Prostaglandin E₂ regulation of amnion cell vascular endothelial growth factor expression: relationship with intramembranous absorption rate in fetal sheep

Cecilia Y. Cheung,1,2 Michael K. Beardall,1 Debra F. Anderson,2 and Robert A. Brace1,2

1Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Oregon Health and Science University, Portland, Oregon; and 2Center for Developmental Health, Knight Cardiovascular Institute, Oregon Health and Science University, Portland, Oregon

Submitted 18 February 2014; accepted in final form 28 May 2014

Cheung CY, Beardall MK, Anderson DF, Brace RA. Prostaglandin E₂ regulation of amnion cell vascular endothelial growth factor expression: relationship with intramembranous absorption rate in fetal sheep. Am J Physiol Regul Integr Comp Physiol 307: R354–R360, 2014. First published June 4, 2014; doi:10.1152/ajpregu.00070.2014.—We hypothesized that prostaglandin E₂ (PGE₂) stimulates amniotic fluid transport across the amnion by upregulating vascular endothelial growth factor (VEGF) expression in amnion cells and that amniotic PGE₂ concentration correlates positively with intramembranous (IM) absorption rate in fetal sheep. The effects of PGE₂ at a range of concentrations on VEGF₁₆₄ and caveolin-1 gene expressions were analyzed in cultured ovine amnion cells. IM absorption rate, amniotic fluid (AF) volume, and PGE₂ concentration in AF were determined in late-gestation fetal sheep during control conditions, isovolumetric fetal urine replacement (low IM absorption rate), or intra-amniotic fluid infusion (high IM absorption rate). In ovine amnion cells, PGE₂ induced dose- and time-dependent increases in VEGF₁₆₄ mRNA levels and reduced caveolin-1 mRNA and protein levels. VEGF receptor blockade abolished the caveolin-1 response, while minimally affecting the VEGF response to PGE₂. In sheep fetuses, urine replacement reduced amniotic PGE₂ concentration by 58%, decreased IM absorption rate by half, and doubled AF volume (P < 0.01). Intramamniotic fluid infusion increased IM absorption rate and AF volume (P < 0.01), while amniotic PGE₂ concentration was unchanged. Neither IM absorption rate nor AF volume correlated with amniotic PGE₂ concentration under each experimental condition. Although PGE₂ at micromolar concentrations induced dose-dependent responses in VEGF and caveolin-1 gene expression in cultured amnion cells consistent with a role of PGE₂ in activating VEGF to mediate AF transport across the amnion, amniotic PGE₂ at physiological nanomolar concentrations does not appear to regulate IM absorption rate or AF volume.

PGE₂ absorption fluid; fetal urine; intramembranous absorption; prostaglandin E₂; sheep

Prostaglandin E₂ (PGE₂) is a potential amniotic fluid component that stimulates transcellular transport of free amino acids across the amnion (10). Moreover, PGE₂ has been shown to increase VEGF gene expression in cultured amnion cells and mediate the transport of solutes across the amnion (15). The exact role of PGE₂ in the regulation of amniotic fluid volume homeostasis is currently poorly understood.

Previous studies have shown that the primary component of IM absorption is a unidirectional vesicular transcytosis of amniotic water and solutes across the amnion independent of osmotic and solute concentration differences. A secondary component is bidirectional and dependent on osmotic and solute concentration differences between AF and fetal blood (1, 5, 22). Vascular endothelial growth factor (VEGF) has been shown to activate vesicular transcytosis in vascular endothelial cells (10). Because the gene expression of VEGF in the amnion is elevated when IM absorption rate is increased above normal under various experimental conditions (12, 15, 19, 29), VEGF may similarly mediate IM transport across the amnion. However, the factors that induce VEGF gene expression in the amnion have not been determined.

We recently demonstrated that fetal urine contains a substance that stimulates IM absorption in vivo (1). The identity of this substance has not been elucidated, although it was shown not to be a major solute such as sodium or chloride, nor was it dependent on urine osmolality (1). Fetal urine contains various proteins, endocrine factors, and cytokines in addition to the major solutes. One possible candidate for the unidentified renal derived IM stimulator is the eicosanoid prostaglandin E₂ (PGE₂). This possibility is supported by several observations. Casey et al. (7) found high concentrations of PGE₂ in human fetal urine and concluded that fetal urine is a source of prostaglandins in AF. Further, Mitchell et al. (32) reported that human AF contains a fetal urine-derived substance that stimulates PGE₂ production in cultured amnion cells and suggested that “Such a process may be of fundamental importance in the regulation of amniotic fluid volume homeostasis.”

Interactions of PGE₂ and VEGF have been reported under a variety of experimental conditions. PGE₂ or COX2 activation has been shown to increase VEGF gene expression in various cell types (11, 18, 26, 33, 38). In addition to its angiogenic properties, VEGF is a potent cell permeability factor that stimulates transcellular transport via caveolae (10, 21). In vascular endothelial cells, VEGF mediates its effect by down-regulating the expression of caveolin-1, the caveolae structural protein (28). Further, caveolin-1 may function as a negative regulator of VEGF receptor 2 (KDR) activity (27). We have previously shown that VEGF and its KDR receptor are expressed in the ovine amnion (2, 3). Further, in fetal sheep subjected to 4 days of hypoxic hypoxia, VEGF gene expression in amniotic epithelial cells was upregulated (29), while caveolin-1 was suppressed (15). Under such conditions, IM absorption rate was greatly increased. These observations are consistent with the notion that caveolin-1 negatively regulates AF transport across amnion cells (15, 35). Thus, it is possible that PGE₂ in the AF would similarly activate VEGF production in...
the fetal membranes to enhance AF transport across the amnion by a caveolae-mediated vesicular process.

To explore the potential role of PGE2 in regulating IM absorption and thus AF volume, we first determined the effects of PGE2 on VEGF and caveolin-1 gene expression in cultured ovine amnion cells. Second, we analyzed the relationship between amniotic PGE2 concentration, IM absorption rate, and AF volume in ovine fetuses under conditions of normal, low, or high IM absorption rates. We hypothesized that PGE2 induces VEGF gene expression in amnion cells leading to downregulation of caveolin-1 expression. We further hypothesized that, in the ovine fetus, PGE2 concentration in AF correlates positively with IM absorption rate and, therefore, negatively with AF volume, presumably through stimulation of the VEGF-mediated transcytotic pathway in the amnion.

MATERIALS AND METHODS

Ethical approval. These studies were approved by our Institutional Animal Care and Use Committee. We followed the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Amnion cell culture and PGE2 experiments. Fresh amnion tissues were obtained from late-gestation sheep [129 ± 1 (SE) days gestation, term = 145–150 days] with single or twin pregnancies. Amnion cells were prepared from minced amniotic membranes using three sequential treatments of 0.625% trypsin, as previously described (16). The final cell pellet was resuspended in DMEM/F12 supplemented with 10% FBS and antibiotics, plated onto 75 mm2 culture flasks, and maintained at 37°C in a humidified atmosphere of air and 5% carbon dioxide. At 90% confluence, the cells were seeded onto 6-well culture plates at 5 × 105 cells per well. For the PGE2 experiments in amnion cells, the culture medium containing serum was withdrawn overnight and replaced with medium supplemented with 1 × serum replacement (Cellgro, Mediatech, Voigt Global Distribution, Lawrence, KS). The cells were treated with 0.01 to 100 μmol/l PGE2 (1 μmol/l = 3.53 ± 106 pg/ml) for 3, 6, 24, or 48 h. At the end of each treatment period, cells were harvested for RNA and protein extraction. For VEGF antagonist experiments, amnion cells were pretreated with a specific mouse monoclonal VEGFR-2 receptor (KDR)-neutralizing antibody at 100 ng/ml (MAb0701; Novus Biologicals, Littleton, CO) for 1 h prior to addition of PGE2. Each amnion cell protocol was replicated in 3 to 6 experiments with triplicate wells per treatment group. Antagonist experiments for VEGFR-1 (Flt-1) blockade were not carried out since we have previously shown minimal expression of this receptor in ovine fetal membranes (3, 14).

RT-PCR analysis of VEGF and caveolin-1. Amnion cell total RNA was extracted using the RNeasy Kit (Qiagen, Valencia, CA) and reverse transcribed using MvUL reverse transcriptase. Ten to fifteen micrograms of cDNA was used in a semiquantitative RT-PCR for determination of VEGF164 mRNA levels with 18S ribosomal RNA as an internal reference, as previously described (15). The procedure was optimized for efficiency to yield amplification products within the linear range of input cDNA (efficiency: 80–85%; linearity: r = 0.97). Because 18S RNA was expressed in high abundance, an 18S competitor (Ambion, Austin, TX) was used in the same PCR reaction to reduce the yield of 18S amplification product to levels similar to that for the target gene. A ratio of 1:29 (18S primer to Competimer) was found to be optimal and used in all subsequent VEGF PCR reactions (15). The VEGF164 primers used were forward: 5’TGTATAATGAC-GAAAGTCTGCAG-3’ and reverse: 5’CACCCGCTCGGTGTGACA-CAACAAG-3’ (13). The reaction was carried out for 28 cycles of 95°C for 15 s and 60°C for 30 s. Caveolin-1 mRNA levels were determined similarly by semiquantitative RT-PCR using L-19 as an internal reference in the same PCR reaction (15, 17). Primers used were the following: for caveolin-1, forward, 5’-AACATCTACAAGCCCAAA-CACAAG-3’ and reverse, 5’-CGCATCAACACGCGAAGA-GAAATA-3’; for L-19, forward, ATCGCATAAGCCAATCTCCC-3’ and reverse, 5’-GTCTGGCTTACGTTGTGG-3’. The reaction, optimized for efficiency and linearity, was carried out for 28 cycles of 55°C for 30 s and 72°C for 30 s. The amplified products were separated in 2% to 3% agarose gel and visualized with ethidium bromide staining. The intensity of the signal was quantified under ultraviolet light and analyzed by ChemiImager 4400 software (Alpha Innotech, San Leandro, CA).

Western immunoblotting for caveolin-1 protein. Protein lysates were obtained from amnion cells using a nondenaturing lysis buffer and quantified by the protein assay kit (Pierce, Rockford, IL). The proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes, as described previously (9, 17). The blots were exposed to rabbit polyclonal anti-human caveolin-1 antibody (N-20, sc-894, Santa Cruz Biotechnology, Santa Cruz, CA) at 1:20,000 dilution. The secondary antibody used was a goat anti-rabbit IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology). The caveolin-1 protein was visualized with ChemiGlO substrate (Alpha Innotech) using the ChemiImager 4400 (Alpha Innotech). For internal reference, the membrane was stripped and reprobed for β-actin using a mouse monoclonal anti-human β-actin antibody (Santa Cruz Biotechnology) at 1:200 dilution. The intensity of the caveolin-1 signal was referenced to the β-actin signal.

Animals and surgical preparations. Eleven near-term pregnant sheep with singleton fetuses were surgically prepared, as previously described (34). Briefly, a carotid artery catheter was placed for monitoring of blood gas status. Fetal urinary bladder and tracheal catheters were placed for sampling and flow rate measurements. A flow probe (Transonic Systems, Ithaca, NY) was placed on the midcervical esophagus for measuring swallowing volume. Multiple catheters were attached to the fetal skin for AF sampling and returning urine and lung liquid to the AF. In vivo data from some of these animals have been presented in part (1, 6). The present study reports new data on PGE2 concentrations and relationships with AF volume and IM absorption rates.

Fetal sheep experimental methods. Experiments were conducted 5 or more days after surgery at a gestational age of 130 ± 2 days. Fetuses were subjected to 2 or 3 successive 2-day experimental protocols in random order: 1) a control period with monitoring only (n = 11); 2) continuous diversion of fetal urine to the exterior and isovolumic replacement with lactated Ringer solution (n = 11); and 3) AV volume expansion by continuous intra-amniotic infusion of lactated Ringer at 2 l/day (1.4 ml/min, n = 6). These conditions were chosen to experimentally alter AF volume and IM absorption rates, while modifying amniotic PGE2 concentrations within the physiological range. Alternative methodologies, such as indomethacin infusion to reduce PGE2 production or PGE2 infusion were avoided because of the associated conditions of ductus arteriosus constriction, fetal hypoxia, and reduction in urine production (8, 36).

At the initiation of each experimental protocol, the AF compartment was emptied by drainage for measurement of AF volume and replaced with 1 liter of warm lactated Ringer solution, such that every protocol began with the same volume and composition of AF (1, 34). During each 2-day protocol, fetal swallowing, urine flow rate, and lung liquid production rate were continuously monitored (34). At the end of the 2-day protocol, samples of AF, fetal urine, and fetal lung liquid were collected for measurement of PGE2 concentration. PGE2 concentrations in fetal plasma were not determined, as PGE2 levels in fetal blood are much lower (less than half) than that in AF (37) and would not have significantly impacted the PGE2 pool in the amniotic compartment. Prior to AF sampling, 20–30 ml of AF were withdrawn from the sampling catheter before collection to ensure a mixed, representative AF sample was obtained. Fetal arterial blood was sampled for blood gases and pH measurements (Radiometer model 725 analyzer; Westlake, OH). AF volume was measured by drainage, and the IM absorption rate was calculated from the time-
Figure 1. Time- and concentration-dependent vascular endothelial growth factor (VEGF) mRNA responses to prostaglandin E2 (PGE2) applied to ovine amnion cells in culture (means ± SE). Cells were grown to 90% confluence in complete medium and incubated in serum-replaced medium (SR) overnight prior to addition of PGE2. Data were analyzed by ANOVA. *P < 0.05 by post hoc test compared with cells in SR at 3 h of incubation in the absence (A) and in the presence (B) of VEGFR-2 neutralizing antibody (100 ng/ml). Time dependence was significant only in the absence of inhibitor (A) at the highest PGE2 concentrations.

PGE2 effects on VEGF gene expression in amnion cells. In amnion cells incubated with control medium containing serum replacement (SR), VEGF mRNA levels were unchanged over time (Fig. 1A). At PGE2 concentrations of 0.1 μmol/l and lower, VEGF mRNA levels were not significantly altered. With increasing concentrations of PGE2, there was a significant increase in VEGF mRNA levels (P < 0.0001, Fig. 1A). PGE2 at concentrations of 1 μmol/l and higher increased VEGF mRNA levels by 3 h (compared to levels in the SR group at the same time point). At PGE2 concentrations of 10 and 100 μmol/l, the increases in VEGF mRNA were both time- (P < 0.05) and concentration- (P < 0.01) dependent, reaching peak levels with 100 μmol/l at 24 h. The VEGF response declined at 48 h of treatment.

PGE2 effects on caveolin-1 expression in amnion cells. Under control conditions, caveolin-1 mRNA levels did not change over time (Fig. 2A). In response to PGE2 treatment at 1, 10, and 100 μmol/l, caveolin-1 mRNA levels in amnion cells decreased significantly compared with the SR group at 3 h, and the effects were time- (ANOVA P < 0.01) and concentration- (ANOVA, P < 0.01) dependent (Fig. 2A). Maximal effect was achieved at PGE2 concentration of 100 μmol/l after 48 h of treatment. The decrease in caveolin-1 mRNA in the presence of increasing PGE2 concentrations was paralleled by both time- and concentration-dependent reductions in caveolin-1 protein levels (P < 0.05, Fig. 3).

Following treatment of amnion cells with a VEGFR-2 neutralizing antibody at 100 ng/μl, PGE2 similarly increased VEGF mRNA levels (ANOVA, P < 0.0001, Fig. 1B). At 1 and 10 μmol/l PGE2, the responses were not different from those in the absence of antibody pretreatment. At 100 μmol/l, the PGE2-induced increase in VEGF mRNA levels was moderately suppressed (ANOVA, P < 0.05) during neutralizing antibody treatment. In contrast, the PGE2-induced decrease in caveolin-1 mRNA levels was completely abolished in the presence of VEGFR-2 blockade at all PGE2 concentrations tested over the 48-h experimental period (Fig. 2B).

In vivo fetal sheep status. During the 2-day control period, fetal arterial pH, carbon dioxide tension, and oxygen tension averaged 7.347 ± 0.006, 53.8 ± 1.3 mmHg, and 22.4 ± 0.8 mmHg, respectively. These values were unchanged during urine replacement and intra-amniotic fluid infusion.

Amniotic fluid, fetal urine, and lung liquid PGE2 concentrations. During the control period, PGE2 concentration in the AF was 887 ± 111 pg/ml, significantly lower than 1,843 ± 256 pg/ml in fetal urine but higher than 113 ± 24 pg/ml in fetal lung liquid (ANOVA, P < 0.001). Fetal urine flow rate was 1,142 ± 108 ml/day, and the calculated renal PGE2 excretion rate was 2,086 ± 254 ng/day. Lung liquid flow rate was 403 ± 67 ml/day, and the calculated lung PGE2 secretion rate was 44 ± 10 ng/day. The lung PGE2 secretion rate was equivalent to 4.8% ± 3.1% of the amount of PGE2 excreted by the kidneys.
During diversion and isovolumic replacement of urine, urinary PGE2 concentration was 1,386 ± 176 pg/ml and was not significantly different from control. Amniotic PGE2 concentration fell significantly to 348 ± 50 pg/ml, which equaled 42% ± 4% of control values (P < 0.05). This suggests that fetal urinary PGE2 contributed 58% ± 4% to the AF PGE2 pool. Lung liquid PGE2 concentration was 120 ± 40 pg/ml, not different from the control value. On the basis of the data obtained during urine replacement, our calculations indicated that the lungs would have contributed only 2.5% ± 1.5% to the amniotic PGE2 pool under control conditions. Expansion of AF volume by continuous intra-amniotic infusion of lactated Ringer solution for 2 days did not significantly alter amniotic or urinary PGE2 concentrations (AF = 1,190 ± 603 pg/ml; urine = 2,355 ± 545 pg/ml). Similarly, fetal lung liquid PGE2 concentration (104 ± 21 pg/ml) was not affected by intra-amniotic fluid infusion.

Amniotic fluid and urinary PGE2 concentration relationships. Fetal urine and amniotic PGE2 concentrations were positively correlated during control conditions and during intra-amniotic infusion. The urinary and amniotic PGE2 concentrations remained positively correlated when urine was diverted and replaced, even though fetal urine did not enter the AF. When the three groups were compared, there were statistically significant shifts in the relationships between amniotic and urinary PGE2 concentrations (ANCOVA, P < 0.05, Fig. 4). These changes were due to decreases in amniotic PGE2 concentrations during urine replacement, resulting in a downward shift in the regression line (ANCOVA, P < 0.01 compared with control). In contrast, during intra-amniotic infusion, neither amniotic PGE2 concentrations nor the relationship between AF and urinary PGE2 concentrations were different from the control (ANCOVA, P = ns). No correlation was found between amniotic and lung liquid PGE2 concentrations or secretion rates under any of the three experimental conditions.

Amniotic fluid volume, intramembranous absorption rate, and amniotic PGE2 concentration relationships. Under control conditions, IM absorption rate (963 ± 118 ml/day) and AF volume (776 ± 134 ml) were negatively correlated (r = −0.61, P < 0.05). During urine diversion and replacement, AF volume doubled (P < 0.001), and IM absorption rate decreased by 57% ± 11% (P < 0.001). However, the negative relationship between AF volume and IM absorption rate (r = −0.58, P < 0.01 for the combined urine replacement and control groups) was not altered (ANCOVA, P = 0.25). During intramniotic fluid infusion, AF volume and IM absorption rate both increased significantly and remained negatively correlated (r = −0.96, P < 0.01). However, in response to fluid infusion, the relationship between AF volume and IM absorption rate shifted upward and to the right due to the large increases in both AF volume and IM absorption rate (ANCOVA, P < 0.0001, Fig. 5).

IM absorption rate and amniotic PGE2 concentration were not significantly correlated under each of the three experimental conditions (Fig. 6A). Similarly, AF volume and amniotic PGE2 concentration were not significantly correlated (Fig. 6B). Further, the changes in neither IM absorption rate nor AF volume doubled (P > 0.25).

Fig. 2. Time- and concentration-dependent caveolin-1 mRNA responses to PGE2 applied to ovine amnion cells in culture (means ± SE). Cells grown to 90% confluence in complete medium and incubated in SR overnight prior to the addition of PGE2. Data were analyzed by ANOVA. *P < 0.05 by post hoc test compared with cells in SR at 3 h of incubation.

Fig. 3. Time- and concentration-dependent caveolin-1 protein responses to PGE2 applied to ovine amnion cells in culture (means ± SE). Cells grown to 90% confluence in complete medium were incubated in SR overnight prior to adding PGE2. Data were analyzed by ANOVA. *P < 0.05 by post hoc test compared with cells in SR at 3 h of incubation.

Fig. 4. Comparison of relationships between amniotic fluid and urinary PGE2 concentrations by analysis of covariance (ANCOVA). • denotes control conditions, ◇ denotes fetal urine replacement, and ■ denotes continuous 2 l/day intra-amniotic infusion of lactated Ringer solution.
Our study demonstrated that PGE2 not only function as the stimulator of IM absorption present in fetal prostanoid abundant in fetal urine, would alter VEGF and sized, but also that the PGE2 effect on caveolin-1 expression in ovine amnion cells in vitro, as hypothesized, was associated with elevated VEGF but reduced caveolin-1 gene expression in the amnion (15).

Further, these findings support our earlier report that increases in IM absorption induced by hypoxia was associated with increased VEGFR-2 receptor. These observations are consistent with the established role of VEGF in stimulating vesicular transcytosis via caveolae (10), and in downregulating caveolin-1 expression observed in vascular endothelial cells (28).

Fig. 5. Comparison of relationships between amniotic fluid volume and intramembranous absorption rate by ANCOVA. ● denotes control conditions, ○ denotes fetal urine replacement, and ■ denotes continuous 2 l/day intra-amniotic infusion of lactated Ringer solution. Data from control and urine replacement groups were combined prior to analysis because the regression relationship between these two groups was unaltered by urine replacement (ANCOVA, $P = 0.25$).

In addition to our original objectives, the present study has provided several unique observations regarding PGE2 levels in amniotic fluid and lung liquid PGE2 concentrations under any of the experimental conditions studied.

**DISCUSSION**

An objective of this study was to explore whether PGE2, a prostanoid abundant in fetal urine, would alter VEGF and caveolin-1 gene expression in amnion cells and potentially function as the stimulator of IM absorption present in fetal sheep urine (1). Our study demonstrated that PGE2 not only upregulated VEGF gene expression and downregulated caveolin-1 expression in ovine amnion cells in vitro, as hypothesized, but also that the PGE2 effect on caveolin-1 was mediated through the VEGFR-2 receptor. These observations are consistent with the established role of VEGF in stimulating vesicular transcytosis via caveolae (10), and in downregulating caveolin-1 expression observed in vascular endothelial cells (28).

Our second objective was to examine the relationship between amniotic PGE2 concentrations and IM absorption rate in chronically catheterized ovine fetuses under experimental conditions of normal, low, or high IM absorption rates. On the basis of our in vitro findings in amnion cells, we expected that amniotic PGE2 concentrations would correlate positively with IM absorption rate in vivo, presumably through the activation of VEGF-mediated transcytotic pathways. However, our present studies in ovine fetuses do not support the functionality of such a pathway because neither amniotic PGE2 concentration, urinary PGE2 concentration, nor renal PGE2 excretion rate correlated with IM absorption rate or AF volume under control and experimentally induced conditions of decreased or increased IM absorption. The lack of relationship likely is due to the in vivo concentrations of PGE2 in AF (250–1,000 pg/ml or 1–4 nmol/l) being lower than the effective dose of PGE2 in the in vitro amnion cell studies (1,000–100,000 nmol/l).

Thus, under normal physiological conditions, amniotic PGE2 would not be involved in the maintenance of IM transport and, thus, of AF volume. However, participation of PGE2 in the regulation of AF dynamics may occur under adverse conditions when amniotic PGE2 is greatly elevated, such as during intrauterine infection when fetal and amniotic PGE2 concentrations reach ~20 times normal in pregnant sheep (24) and ~120 times normal in Rhesus monkeys (25).

**Fig. 6.** ANCOVA comparison of relationships between amniotic fluid PGE2 concentration and intramembranous (IM) absorption rate (A) and amniotic fluid volume (B). ● denotes control conditions, ○ denotes fetal urine replacement, ■ denotes 2 l/day intra-amniotic infusion of lactated Ringer solution. With the one outlier excluded, $P < 0.001$ for panels A and B. When log transformed to normalize variances, $P < 0.01$ for panels A and B, after excluding the one negative IM absorption rate in panel A (log data not shown).
the AF compartment. In near-term ovine fetuses under normal conditions, amniotic PGE2 concentrations are high, while urinary levels are twice amniotic levels. The fetal kidneys normally excrete more than 40 times as much PGE2 as the fetal lungs secrete into the AF. With high renal excretion rates, it is not unexpected that a positive correlation existed between urinary and amniotic PGE2 concentrations under control conditions and during intra-amniotic fluid infusion. These results are consistent with the notion that fetal urinary PGE2 is a primary contributor to the PGE2 pool in AF. However, the observation that amniotic and urinary PGE2 concentrations remained positively correlated but shifted downward when fetal urine was replaced with Ringer solution was not expected and suggests that amniotic PGE2 and urinary PGE2 levels are regulated by a common endogenous factor presumably present in the fetal circulation. This observation requires further investigation.

Because fetal urine contributes about 58% to the amniotic PGE2 pool, while lung liquid contributes 3%, the remaining PGE2 in AF must be derived from other sources. Because PGE2 concentration in ovine fetal blood is low compared with AF concentrations (37), the fetal vascular system would be a sink rather than source of amniotic PGE2. The most likely source would be endogenous production by the fetal membranes and, to a lesser extent, the umbilical cord (30, 31). Our finding that amniotic PGE2 concentration during urine replacement was much higher than could be explained by a pulmonary source alone also supports this possibility.

Perspectives and Significance

Although it has long been recognized that AF volume is regulated by the rate of IM absorption, presently there is no detailed knowledge of the factors involved in modulating IM transport across the amnion and into the underlying fetal vasculature. Over the past decade, the most significant advance in this understanding has been the observations that IM transport across the amnion has two components: a primary unidirectional vesicular transcytosis of AF mediated by VEGF and a secondary passive bidirectional diffusion of water and solutes down concentration and osmotic gradients between AF and fetal blood through aquaporin water channels. The recent observation that fetal urine contains a stimulator of IM absorption (1) raised the possibility that PGE2 is the unidentified renal stimulator of IM absorption across the amnion. This is because PGE2 is present in fetal urine in high concentrations, and PGE2 is known to stimulate cellular VEGF expression and function. However, in contrast to our hypothesis, PGE2 does not appear to be involved in the regulation of AF volume under physiological conditions. Thus, the stimulator of IM absorption present in fetal urine remains to be identified.

GRANTS

This work was supported in part by a grant from the National Institutes of Health/National Institute of Child Health and Human Development, R01 HD061541.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

22. Gesteland KM, Anderson DF, Davis LE, Robertson P, Faber JJ, Brace RA. Intramembranous solute and water fluxes during high in-


