatory programming.

In design of the study, we recognized that maternal dehydration-induced anorexia would likely result in reduced maternal nutrient intake and potential fetal nutrient stress. Although maternal food intake and weight were not quantified, relative anorexia was observed among thePre-Dehy ewes, consistent with the reduced newborn weight. Our laboratory recently demonstrated (28) that twin lambs are significantly smaller than singletons at birth and demonstrate a further relative weight reduction when both twins are nursed by the maternal ewe. At 3 wk of age, the twin lambs demonstrated significant hypertension and hypernatremia with no change observed in plasma osmolality or hematocrit (28). Thus it appears that there are specific effects of prenatal nutrient restriction compared with prenatal dehydration.

By 3 wk of age, Pre-Dehy lambs were similar in weight to controls. Whereas water restriction during lactation decreases milk yield by 25–35%, there are no reports of the impact of maternal dehydration during pregnancy on subsequent lactation milk yield or composition. In the present study, the catch-up growth demonstrated by Pre-Dehy lambs suggests that maternal milk yield and newborn lactation were adequate, if not excessive, in the Pre-Dehy group. Potentially, growth restriction resulting from dehydration anorexia during the last 25% of gestation may have an impact on the expression of...
hyperphagia after birth. Thus the offspring effects of maternal water restriction may be mediated by both perinatal osmoregulatory and nutrient deprivation mechanisms. Although in utero plasma hypertonicity was the primary experimental comparison, environmental factors also may have contributed to the observed differences in offspring phenotype. The laboratory environment of the Pre-Dehy offspring during the last 25% of gestation differed from the free-range conditions of the euhydrated ewes, and only the water-restricted ewes received vascular catheters. However, maternal vascular catheters alone have no demonstrated effects on fetal growth.

In summary, these studies provide evidence in a preclinical species that maternal plasma hypertonicity may program newborn physiology. Offspring of water-restricted ewes demonstrated a syndrome of hypernatremic hypertension, with significant hematologic and cardiovascular alterations. Although the long-term effects and underlying physiological, molecular, and cellular mechanisms for these changes are unknown, these abnormalities, if persistent, may have adverse clinical effects in adult offspring. Furthermore, a transgenerational impact may result should female offspring demonstrate impaired osmoregulatory and cardiovascular adaptations (i.e., plasma volume expansion) in their subsequent pregnancies.

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