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Vasopressin Concentration in Amniotic Fluid as an Index of Fetal Hypoxia: Mechanism of Release in Sheep

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Summary

Hypoxia is a potent stimulus to the release of vasopressin in fetal sheep, and plasma concentrations of the hormone correlate inversely with fetal oxygenation. Since the fetal kidney contributes to vasopressin clearance, we propose that measurement of

increased amounts of vasopressin in amniotic fluid would be indicative of fetal hypoxia. Therefore, we measured concentrations of vasopressin in amniotic fluid under resting conditions, during and after fetal hypoxia, and with intravenous and intra-amniotic administration of vasopressin in 15 chronically instrumented fetal lambs between 111 and 141 days gestation. In the resting state, mean ($\pm$ SE) vasopressin concentrations in amniotic fluid (1.6 ± 0.3 pg ml⁻¹) did not differ from those in maternal (1.4 ± 0.4 pg ml⁻¹) or fetal (1.8 ± 0.2 pg ml⁻¹) plasma. Following exposure of the ewe to 10% O₂ or partial occlusion of the umbilical cord, vasopressin concentrations in fetal plasma in-

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creased significantly ($P < 0.001$) to 200 ± 59 pg ml⁻¹ with a delayed increase in amniotic fluid concentrations ($P < 0.03$) to 15.8 ± 4.5 pg ml⁻¹. This rise in concentration of vasopressin in amniotic fluid was sustained for at least 24 h and levels at that time were highly correlated with peak plasma concentrations ($r = 0.83$; $P < 0.001$). Intravenous infusion of vasopressin into the fetus was accompanied by an equally significant ($P < 0.02$) and sustained increase of vasopressin in amniotic fluid. Following intra-amniotic injection of vasopressin, levels remained increased for at least 24 h.

Thus, in the third trimester of ovine gestation, amniotic fluid vasopressin concentration was a reliable indicator of prior fetal hypoxia. Parallel amounts of antidiuretic activity measured by bioassay and vasopressin measured by radioimmunoassay confirmed the presence of active hormone in amniotic fluid. Hypoxia alone was not a stimulus to passage of meconium, and increased concentrations of vasopressin in fetal plasma were not associated with expulsion of meconium *in utero*. Despite elevated concentrations of vasopressin in amniotic fluid, no associated changes in intrauterine pressure were discerned.

Evidence from a detailed review of long-term follow-up of infants by Paneth and Stark (19) supports the conclusion that asphyxia as indicated by clinical markers during the process of birth accounts for a smaller proportion of cerebral palsy and severe mental retardation than had been suspected. A major part of this discrepancy could be the result of asphyxial episodes occurring prior to the onset of labor. These episodes may not be reflected in the condition of the infant at birth, but could still lead to severe handicapping conditions. We have therefore endeavored to develop techniques for antipartum fetal surveillance.

Data from both animal and human studies have shown that hypoxic stress is a potent stimulus for the release of vasopressin as well as several other peptide hormones from the fetal pituitary. In the present study, we investigated the potential utility of the measurement of vasopressin concentration in the amniotic fluid as an index of hypoxic stress to the fetus during the last quarter of gestation in sheep.

Of further interest are the potential pathophysiologic consequences for the fetus of the release of vasopressin induced by hypoxia. De Vane *et al.* (10, 11) have suggested that increases in vasopressin during fetal asphyxia could be important in the fetal expulsion of meconium into the amniotic sac, thereby establishing the situation necessary for meconium aspiration. Still other investigators have proposed a role for vasopressin in the processes initiating labor (5, 17) and possibly the onset of premature delivery. Thus, in addition to assessment of vasopressin as a marker of hypoxia, we designed experiments to examine the relationship of the release of vasopressin by the fetus to the passage of meconium *in utero* and the stimulation of regular uterine contractions.

MATERIALS AND METHODS

Surgical Procedures. Fifteen mixed breed ewes of 95 to 105 days of gestation, defined by day of mounting, were acclimatized to the animal care facility and feed for at least 1 week prior to surgery. At 102 to 119 days of gestation, the animals were given spinal anesthesia with 10 mg tetracaine. After cannulation of the maternal femoral artery and vein, anesthesia was supplemented with an IV infusion of 2 mg ml⁻¹ sodium thiopental in 5% dextrose (150–450 mg total dose). With appropriate sterile preparation and draping, the uterus was exposed through midline abdominal incision. The fetal neck was marsupialized to an antimesometrial portion of the uterine wall free of cotyledons of major vessels. Polyvinyl catheters were placed in the fetal carotid artery and jugular vein. Two polyvinyl catheters (0.9 mm ID $\times$ 1.4 mm OD) with sumps fixed to the end to prevent occlusion by fetal membranes or particulate material were placed in the amniotic cavity and fixed to the fetal skin. Through a separate uterine incision, an inflatable silicon rubber cuff constrictor was

placed around the umbilical cord. Care was taken to ensure that the constrictor did not occlude the umbilical cord in the deflated state. All catheters and activating tubing were tunneled subcutaneously and exteriorized into a pouch affixed to the ewe's flank. Benzathine penicillin (2.5 million units) was administered to the ewe on the day of surgery and ampicillin (25 mg kg⁻¹) was given to the fetus and the ewe every 12 h for 3 days postoperatively. All vascular catheters were flushed on alternate days and filled with normal saline containing 200 U ml⁻¹ heparin.

Condition of Animals. Experiments were conducted at least 6 days postoperatively. When more than one experiment was done on an animal, an interval of at least 6 days was allowed between studies. Prior to assignment to an experimental protocol, it was ascertained that fetal pH and blood gas tensions were within the normal range for our laboratory (pH_a 7.37 ± 0.01 ; PaCO₂ 43.5 ± 0.9 mm Hg; PaO₂ 20 ± 1.0 mm Hg). Eight ewes delivered lambs by spontaneous vaginal delivery at 144 ± 1.2 days (normal gestation in these animals is 148 days) and birth weight was 3.8 ± 0.2 kg. Four fetuses died during labor or at unattended delivery at 144 ± 1.4 days, two of whom had umbilical cord occluders. Three fetuses died *in utero* at 131 ± 2.5 days, deaths occurring 2, 5, and 6 days after an experimental protocol.

Experimental Protocol. Pregnant ewes were maintained in portable pens with free access to feed and water throughout the study period. Amniotic fluid pressure was monitored continuously through one amniotic catheter with a Statham p 23 Db (Hato Rey, Puerto Rico) strain gauge transducer and recorded on a Beckman Model 49 (Chicago, IL) paper chart recorder. Fetal heart rate was derived with a tachometer triggered from the blood pressure wave obtained and recorded with similar techniques. The animals were allowed at least 2 h to acclimatize to the study facility, prior to experimental interventions.

To examine the effects of fetal hypoxia on amniotic fluid vasopressin, ewes were given 10% O₂ in N₂ to breathe for 30 min. The gas mixture was administered at flow rates of 6 to 8 liters min⁻¹ into a specifically fitted head piece covered with a polyethylene bag taped loosely around the neck of the ewe. Results from these hypoxia studies were compared to control experiments conducted in a similar fashion except that room air was passed into the bag. To study the transfer of vasopressin from fetus to amniotic fluid, synthetic vasopressin (Parke-Davis, Morris Plains, NJ) in 0.9% saline was infused intravenously into the fetal jugular vein for 30 min. The rate of infusion was 0.1 cc min⁻¹ and the dose 0.5 mU min⁻¹. To examine the rate of clearance of vasopressin from amniotic fluid, a bolus of synthetic vasopressin, 1 to 10 mU in 2 ml of 0.9% saline, was injected into the amniotic sac. Finally, to examine the rate of accumulation of endogenous fetal vasopressin in amniotic fluid as standardized in previous studies, the umbilical cord occluder was partially inflated until a 10 to 20% reduction in fetal heart rate was obtained and that degree of occlusion was maintained for 30 min (9). The number of studies and gestational age of the animals are summarized in Table 1.

Fetal arterial blood samples were drawn at 30 and 0 min before and 30, 60, 90, and 120 min, and 24 h after each of the above experiments. Amniotic fluid samples were obtained on the same schedule with additional samples at 3, 4, 6, 18, and 24 h. The presence or absence of meconium in each sample was noted. Samples were not obtainable from all animals at all time intervals. If both amniotic fluid catheters were obstructed, they were flushed with 2.5 ml 0.9% saline and the subsequent scheduled sample was obtained. It was only with an animal at greater than 135 days of gestation that this problem was encountered.

Biochemical Analysis. Samples of 2.5 ml of blood and amniotic fluid were withdrawn anaerobically in heparinized syringes; 1.5 ml was immediately transferred to chilled tubes containing EDTA for determination of vasopressin. Blood and amniotic fluid samples were centrifuged at 4°C for 20 min and plasma and amniotic fluid supernatant were stored at -20°C until analyzed. The remainder of the blood sample was used for determination of pH, PCO₂, and PO₂ with a Radiometer analyzer

Table 1. Summary of experimental protocols, number of animals, and studies performed, and presence of meconium before and after a study

	Intervention	Gestational age (days)		Animals (n)	Studies (n)	Amniotic fluid meconium (n)	
		Mean	Range			Before	After
Control	Room air $\times$ 30 min	129 $\pm$ 2.1	124–136	4	5	0	0
Hypoxia	10% O ₂ in N ₂ $\times$ 30 min	128 $\pm$ 2.0	121–141	10	16	2	3
Umbilical cord occlusion	Partial inflation $\times$ 30 min	131 $\pm$ 4.6	123–139	2	3	0	3
Intravenous vasopressin	0.5 mU/min $\times$ 30 min	127 $\pm$ 3.0	111–138	6	9	1	1
Intra-amniotic vasopressin	1 to 10 mU bolus	128 $\pm$ 4.3	118–138	4	5	0	0

and microelectrodes. Base excess was subsequently calculated (30).

Vasopressin was extracted from 0.5 ml of plasma with acetone and petroleum ether and measured by radioimmunoassay using methods that have been previously described (14, 31). The antibody employed in this assay has essentially no cross-reactivity with oxytocin or vasotocin. The method has a lower limit of sensitivity of better than 0.5 pg tube⁻¹ and an interassay variability of 8.4%. The standards used in the assay were made from a synthetic arginine vasopressin obtained from Schwarz/Mann. Recovery of known amounts of vasopressin added before extraction was 67 $\pm$ 4% and when added after extraction was 98 $\pm$ 3%.

Amniotic fluid vasopressin concentrations were determined by a modification of the plasma assay. Samples of 0.5 ml were extracted with 2:1 acetone and dried under air. When vasopressin was added before extraction, recovery was 80 $\pm$ 4%. With 0.5 ml extracted amniotic fluid, the lower limit of sensitivity of the assay was 1.0 pg tube⁻¹. Four pools of amniotic fluid containing 2, 7, 17, and 28 pg ml⁻¹ were assayed $\times$ 10 in a single assay and gave an intra-assay variability of 8, 4, 3, and 3%, respectively. Two pools of amniotic fluid containing 4 and 25 pg ml⁻¹ were assayed in six different assay runs and gave an interassay variability of 8 and 10%, respectively. In general, all samples from a single experiment were analyzed in a single radioimmunoassay run.

To aid in establishing that the immunoreactive material in amniotic fluid was vasopressin, values determined by radioimmunoassay were compared with those obtained from the anti-diuretic bioassay. Bioassays were kindly performed by Dr. Wilbur H. Sawyer, College of Physicians and Surgeons, Columbia University, using an alcohol-anesthetized rat anti-diuretic assay (29). Three pools of amniotic fluid obtained following fetal hypoxia were used for this comparison.

Statistical Analysis. Data were expressed for purposes of summarization as mean $\pm$ SEM except where otherwise indicated. Mean values were compared by one-way analysis of variance techniques to determine differences within experimental groups. The significance of differences at given time intervals was obtained using a modification of the Tukey transformation. Linear regression analysis using least mean squares methods was performed when correlating individual values. Finally, an unpaired *t* test was used to compare mean values in control conditions. Standard techniques for determination of coefficient of correlation and per cent variation were also employed.

RESULTS

Resting Condition. In 13 animals, 59 matched samples of amniotic fluid and fetal plasma were obtained at 115 to 147 days of gestation. These samples were taken at least 5 days after any study protocol and animals were considered to be in a stable condition as defined by fetal pH 7.381 $\pm$ 0.005, PCO₂ 41.5 $\pm$ 0.5 mm Hg, and PO₂ 22 $\pm$ 0.9 mm Hg. The mean amniotic fluid vasopressin of 1.6 $\pm$ 0.3 pg ml⁻¹ (range, 0.5 to 7.5) did not differ significantly from fetal plasma vasopressin of 1.8 $\pm$ 0.2 (range,

0.5 to 6.0) or maternal plasma vasopressin of 1.4 $\pm$ 0.4 (range, 0.5 to 4.3). No differences in amniotic fluid or fetal plasma vasopressin were observed with advancing gestation.

Amniotic fluid pH determinations were made on 48 of these samples. The mean pH 6.89 $\pm$ 0.04 did not vary significantly with gestational maturation from 115 to 141 days of gestation, although there was a trend toward decreased pH close to term.

Experimental Conditions. Fetal arterial pH, blood gas tensions, and base excess values during the various experimental protocols are summarized in Table 2. No significant differences were noted with exposure to room air (control) in contradistinction to the development of hypoxia and metabolic acidosis in the fetuses whose mothers were exposed to 10% O₂. In these fetuses, mean PO₂ decreased significantly from 22.6 $\pm$ 0.7 to 13.2 $\pm$ 0.6 mm Hg during the exposure while pH and base excess decreased both during and after the exposure. The only measurable change in pH or blood gas tensions with intravenous or intra-amniotic vasopressin administration was a significant increase in PO₂ during IV vasopressin infusion.

Control and hypoxia studies were undertaken to measure changes of vasopressin concentration in amniotic fluid and fetal plasma and are summarized in Figure 1. Exposure of the ewe to room air for 30 min (control) was not associated with any distinct change in the concentration of vasopressin in either amniotic fluid or fetal plasma. Maternal plasma vasopressin values 1.3 $\pm$ 0.2 pg ml⁻¹ (range, 1.0 to 3.5) were equally stable.

Exposure of the ewe to 10% O₂ in N₂ for 30 min (hypoxia) produced an increase of plasma vasopressin in all fetuses (F = 9.00; P < 0.001). There was a rapid decrease in mean concentrations during the 90 min following return to room air and 24 h later values were similar to those obtained prior to exposure (Fig. 1). Maternal plasma vasopressin concentrations increased in only one animal (1.3 to 4.0 pg ml⁻¹) and the mean concentration during hypoxia (1.8 $\pm$ 0.6 pg ml⁻¹) was not different from that preceding hypoxia (1.5 $\pm$ 0.4 pg ml⁻¹). Amniotic fluid concentrations of vasopressin increased in the hour following hypoxia and remained elevated at 24 h (F = 3.26; P < 0.03). Amniotic fluid vasopressin concentrations at both 4 and 24 h of recovery were highly correlated with peak fetal plasma concentrations (r = 0.78, P < 0.001 and r = 0.83, P < 0.001). When peak fetal and amniotic fluid concentrations were grouped with respect to gestational age at the time of hypoxia there was a trend toward increased mean values with advancing gestation; at 116 to 122 days (n = 6) fetal plasma mean was 124 $\pm$ 58 and amniotic fluid mean was 13.3 $\pm$ 3.8 pg ml⁻¹, at 125 to 129 days (n = 5) 171 $\pm$ 79 and 18.2 $\pm$ 8.7 pg ml⁻¹, and at 134 to 141 days (n = 5) 345 $\pm$ 149 and 21 $\pm$ 7.4 pg ml⁻¹.

When vasopressin was infused into the fetal jugular vein at a dose of 0.5 mU min⁻¹ for 30 min, fetal plasma vasopressin concentrations after infusion ranged from 29 to 180 pg ml⁻¹. The appearance of increased vasopressin concentrations (F = 2.67; P < 0.02) in amniotic fluid followed a pattern similar to that seen with hypoxia (Fig. 1). Mean maternal plasma vasopressin did not change, being 1.5 $\pm$ 0.2, 1.8 $\pm$ 0.4, and 1.5 $\pm$ 0.4 pg ml⁻¹ before, during, and after infusion into the fetus, respectively.

Table 2. Arterial pH, blood gas, and base deficit (BE) of fetal lambs at times before, during, and after control (exposure to room air $\times 30$ min) hypoxia (exposure to 10% O₂ in N₂ $\times 30$ min), intravenous infusion of vasopressin to the fetus (0.5 μ U min⁻¹ $\times 30$ min), and intra-amniotic bolus injection of vasopressin (1 to 10 mU) (significant difference from control, $P < 0.01^{**}$; $P < 0.05^{*}$)

	Before (-30 min)	During (+30 min)	After	
			(+60 min)	(+180 min)
Control (room air)				
pH	7.377 $\pm$ 0.016	7.370 $\pm$ 0.017	7.384 $\pm$ 0.019	7.379 $\pm$ 0.015
PO ₂	23.5 $\pm$ 0.5	24.6 $\pm$ 0.6	23.9 $\pm$ 0.5	24.5 $\pm$ 0.6
PCO ₂	43.9 $\pm$ 1.1	44.6 $\pm$ 1.2	41.0 $\pm$ 0.7	43.8 $\pm$ 1.6
BE	+0.9 $\pm$ 0.7	-0.2 $\pm$ 1.5	-0.2 $\pm$ 0.9	+0.6 $\pm$ 1.3
Hypoxia (10% O ₂)				
pH	7.383 $\pm$ 0.006	7.352 $\pm$ 0.022	7.331 $\pm$ 0.015*	7.361 $\pm$ 0.016
PO ₂	22.6 $\pm$ 0.7	13.2 $\pm$ 0.6**	24.5 $\pm$ 1.4	23.9 $\pm$ 0.9
PCO ₂	44.3 $\pm$ 0.8	41.8 $\pm$ 2.6	42.8 $\pm$ 1.4	42.1 $\pm$ 1.0
BE	+0.6 $\pm$ 0.6	-2.2 $\pm$ 1.3*	-3.2 $\pm$ 1.0**	-1.5 $\pm$ 0.9
Intravenous vasopressin				
pH	7.381 $\pm$ 0.008	7.379 $\pm$ 0.009	7.381 $\pm$ 0.010	7.373 $\pm$ 0.010
PO ₂	22.7 $\pm$ 1.3	25.4 $\pm$ 2.0*	24.4 $\pm$ 1.7	24.2 $\pm$ 1.1
PCO ₂	41.2 $\pm$ 0.7	41.0 $\pm$ 0.5	41.4 $\pm$ 0.9	42.3 $\pm$ 1.1
BE	-0.8 $\pm$ 0.6	-0.8 $\pm$ 0.7	-0.4 $\pm$ 0.6	-0.4 $\pm$ 0.7
Intra-amniotic vasopressin				
pH	7.383 $\pm$ 0.007	7.376 $\pm$ 0.007	7.373 $\pm$ 0.007	7.375 $\pm$ 0.008
PO ₂	22.6 $\pm$ 1.3	20.6 $\pm$ 1.8	22.1 $\pm$ 1.4	22.6 $\pm$ 2.1
PCO ₂	44.8 $\pm$ 1.5	44.9 $\pm$ 1.1	42.4 $\pm$ 1.7	42.9 $\pm$ 1.3
BE	1.5 $\pm$ 0.6	1.2 $\pm$ 1.0	0.5 $\pm$ 1.2	0.2 $\pm$ 0.7

Fig. 1. Vasopressin concentrations (mean $\pm$ SE) in fetal plasma and amniotic fluid at times before, during (shaded bar), and after 30-min exposure to room air ($\bullet$), 10% O₂ in N₂ (Δ), and intravenous vasopressin, 0.5 mU min⁻¹ ($\circ$).

Partial occlusion of the umbilical cord for 30 min not only caused a decrease from resting values of PO₂ but also a large decrease in pH and base excess and increase in PCO₂ (Table 3). This asphyxial stress was associated with a marked release of vasopressin into fetal plasma (Fig. 2). Vasopressin began to accumulate in amniotic fluid in the hour after occlusion and increased at a rate of 12.5 pg h⁻¹ over the subsequent 3 h.

Intra-amniotic injections of vasopressin were given to examine the pattern of disappearance of vasopressin from amniotic fluid (Fig. 3). Concentrations of vasopressin remained at a constant elevated level for at least 6 h after bolus doses ranging from 1 to 10 mU. Neither fetal (1.8 ± 0.6 pg ml⁻¹) nor maternal (1.4 ± 0.3 pg ml⁻¹) plasma concentrations varied over this interval. At 24 h after injection, amniotic fluid vasopressin concentrations were consistently higher than those in the resting state.

The presence or absence of meconium in amniotic fluid before and 6 h after each study was determined macroscopically and observations are summarized in Table 1. No consistent pattern of meconium passage was observed except in those animals subjected to cord occlusion where meconium was found after but not before each study.

Intra-amniotic pressure was recorded for at least 2 h before and 6 h after each study. No discernible change in baseline intra-amniotic pressure was defined except during and immediately following exposure of the ewe to 10% O₂ in N₂. This increase in pressure was associated with maternal tachypnea and bleating. No sustained episodes of tonic increases in intra-amniotic pressure (uterine contractions) were recorded during the observation period in any animal. No animal delivered within 3 days of an experiment. A typical intra-amniotic pressure tracing during intravenous infusion of vasopressin to the fetus is shown in Figure 4. There was no tendency toward increased intra-amniotic pressure despite large increases in the concentration of vasopressin in amniotic fluid.

DISCUSSION

The fetal hypothalamo-hypophyseal system is activated by hypoxia with release of both anterior and posterior pituitary hormones. Because of this, we proposed that measurements of the concentrations of peptide hormones in amniotic fluid might serve as indicators of fetal condition (Fig. 5). Vasopressin was chosen for evaluation as an indicator of fetal hypoxia because numerous investigators have described increased concentrations of the peptide in the umbilical cord blood of human infants (5, 11–13, 20, 21, 23). Taken together, data from these studies suggest that fetal plasma vasopressin is elevated by the stress of labor and delivery. Although specific correlations were not apparent between Apgar score, pH, or PO₂ of umbilical arterial blood and concentrations of vasopressin in our group (21), most

Table 3. Arterial pH, blood gas tension, and base excess before and after a 30-min partial occlusion of the umbilical cord in the fetal lamb at three gestational ages

Animal	Gestational age (days)	pH		PO ₂ mm Hg		PCO ₂ mm Hg		Base excess	
		Before	After	Before	After	Before	After	Before	After
1	123	7.370	7.067	22	14	42	80	-1.0	-13.0
2	131	7.399	6.870	21	11	47	117	+3.8	-15.9
3	139	7.397	7.125	23	14	46	58.5	+3.0	-12.1

Fig. 2. Vasopressin concentrations in fetal plasma and amniotic fluid at times before, during (shaded bar), and after 30-min partial occlusion of the umbilical cord.

investigators presumed that hypoxia was a major stimulus to vasopressin release. The validity of this presumption has been clearly demonstrated in the fetal lamb (2, 9, 10, 24, 28, 33) where hypoxia alone or in combination with asphyxia was found to be a potent stimulus to release of vasopressin by the fetus. Increases in fetal plasma vasopressin concentrations were strongly correlated with decreases in PO₂ and pH (28, 33). Other stressful processes including umbilical cord occlusion (9), fetal hemorrhage (1, 25), decreased uterine perfusion (10), and fetal hypotension (26) have equally been associated with increased concentrations of vasopressin in fetal plasma. We found both spontaneous and prematurely induced labor to be potent stimuli to fetal vasopressin secretion (31, 32) levels rising only after the onset of labor. Finally, we have recently demonstrated that during and after hypoxia renal excretion of vasopressin increases 10- to 20-fold (8). Thus, existing data strongly suggest that measurement of amniotic fluid vasopressin concentrations would serve as an index of fetal hypoxia.

In the present study, we found that concentrations of vasopressin in amniotic fluid of sheep rose following fetal hypoxia (Fig. 1). The time course of change in concentrations of vasopressin in fetal plasma and delayed accumulation in amniotic fluid follows a pattern expected of a hormone released into the fetal plasma and excreted with fetal urine into the amniotic cavity (Fig. 5). It is noteworthy that although the serum of

Fig. 3. Vasopressin concentrations in amniotic fluid before and after a bolus injection of synthetic vasopressin into the amniotic sac. Dosages are indicated on the right.

pregnant woman was found to contain increasingly large amounts of vasopressinase activity as gestation advances, umbilical cord plasma and amniotic fluid have several orders of magnitude less activity (27). Vasopressinase activity, a measure of the degradation of vasopressin, is not detectable in the plasma or amniotic fluid of sheep (27). In the absence of intrinsic mechanisms for enzymatic degradation, we found that increased concentrations of vasopressin in sheep amniotic fluid persist for at least 24 h. Thus, we have data confirming the validity of the measurement of vasopressin as an index of fetal hypoxia in sheep. Further, studies are in progress to confirm the utility of this measurement and that of other products of the hypothalamo-hypophyseal system in human pregnancy.

The potential pathogenic consequences to the fetus of vasopressin released as a result of hypoxia have been examined from several points of view. With data derived from the umbilical cord plasma of human fetuses, DeVane and Porter (11) have described markedly increased concentrations of vasopressin following intrauterine bradycardia and meconium passage *in utero*. These data led them to conclude that passage of meconium *in utero* might be mediated by vasopressin released by the asphyxiated fetus. In subsequent studies with the fetal lamb, they report meconium passage into the amniotic sac during profound asphyxial stress but not with milder forms of oxygen deprivation (10). In the present experiments, no consistent pattern of meconium passage was observed with moderate fetal hypoxia induced by exposure of the ewe to 10% O₂. More severe hypoxia and asphyxia achieved by umbilical cord occlusion was associated with meconium passage (Table 3). However, if the *in utero* passage of meconium were mediated by an increase in circulating vasopressin, as has recently been suggested by Wiriathian *et al.* (37), then passage of meconium might be expected with intravenous infusion of vasopressin to the fetus. We found no such association. Despite 10- to 100-fold increases in fetal plasma vasopressin, levels comparable to those attained with umbilical cord occlusion, amniotic fluid remained free of meconium, unless meconium were present prior to the study (Fig. 3 and Table 1). These discrepancies may be related to the consistency of sampling following hypoxia or to differences in the gestational age of the animals. Nevertheless, these observations support the long held clinical conviction of obstetricians that meconium

Fig. 4. Representative changes in intra-amniotic pressure (mm Hg) in 15-min epochs before, during, and after intravenous vasopressin infusion ($0.5 \text{ mU min}^{-1} \times 30 \text{ min}$) to a fetal lamb on day 128 of gestation. Arrows mark the time of sampling and concentration of amniotic fluid vasopressin.

Fig. 5. Rationale for measurement of hypothalamo-hypophyseal hormones in fetal plasma at birth and amniotic fluid during gestation.

passage is an indication of fetal distress, but deny a role for vasopressin in the mechanisms leading to meconium passage.

Two mechanisms have been suggested by which fetal vasopressin may exert an effect on the initiation or maintenance of parturition. First, the activity of corticotropic peptides released from fetal pituitary is thought to be of central importance in the triggering mechanism of parturition in sheep; yet the definition of the specific tropic factor or factors remains controversial as does the importance of maturation of fetal adrenal sensitivity to tropic stimulation (4). Nonetheless, vasopressin has recently been shown to potentiate the activity of corticotropin-releasing factor with respect to adrenocorticotropin release (35). Thus, enhanced

vasopressin release by the hypoxic fetus has the potential to function in an ancillary role in the cascade of events leading to labor. Adding further credence to the possibility is our recent demonstration that not only plasma but also cerebrospinal fluid concentration of vasopressin is increased by fetal hypoxia (34). The transient increases in fetal plasma vasopressin concentrations achieved by hypoxic stimulation or intra-venous infusion in the present study had no demonstrable effect on uterine activity as measured by the absence of induced preterm parturition. What effect protracted stimulation of vasopressin secretion might have remains to be determined.

Alternately, vasopressin might stimulate uterine contractility indirectly. Vasopressin has the capacity to enhance the synthesis of prostaglandins (22) and prostaglandin generation in the amniotic membranes and decidua is thought to be the final common pathway for initiation of labor (15). The present experiments have defined a means by which fetal vasopressin can gain access to the fetal membranes. However, even 100-fold increases in amniotic fluid vasopressin concentration sustained for at least 24 h had no demonstrable effect on myometrial activity as measured by intra-amniotic pressure.

Finally, there have been few experiments examining the humoral factors influencing amniotic fluid volume and composition. Work by ourselves and others suggests that the fetal neurohypophysis participates in the maintenance of fetal osmolar and volume homeostasis through action on the fetal kidney and urinary concentration mechanisms (7, 36), thereby affecting amniotic fluid dynamics. Furthermore, vasopressin has been shown to have a direct effect on water metabolism of the amniotic membranes (16). Data from the present study demonstrate the release of significant amounts of biologically active vasopressin into amniotic fluid following fetal hypoxia. The humoral mechanism regulating amniotic fluid volume and composition may include a role for vasopressin and may be altered by fetal hypoxia.

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