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

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Abstract

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.

BACKGROUND AND OBJECTIVE

Studies in late-gestation fetal sheep have shown that when the amniotic fluid (AF) volume is increased via either experimentally increased fetal urine production or intraamniotic fluid infusion, intramembranous absorption increases from a basal rate of a few hundred milliliters per day to several thousand milliliters per day.

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. This is important, because perturbations in these purported mechanisms may underlie the pathophysiology of oligohydramnios and hydramnios. Further, although AF washout studies indirectly suggest that fetal lung liquid may contain 1 or more substances that modulate the rate of intramembranous absorption, 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 absorption rates are increased. We also determined whether diversion of lung liquid would alter intramembranous solute absorption rates.

MATERIALS AND METHODS

Eight pregnant sheep with single fetuses were included in this study. 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; or (4) a supplementation/diversion 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.

The rates of urine flow, lung liquid flow, and swallowing were measured continuously for each 3-day experimental period. Three-milliliter samples of fetal blood, urine, lung liquid, and AF were obtained at the start and end of each 3-day experimental period. Samples were run on a Radiometer ABL analyzer that uses ion-sensitive electrodes to determine free concentrations of sodium, potassium, chloride, calcium, glucose, and lactate.

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.

RESULTS

AF volume and intramembranous volume absorption rates did not change with diversion and isovolumetric replacement of lung liquid. At the end of the 3-day period without intraamniotic supplementation, the mean AF volume was 648 ± 131 mL. After the 3-day period with intraamniotic supplementation with 4 L/d of lactated Ringer's solution, the mean AF volume increased to 2710 ± 540 mL. Intramembranous absorption increased from its nonsupplemented mean of 1120 ± 120 mL/d ($n = 14$) to a supplemented mean of 5110 ± 390 mL/d ($n = 12$; $P < .0001$).

In the absence of intraamniotic supplementation of lactated Ringer's solution, the intramembranous flux from the AF and into fetal plasma for each of the 6 solutes was significantly greater than zero. During intraamniotic supplementation, the flux of each solute increased significantly.

The bivariate regression relationships between intramembranous solute and volume absorption rates were highly linear for each of the solutes. The Y-axis intercept was significantly less than zero for sodium and chloride and not different from zero for the other solutes. The regression relationship between intramembranous sodium flux and volume flux was independent of whether fetal lung liquid entered the amniotic sac or was diverted and replaced with an equal volume of lactated Ringer's solution (Figure). The independence of lung liquid entry into the amniotic sac was true for all solutes, except for a small but significant shift to the right for the chloride flux (ANCOVA $P = .011$).

During the nonsupplementation period, the AF clearances of sodium and chloride were similar, whereas the AF clearances of calcium, glucose, and lactate were higher than that of sodium or chloride (ANOVA $P = .0004$). The potassium clearance was not significantly greater than sodium clearance with post hoc testing. Only the glucose and lactate clearances were statistically greater than the volume absorption rate. During the supplementation period, all

AF solute clearances were significantly higher than those during the nonsupplementation period. All clearances were similar during the supplementation period, except that lactate clearance was approximately twice that of the other solutes ($P < .0001$).

COMMENT

The primary objective of this study was to determine the mechanisms that mediate the large increases in intramembranous absorption during intraamniotic supplementation with lactated Ringer's solution. Passive solute diffusion is not the primary mechanism that mediates their intramembranous absorption.

Intramembranous absorption occurs against rather than with hydrostatic gradients. Osmotically driven movement through water channels or intercellular junctions is not the major mechanism involved in intramembranous water and solute transport.

In the current study, the intramembranous flux of each of the monitored solutes varied linearly with the volume flow rate. This finding suggests that the solute movements and water movements were occurring via the same mechanism as would occur with bulk flow because of transcellular vesicular transport. The observation that clearances equaled the rate of intramembranous volume absorption during the AF supplementation suggests that bulk transcellular vesicular transport was the only transport mechanism augmented during intraamniotic supplementation. The regression equations provide insight into passive permeation and the extent to which passive diffusion was occurring.

We conclude that our data do not support the hypothesis that lung liquid actively modulates the rate of intramembranous solute absorption. The increases in solute absorption above basal levels during high-volume absorption rates appear to be due entirely to an increase in bulk flow across the intramembranous pathway. The most likely explanation is vesicular transport without discrimination of solutes.

CLINICAL IMPLICATIONS

- Modulation of the rate of intramembranous absorption of amniotic fluid water and solutes by transcellular bulk vesicular transport may underlie the pathogenesis of oligohydramnios and polyhydramnios.

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FIGURE. Sodium absorption

Open circles, lung liquid entered the amniotic fluid; *closed circles*, lung liquid diverted and replaced with an equal volume of lactated Ringer's solution. There was no difference in sodium absorption with and without lung liquid diversion (ANCOVA $P = .093$), so the data were combined. *Solid line*, regression line; *dotted lines*, 95% confidence interval about regression line.