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, in turn, plasma concentrations of the hormone correlate inversely with fetal oxygenation. Because 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. We therefore 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 111–141 d 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. After exposure of the ewe to 10% O₂ or partial occlusion of the umbilical cord, vasopressin concentrations in fetal plasma increased 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. After intraamniotic injection of vasopressin, levels remained increased for at least 24 h.

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 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 intra-uterine 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 before the onset of labor. These episodes may not be reflected in the condition of the infant at birth, but could still lead to severe handicapped conditions. We have therefore endeavored to develop techniques for antipartum fetal surveillance.

Data from both clinical 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. DeVane *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 95–105 d of gestation, defined by day of mounting, were acclimatized to the animal care facility and feed for at least 1 wk before surgery. At 102–119 d of gestation the animals were given spinal anesthesia with 10 mg tetracain. 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 or major vessels. Polyvinyl catheters were placed in the fetal carotid artery and jugular vein. Two polyvinyl catheters [0.9 (internal diameter) $\times$ 1.4 mm (outer diameter)], 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 assure 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 U) 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 d 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 d postoperatively. When more than one experiment was done

on an animal, an interval of at least 6 d was allowed between studies. Before assignment to an experimental protocol it was ascertained that fetal pH and blood gas tensions were within the normal range for our laboratory (pHa 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 d (normal gestation in these animals is 148 d) and birth weight 3.8 ± 0.2 kg. Four fetuses died during labor or unattended delivery at 144 ± 1.4 d. Two of these had umbilical cord occluders. Three fetuses died *in utero* at 131 ± 2.5 d, the deaths occurring 2, 5, and 6 d 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, PR) 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 to acclimatize for at least 2 h to the study facility before 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 6–8 L·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 with 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, 1–10 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 10–20% reduction in fetal heart rate was obtained and that degree of occlusion maintained for 30 min (9). The number of studies and gestational age of the animals is summarized in Table 1.

Fetal arterial blood samples were drawn at 30 and 0 min before and 30, 60, 90, 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, and 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 obtained. It was only with animals greater than 135 d 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 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 and micro-electrodes. 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 per tube 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 $98 \pm 3\%$.

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 per tube. 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 after 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 square 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 percentage variation were also employed.

RESULTS

Resting condition. In 13 animals 59 matched samples of amniotic fluid and fetal plasma were obtained at 115–147 d of gestation. These samples were taken at least 5 d after any study

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

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

protocol and animals were considered to be in a stable condition as defined by fetal pH, 7.381 ± 0.005 ; PCO_2 , 41.5 ± 0.5 mm Hg; and PO_2 , 22 ± 0.9 mm Hg. The mean amniotic fluid vasopressin of 1.6 ± 0.3 pg·ml⁻¹ (range, 0.5–7.5) did not differ significantly from fetal plasma vasopressin of 1.8 ± 0.2 (range, 0.5–6.0) or maternal plasma vasopressin of 1.4 ± 0.4 (range, 0.5–4.3). 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 ± 0.04 , did not vary significantly with gestational maturation from 115–141 d 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% O_2 . In these fetuses, mean PO_2 decreased significantly from 22.6 ± 0.7 to 13.2 ± 0.6 mm Hg during the exposure whereas 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 PO_2 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 ± 0.2 pg·ml⁻¹ (range, 1.0–3.5) were equally stable.

Exposure of the ewe to 10% O_2 in N_2 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 after return to room-air and 24 h later, values were similar to those obtained before exposure (Fig. 1). Maternal plasma vasopressin concentrations increased in only one animal (1.3 – 4.0 pg·ml⁻¹) and the mean concentration during hypoxia (1.8 ± 0.6 pg·ml⁻¹) was not different from that preceding hypoxia (1.5 ± 0.4 pg·ml⁻¹). Amniotic fluid concentrations

of vasopressin increased in the hour after 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 116–122 d ($n = 6$) fetal plasma mean was 124 ± 58 and amniotic fluid mean 13.3 ± 3.8 pg·ml⁻¹, at 125–129 d ($n = 5$) 171 ± 79 and 18.2 ± 8.7 pg·

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 (closed circles), 10% O_2 in N_2 (triangles) and intravenous vasopressin 0.5 mU·min⁻¹ (open circles).

Table 2. Arterial pH, blood gas, and base-deficit of fetal lambs at times before, during and after control (exposure to room-air $\times$ 30 min), hypoxia (exposure to 10% O_2 in N_2 $\times$ 30 min), intravenous infusion of vasopressin to the fetus (0.5 mU·min⁻¹ $\times$ 30 min), and intra-amniotic bolus injection of vasopressin (1 – 10 mU)*

	Before (–30 min)	During (+30 min)	After	
			(+60)	(+180)
Control (room air)				
pH	7.377 ± 0.016	7.370 ± 0.017	7.384 ± 0.019	7.379 ± 0.015
PO_2	23.5 ± 0.5	24.6 ± 0.6	23.9 ± 0.5	24.5 ± 0.6
PCO_2	43.9 ± 1.1	44.6 ± 1.2	41.0 ± 0.7	43.8 ± 1.6
BE	0.9 ± 0.7	-0.2 ± 1.5	-0.2 ± 0.9	0.6 ± 1.3
Hypoxia (10% O_2)				
pH	7.383 ± 0.006	7.352 ± 0.022	$7.331 \pm 0.015^\dagger$	7.361 ± 0.016
PO_2	22.6 ± 0.7	$13.2 \pm 0.6^\ddagger$	24.5 ± 1.4	23.9 ± 0.9
PCO_2	44.3 ± 0.8	41.8 ± 2.6	42.8 ± 1.4	42.1 ± 1.0
BE	0.6 ± 0.6	$-2.2 \pm 1.3^\ddagger$	$-3.2 \pm 1.0^\ddagger$	-1.5 ± 0.9
Intravenous vasopressin				
pH	7.381 ± 0.008	7.379 ± 0.009	7.381 ± 0.010	7.373 ± 0.010
PO_2	22.7 ± 1.3	$25.4 \pm 2.0^\ddagger$	24.4 ± 1.7	24.2 ± 1.1
PCO_2	41.2 ± 0.7	41.0 ± 0.5	41.4 ± 0.9	42.3 ± 1.1
BE	-0.8 ± 0.6	-0.8 ± 0.7	-0.4 ± 0.6	-0.4 ± 0.7
Intraamniotic vasopressin				
pH	7.383 ± 0.007	7.376 ± 0.007	7.373 ± 0.007	7.375 ± 0.008
PO_2	22.6 ± 1.3	20.6 ± 1.8	22.1 ± 1.4	22.6 ± 2.1
PCO_2	44.8 ± 1.5	44.9 ± 1.1	42.4 ± 1.7	42.9 ± 1.3
BE	1.5 ± 0.6	1.2 ± 1.0	0.5 ± 1.2	0.2 ± 0.7

* Significant difference from control

$^\dagger P < 0.05$ and $^\ddagger P < 0.01$.

ml⁻¹ and at 134–141 d ($n = 5$) 345 ± 149 and 21 ± 7.4 pg·ml⁻¹.

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

Partial occlusion of the umbilical cord for 30 min not only caused a decrease from resting values of PO_2 but also a large decrease in pH and base excess and increase in PCO_2 (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 $12.5 \text{ pg} \cdot \text{h}^{-1}$ 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 1–10 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 after exposure of the ewe to 10% O_2 in N_2 . This increase in pressure was associated with maternal tachypnea and bleeding. No sustained episodes of tonic increases in intra-amniotic pressure (uterine contractions) or contractures (18) were recorded during the observation period in any animal. No animal delivered within 3 d 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 hypothalmo-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 (6, 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 PO_2 of umbilical arterial

blood and concentrations of vasopressin to our group (21), most 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 PO_2 and pH (28, 33). Other stressful

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.

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.

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

Gestational age (d)		pH		PO ₂		PCO ₂		Base excess	
		Before	After	Before (mm Hg)	After	Before (mm Hg)	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. 4. Representative changes in intra-amniotic pressure (mm Hg) in 15-min epochs, before, during, and after intravenous vasopressin infusion ($0.5 \text{ mU} \cdot \text{min}^{-1} \times 30 \text{ min}$) to a fetal lamb on d 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.

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 10–20-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 after 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 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 after 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% O_2 . More severe hypoxia and asphyxia achieved by umbilical cord occlusion was associated with meconium passage (Table 3). But, if the *in utero* passage of meconium was 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 10–100-fold increases in fetal plasma vasopressin, levels comparable to those attained with umbilical cord occlusion, amniotic fluid remained free of meconium unless meconium was present before the study (Fig. 3 and Table 1). These discrepancies may be related to the consistency of sampling after hypoxia or to differences in the gestational age of the animals. Nevertheless, these observations support the long-held clinical conviction of obstetricians that meconium 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 corticotrophic 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 trophic factor or factors remains controversial as does the importance of maturation of fetal adrenal sensitivity to trophic stimulation (4). Nonetheless, vasopressin has recently been shown to potentiate the activity of corticotrophin releasing factor with respect to adrenocorticotrophin release (35). Enhanced vasopressin release by the hypoxic fetus, thus, 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 intravenous 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 (3). 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 effecting 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 demonstrates the release of significant amounts of biologically active vasopressin into amniotic fluid following fetal hypoxia. The humor mechanism regulating amniotic fluid volume and composition may include a role for vasopressin and may be altered by fetal hypoxia.

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Ontogeny of Insulin and Glucagon Receptors and the Adenylate Cyclase System in Guinea Pig Liver

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Summary

To gain insight into the mechanisms responsible for the impaired glycogenolytic response to glucagon and the diminished ketogenic capacity of newborn guinea pig, we studied the ontogeny of insulin and glucagon receptors, and the responsiveness of the adenylate cyclase complex to glucagon, PGE₁, NaF, and cholera toxin in liver plasma membrane from fetal (58 d, late gestation, and 65 d, term) and adult guinea pigs. The number of insulin receptors ($\times 10^{-10}$ M/L) was least in 58-d fetus (3.0 ± 0.4 ; mean $\pm$ SEM) and increased 3-fold in 65 d fetus (8.8 ± 0.6 ; $P < 0.01$). In adult guinea pig, both insulin receptor number (6.0 ± 0.7) and average affinity constant ($1.20 \pm 0.08 \times 10^8$ M⁻¹) were significantly lower ($P < 0.01$) compared with 65-d fetus. The number of glucagon receptors remained unchanged between 58-d and 65-d fetuses, but both average and high affinity association constants were significantly higher at d 65. In contrast to the lower capacity and affinity of insulin receptors in the adult compared with term fetus, the total glucagon receptor number ($\times 10^{-10}$ M/L) in adults (7.2 ± 0.8) was twice that of the 58 d (3.2 ± 0.2) and 65 d (3.2 ± 1.0) fetuses. The average affinity constant ($\times 10^8$ M⁻¹) in adult (3.8 ± 0.2) was, however, significantly lower than the two fetal groups (58 d, 5.0 ± 0.3 ; $P < 0.05$ and 65 d, 8.1 ± 1.0 ; $P < 0.05$). Although the total glucagon receptor number at d 58 was approximately 50% of that present in the adult, glucagon did not stimulate cAMP production above basal; all other stimuli evoked significant increases. By 65 d, the fetal membranes responded to glucagon as well as to all other agents with appropriate cAMP production. In the presence of 0.5 mM guanosine-5'-triphosphate, the cAMP response to prostaglandin E₁ was similar in all three groups. These results demonstrate the following in liver plasma membrane from the guinea pig. 1) At term, insulin receptor numbers are higher than in less mature fetus or in adult. 2) There is a delay in maturation of functional glucagon receptors coupled to the adenylate cyclase complex. 3) Apart from failure of response to glucagon, the adenylate cyclase complex is otherwise functionally mature even at 58 d because it responds to PGE₁ and other agents.

Abbreviations

BSA, bovine serum albumin
G/F, GTP binding protein
LPM, liver plasma membrane
M/L, moles per liter
PGE₁, prostaglandin E₁

A number of peptide hormones including insulin and glucagon are present in fetal mammals at an early gestational age (3, 15, 29, 39, 41). The functional significance of these hormones during maturation of the developing fetus is, however, not adequately understood. There have been several reports regarding the ontogeny and development of insulin receptors in tissues such as liver, lung, heart, or circulating cells such as erythrocytes and monocytes (3, 14, 17, 21, 22, 33); glucagon receptor development in liver and heart has also been reported (4, 5, 26, 27, 42, 46). There seems to be general agreement that at term and in the immediate newborn period, insulin receptor number and/or affinity are greater than in corresponding adult tissues (14, 21, 22, 41). Conversely, glucagon receptor number seems to be lower in fetus and in newborn and increases gradually to reach adult levels during the first few weeks of life (4, 5, 26, 27, 42, 46).

In spite of the general agreement regarding the ontogenic pattern of insulin and glucagon receptors in various studies, there is inconsistency regarding the maturation of the post-receptor axis for these hormones (4, 7, 12, 26, 27, 42, 46). For glucagon, this post-receptor complex involves the adenylate cyclase complex. The maturation of the adenylate cyclase complex and its expression as a stimutable, functional unit and its ability to be coupled to the surface receptors seems to vary with type of tissue, surface receptor, and species (6, 7, 11, 18, 24, 25, 43, 44, 46). During fetal and neonatal development of lung, the adenylate cyclase complex develops in parallel with the catecholamine receptors in most species examined (44, 45); however, maturation of brain adenylate cyclase activity lags behind the appearance of the catecholamine receptors (11, 18). Appropriate cou-