BASIC SCIENCE: OBSTETRICS

Intramembranous solute and water fluxes during high intramembranous absorption rates in fetal sheep with and without lung liquid diversion

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OBJECTIVE: To examine mechanisms that mediate increased intramembranous solute and water absorption.

STUDY DESIGN: Intramembranous solute and water fluxes were measured in fetal sheep under basal conditions and after intraamniotic infusion of lactated Ringer's solution of 4 L/d for 3 days with and without lung liquid diversion.

RESULTS: Intramembranous sodium, potassium, chloride, calcium, glucose, and lactate fluxes increased 2.5- to 7.9-fold, were linearly related to volume fluxes ($r = 0.83$-0.99), and were unaffected by lung liquid. All clearance rates, except that of lactate, increased to equal the intramembranous volume absorption rate during infusion.

CONCLUSION: Under basal conditions, passive diffusion makes a minor and bulk flow a major contribution to intramembranous solute absorption. During high absorption rates, the increase in solute absorption above basal levels appears to be due entirely to bulk flow and is unaffected by lung liquid. The increased bulk flow is consistent with vesicular transcytosis.

Key words: bulk flow, fetal sheep, intramembranous water and solute absorption, passive diffusion, vesicular transport

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Past experimental studies have shown that amniotic fluid (AF) volume is regulated primarily by modulation of the rate of intramembranous absorption of amniotic water and solutes with transfer directly into fetal blood.[1-9] In humans[10,11] and nonhuman primates,[12] this absorption occurs at the fetal surface of the placenta. In species that have vascularized fetal membranes, such as sheep,[13] intramembranous absorption occurs at the fetal surface of the placenta, in the vascularized chorion, and at the outer surface of the amnion.[1,13]

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Studies in late-gestation fetal sheep have shown that when the AF volume is increased, either via experimentally increased fetal urine production[14,15] or intraamniotic fluid infusion,[1,8] intramembranous absorption increases from a basal rate of a few hundred milliliters per day[1,4,16] to several thousand milliliters per day.[17] This change in absorption minimizes the increase in AF volume that would otherwise occur.

The transport mechanisms responsible for the increase in water and solute absorption and their associated regulation remain unknown. Theoretically, at least 6 mechanisms may be involved: (1) passive bulk flow mediated by an osmotic or hydrostatic gradient, (2) passive diffusion of solutes down their concentration gradients, (3) active transcytotic bulk transfer through a vesicular transport system,[18] (4) facilitated diffusion, (5) active transfer of individual solutes, and (6) lymphatic drainage.[9] One approach to understanding the relative contribution of each transport mechanism is to determine the relationships between solute transport rates and their potential driving forces. To date, only 2 studies have taken this approach; 1 was performed over a range of osmolalities,[9] whereas the other 1 was performed under basal conditions.[18] Although these studies suggest that osmosis, passive solute diffusion, bulk flow of water and solutes by transcellular vesicular transport, water channels, and lymphatic-like transport may all be involved, the relative contribution of each of these mechanisms to the increase in intramembranous absorption has not been determined. This is important, because perturbations in these purported mechanisms might underlie the pathophysiology of oligohydramnios and hydramnios. Further, although AF washout studies indirectly suggest that fetal lung liquid may contain a substance(s) that modulates the rate of intramembranous absorption,[19,20] it is not known which of the transport mechanisms may be affected.

The purpose of the current study was to examine the relationships between intramembranous solute absorption rates, solute concentration gradients, and volume flow rates under conditions of basal and high intramembranous absorption to gain insight into the transport mechanisms that may be involved when ab-

sorption rates are increased. We also determined whether diversion of lung liquid would alter intramembranous solute absorption rates.

MATERIALS AND METHODS

Ethical approval

The animal use and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Oregon Health and Science University. In addition, we followed the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Experimental procedures

Eight pregnant sheep with single fetuses were included in this study. The AF volumes and intramembranous volume absorption rates have been recently reported.[21] This article focuses on new data describing intramembranous solute absorption and the associated potential transport mechanisms.

Surgeries were performed at 119-121 days' gestation, as detailed elsewhere.[17,21] Briefly, with inhalation anesthesia (oxygen and nitrous oxide 3:1 with 2% isoflurane) and aseptic technique, polyvinyl catheters were placed in a fetal jugular vein and carotid artery. Catheters were also placed into the fetal trachea and urinary bladder. A paravascular flow probe (Transonic Systems, Ithaca, NY) was secured around the esophagus to monitor fetal swallowing. The urachus was ligated at the cranial pole of the urinary bladder to prevent urine inflow into the allantoic sac. Three additional catheters were placed to allow access to the AF.

Experiments began 5-6 days after catheterization. Animals were randomly assigned to 1 of 4 successive experimental periods: (1) a control period, during which lung liquid entered the amniotic cavity as normally occurs; (2) a diversion period, during which lung liquid was diverted continuously away from the AF to the exterior and replaced with an equal volume of lactated Ringer's solution; (3) a supplementation period, during which 4 L/d of lactated Ringer's solution were infused continuously into the AF while lung liquid was diverted and replaced with an equal volume of lactated Ringer's solution. Each protocol lasted 3 days. At the beginning of each protocol, the AF was drained through the 3 amniotic catheters into evacuated bottles. We have previously shown that AF drainage removes all AF.[9,17,20] After drainage, 1 L of warm lactated Ringer's solution (130 mEq/L sodium, 109 mEq/L chloride, 28 mEq/L lactate, 4 mEq/L potassium, 3 mEq/L calcium) containing 250,000 units of penicillin or 5 mg of gentamicin was infused into the amniotic cavity. This initial replacement of AF with 1 L of lactated Ringer's solution was used so that all experimental periods began with the same AF volume and composition. There was no recovery period between the experimental periods. During each protocol, fetal urine entered the amniotic sac and fetal swallowing continued.[21] Five sheep underwent all 4 of the 3-day experimental periods, whereas 3 sheep underwent 2 of the 3-day experimental periods.

On completion of the experiments, the animals were given an IACUC-approved intravenous injection of euthanasia solution (Euthasol; Virbac AH, Inc, Fort Worth, TX).

Measurements

The rates of urine flow, lung liquid flow, and swallowing were measured continuously for each 3-day experimental period. Urine and lung liquid flow rates were measured using a previously described electronic technique.[19,21] The fluids were continuously drained into a sterile flask and when the fluid level rose to a given level, it triggered sensors to return the fluids to the fetus while a parallel circuit determined volumes. To measure swallowing of AF, the area under the esophageal flow curve was integrated with baseline correction every 20 minutes.[21]

Three-milliliter samples of fetal blood, urine, lung liquid, and AF were obtained at the start and end of each 3-day experimental period. Fetal blood gases and pH remained stable throughout these experiments.[21] Plasma was separated by centrifugation. The samples were stored frozen in sealed tubes at -12°C up to 1 year before analysis. Samples were run on a Radiometer ABL analyzer (model 725; Radiometer, Copenhagen, Denmark) that uses ion-sensitive electrodes to determine free concentrations of sodium, potassium, chloride, calcium, glucose, and lactate. Samples from cracked tubes were discarded, as were samples that did not yield reproducible values, typically because of high mucus content or precipitate in the tubes (approximately 5%).

Terminology and calculations

Intramembranous volume flow was calculated as the change in AF volume over the 3-day period plus the net volume from the 4 flows (infused lactated Ringer's solution, urine, lung liquid, and swallowing). Intramembranous solute absorption rates were calculated similarly by determining the difference between the initial and final amniotic solute masses and adding the solute masses of the 4 flows. The total amount (mass) of each solute within the AF at the initial and final time points was calculated as the product of AF volume and AF solute concentration. Solute absorption from the AF to fetus was considered positive, whereas solute transport from the fetus to the AF was considered negative. The AF clearance for each solute was calculated as the solute absorption rate divided by the mean AF concentration. The AF to fetal plasma concentration gradients were calculated by subtracting the AF concentration from the fetal plasma concentration.

Data presentation and statistics

Data are presented as the mean ± standard error of the mean (SEM). The letter "n" represents the number of measurements within each mean. Bivariate and multivariate least squares regression were used to determine statistical relationships between and among variables. A *t* test was used to compare AF volumes and intramembranous volume absorption rates under basal conditions with those during supplementation with lactated Ringer's solution. Solute concentrations and concentration gradients with and without intraamniotic supplementation were compared with a paired *t* test or Wilcoxon signed-rank test when