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Vasopressin and Catecholamine Secretion during Metabolic Acidemia in the Ovine Fetus[1]

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ABSTRACT. It has been suggested that the substantial rise in fetal plasma arginine vasopressin (AVP) during intrauterine hypoxia/asphyxia reflects decreases in PaO_2 and/or pHa; however, the components of these "stresses," *i.e.* PO_2, PCO_2, and pH, have not been controlled. Recently, only modest increases in fetal AVP secretion were seen during hypoxia independent of changes in pH and PCO_2. Since the independent effects of metabolic acidosis on fetal AVP secretion are unknown, we induced acute metabolic acidemia in fetal sheep at 137 ± 4 (mean ± SD) days gestation with 1 M NH_4Cl, while monitoring mean arterial pressure, heart rate, PaO_2, $PaCO_2$, pHa, plasma osmolality, and blood concentrations of electrolytes, AVP, dopamine, norepinephrine, and epinephrine. Mean arterial pressure, PaO_2, $PaCO_2$, and plasma osmolality and sodium were unchanged; pHa decreased from 7.37 ± 0.01 to 7.04 ± 0.05 ($p < 0.05$) during NH_4Cl and did not return to control levels until 24 h later. AVP increased from 2.85 ± 0.23 to 5.26 ± 1.11 μU/ml ($p < 0.05$) at the time of maximum acidosis, correlating with the fall in pHa ($r = -0.67$, $p = 0.001$); however, after stopping NH_4Cl, AVP returned to baseline levels although pHa remained <7.15. In control studies using the same osmolar load, volume, and rate of infusion, AVP levels were unchanged. Only epinephrine was significantly ($p < 0.05$) elevated during acidosis, but did not correlate with pHa or plasma AVP. Marked metabolic acidemia appears to have little or no effect on fetal AVP secretion, and fetal catecholamine secretion is variable. (*Pediatr Res* 21: 38–43, 1987)

Abbreviations

AVP, arginine vasopressin
DA, dopamine
NE, norepinephrine
E, epinephrine
MAP, mean arterial pressure
HR, heart rate
ANOVA, analysis of variance

Elevated plasma concentrations of AVP have been observed in adult animals during various forms of "stress" (1). Similarly, increased AVP levels have been reported in the umbilical cord plasma of human infants (2–5) and shown to reflect not only the route and "stresses" of delivery, but also the occurrence of additional intrauterine "stresses" (2, 5, 6). Furthermore, elevated plasma levels of AVP are seen in fetal sheep during hemorrhage (7–9) and hypoxia/asphyxia (10–14). Thus it appears that, as in the adult, AVP may be considered a "stress" hormone in the fetus.

Whereas the stimulus for fetal hypersecretion of AVP during hemorrhage is clear, *i.e.* hypovolemia and baroreceptor mechanisms (9), the mechanisms(s) responsible for fetal AVP release during hypoxia/asphyxia is less clear. For example, although it has been suggested that during hypoxic insults (11, 13, 15) and parturition (14) the fall in PO_2 is responsible for the elevated plasma levels of AVP, neither pH nor PCO_2 were controlled. Recently, we (12) reported that during mild to moderate fetal hypoxemia (a fall in PaO_2 from 20 to 11 mm Hg) in the absence of alterations in either pHa or $PaCO_2$, plasma AVP levels rose from 2 to 10 μU/ml (2.5 pg/ml = 1.0 μU/ml) in contrast to the substantially greater increases reported by others and the nearly 200-fold rise observed during asphyxia, *i.e.* in the presence of hypoxemia, hypercapnia and acidosis (12). Moreover, we observed that maximum AVP levels were not reached until 30 min after the induction of hypoxemia, whereas maximum cardiovascular responses were seen within 5 min. From these observations we hypothesized that hypoxemia, as an independent variable, acts only as a modest stimulant of fetal AVP release, and thus, might not account for the substantially greater increases in AVP previously reported, that AVP might not be the primary hormonal response to hypoxemia, and finally, that the rise in plasma AVP could not account for the immediate cardiovascular changes that were seen. Moreover, it appeared that the role of PCO_2 and pH in fetal AVP release remained to be determined.

Catecholamines also are released during various "stress" states (16), *e.g.* plasma E concentrations increase 20-fold during the first 5 min after induction of hypoxemia in fetal sheep (17). However, in contrast to that seen with AVP, the rise in plasma catecholamines appears to coincide with the cardiovascular alterations (17). Therefore, an interaction may exist between neurotransmitters, such as catecholamines, and the magnocellular system (1).

Inasmuch as the mechanism(s) responsible for the hypersecretion of AVP and its relationship with catecholamine secretion is unclear, we sought to ascertain in the present investigation the effects of pure metabolic acidemia on fetal secretion of this peptide hormone, and the relationship that might exist between AVP and the changes in plasma levels of DA, NE, and E.

MATERIALS AND METHODS

Animal preparation. Six ewes of mixed Western breed bearing singleton fetuses were used to study the effects of NH_4Cl infusion. Surgery and studies were performed at 126 ± 2.1 (SD) and 137

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± 4.3 days of gestation (term ~145 days), respectively. The surgical procedure for the preparation of the chronically instrumented sheep model has been previously described in detail (18, 19). In brief, after sedation with intravenous ketamine hydrochloride (2 mg/kg), spinal anesthesia was induced with hyperbaric pontocaine hydrochloride (12 mg) and supplemented with intravenous sodium pentobarbital as needed (15 mg/kg). The uterus was exposed through a midline abdominal incision, withdrawn from the abdominal cavity, and wrapped in warm, moist towels. One fetal hindlimb was brought out through a small uterine incision, and polyvinyl catheters were inserted into the femoral artery (the tip lying in the lower aorta) and the femoral vein (the tip lying in the high vena cava). Another catheter was placed in the amniotic sac near the external meatus of the fetal urethra. Ampicillin (250 mg) was instilled in the amniotic cavity. The uterine incision was closed, and the uterus was replaced in the abdominal cavity of the ewe.

Electromagnetic flow probes (Micron Instruments, Los Angeles, CA) of appropriate internal diameter were implanted around both main uterine arteries proximal to their bifurcation, enabling us to measure blood flow continuously during the experimental protocol. All intraabdominal catheters and the flow probe leads were brought out of the abdomen through a stab wound in the fascia, and the abdomen was closed. Through a separate inguinal incision a catheter was inserted into the maternal femoral artery (the tip lying just distal to the aortic bifurcation) and another into the maternal femoral vein (the tip lying just below the diaphragm in the inferior vena cava). All catheters and flow probe leads were brought out to the flank through a subcutaneous tunnel and placed in a canvas pouch that was attached to the skin with steel pins. The animal was then returned to the laboratory and provided feed *ad libitum* (Purina Commercial Creep Chow II, G, St. Louis, MO). The catheters were flushed daily with 0.15 M NaCl containing heparin (250 U/ml) and sealed with sterile metal pins. The ewes were given penicillin G (600,000 U) and streptomycin (0.5 g) on the day of surgery and for the next 2 postoperative days. The fetuses were given ampicillin (50 mg) into the amniotic sac every other day. Each animal was allowed to recover from surgery and anesthesia for at least 6 days before studies were initiated. Fetal sheep with abnormal blood gases or pH were not studied.

Experimental protocol. Ammonium chloride (NH_4Cl) was prepared under aseptic conditions immediately before each experiment. Six animals were used in nine different experiments; all were infused with 1 M NH_4Cl through the fetal femoral venous catheter. The length of infusion was 120 min or a time necessary to reach an arterial pH of ~7.10; two preparations were infused 80 min, one 100 min, and six 120 min. All animals were infused at a rate of 0.382 ml/min, or 0.03 to 0.07 mEq/min·kg; fetal weight at the time of study was estimated at birth or sacrifice by the method of Gresham *et al.* (20). Control experiments were performed in five additional animals by infusing either saline ($n = 4$) or glucose ($n = 5$) in the same concentration, volume, and rate as with NH_4Cl. Animals were allowed to rest a minimum of 3 days between experiments.

MAP in the lower abdominal aorta of the ewe and fetus was continuously monitored using pressure transducers (type 4-327-0109, Bell and Howell, Pasadena, CA) and the output was recorded by means of a two-channel pen-recorder (model 220, Brush Instruments, Div. Clevite Corp., Cleveland, OH). HR was obtained at intervals from a direct recording of the phasic signal from the arterial catheter. Uterine blood flow was monitored continuously with square-wave electromagnetic flowmeters (model RC-1000, Micron Instruments) and recorded on a second two-channel pen-recorder electronically integrated with the former.

Heparinized blood for the measurement of plasma AVP (2.0 ml) and catecholamines (1.0 ml) was obtained from the fetal femoral artery catheter before starting the infusion of NH_4Cl, at 20-min intervals during the infusion, and at 30, 60, 90, 120, 150, 180 min, and 4, 8, and 24 h after the infusion was stopped. Arterial blood was obtained for blood gas analysis at similar times. Maternal blood was obtained from the femoral artery catheter for blood gas analysis before the infusion, at 80 min after the beginning of the infusion, and 1, 4, and 24 h after completing the infusion. Each sample of blood collected for AVP and catecholamines was centrifuged immediately, the plasma removed, the erythrocytes resuspended in a volume of sterile isotonic saline equal to the plasma volume, and reinfused into the fetus in the manner previously reported (19).

Samples of amniotic fluid (2 ml) were collected before starting the NH_4Cl infusion, at 20 and 60 min during the infusion, at the end of the infusion, and 1, 2, 3, 8, and 24 h after stopping the infusion in five experiments.

Assays. Arterial blood samples (0.5 ml from the fetus and 0.8 ml from the ewe) for blood gas and pH determinations were collected in 1.0-ml heparinized glass syringes and kept on ice until analyzed using a pH/gas analyzer (model 113, Instrumentation Laboratory, Lexington, MA). The hematocrit was measured on these samples by the micromethod of Wintrobe. Osmolality of fetal plasma was measured with an automatic osmometer (Osmette A, model 5002, Precision Systems, Sudbury, MA). Fetal plasma concentrations of sodium and potassium were determined by flame photometry using lithium as an internal standard (IL 443 flame photometer).

Blood samples for measurement of AVP were collected in chilled heparinized syringes and immediately transferred to sterile plastic centrifuge tubes. After centrifugation at 10,000 × *g* for 60 s in a Beckman Microfuge, the plasma was removed, and a 1.0-ml aliquot of the plasma was mixed with 2.0 ml of cold acetone (for amniotic fluid, 1.0 ml was mixed with 2.0 ml of cold acetone). The acetone mixture, in which we have found AVP to be stable for at least 4 wk, was immediately placed on dry ice and later stored at −20° C. At the time of assay the plasma-acetone or amniotic fluid-acetone mixture was centrifuged at 3000 × *g* for 2 min to sediment the denatured proteins. The supernatant fluid was removed and extracted with diethyl ether with a modification of the procedure of Robertson *et al.* (21). The aqueous phase was evaporated to dryness under a stream of nitrogen. The residue was suspended in 400 μl of 0.15 M phosphate buffer (pH 7.2), and 50 or 100 μl of the solution were assayed for AVP. AVP in extracts was measured using a radioimmunoassay procedure described by Skowsky *et al.* (22), as modified by Weitzman *et al.* (23). The use of this assay in our laboratory has been described and validated by DeVane and associates (5, 12). The sensitivity is 0.8 μU/ml; the inter- and intra-assay variability are 7.9 and 8.9%, respectively. The mean recovery of AVP from sheep plasma was 65–70%. The data presented have not been corrected for recovery.

Blood samples for measurement of catecholamines were centrifuged at 10,000 × *g* for 1 min and the fetal plasma mixed with an equal volume of 0.6 M perchloric acid containing 0.1% EDTA. The supernate was stored at −20° C until the time of assay for measurement of DA, NE, and E. The catecholamines were quantified using the method of Ben-Jonathan and Porter (24). The assay is linear from 0 to 5 μg for each catecholamine and has a sensitivity of 10–30 pg.

Data analysis. Repeated measures ANOVA was used to analyze changes over time; where significance was observed ($p < 0.05$), this was further analyzed by Duncan's multiple range test for each variable and/or Student's paired *t* test. Regressions lines were obtained by the least-squares method. Data are presented as the mean and 1 SE unless otherwise specified.

RESULTS

Baseline data for fetal and maternal arterial blood gases, hematocrit, HR, MAP, and uterine blood flow are presented in Table 1. Maternal heart rate, MAP, uterine blood flow, and arterial blood gases were unchanged during the fetal infusion of NH_4Cl.

Table 1. *Baseline maternal and fetal arterial blood gases and hemodynamic variables (mean ± SE)*

	Maternal	Fetal
Arterial blood gases		
PO_2 (mm Hg)	103 ± 2.2	18.5 ± 0.5
PCO_2 (mm Hg)	29 ± 0.7	44.8 ± 2.0
pH	7.50 ± 0.02	7.37 ± 0.01
Base deficit	+1.6 ± 1.3	+0.1 ± 1.2
Hematocrit	28 ± 1.5	37 ± 2.4
Heart rate (beat/min)	105 ± 7.4	154 ± 5.8
Mean arterial pressure (mm Hg)	84 ± 2.3	51 ± 1.9
Total uterine blood flow (ml/min)	761 ± 86.0	

As illustrated in Figure 1, the fetal arterial pH significantly decreased during the infusion of NH_4Cl, gradually falling to 7.04 ± 0.05 by 120 min ($p < 0.05$). After the NH_4Cl infusion, pHa rose quite slowly, reaching a value of only 7.24 ± 0.03 by 8 h postinfusion; at 24 h postinfusion, the pHa was not significantly different from control. The fall in pHa at 120 min was associated with a base deficit of −19 ± 2.1 ($p < 0.05$). Although fetal PaO_2 rose during the development of acidemia (Figure 2) and remained ~12% above control values during the NH_4Cl infusion, this increase did not achieve statistical significance. However, this rise in PaO_2 correlated, although weakly, with the fall in pHa ($r = -0.294$, $n = 45$, $p < 0.05$). Neither the fetal $PaCO_2$ nor the hematocrit were significantly changed during and after the NH_4Cl infusion.

Serial measurements of fetal plasma osmolality were obtained in seven experiments. We did not observe any change in plasma osmolality over the entire experimental protocol; values before and at 120 min of the NH_4Cl infusion were 269 ± 5 and 272 ± 4 mosmol/kg H_2O, respectively.

Although the plasma concentration of sodium did not change, the mean plasma potassium concentration increased from 3.45 ± 0.14 to 4.24 ± 0.14 mEq/liter ($p < 0.05$) by the end of the infusion and gradually decreased thereafter, reaching a value of 3.69 ± 0.23 mEq/liter by 24 h postinfusion.

As illustrated in Figure 1, despite the development of rather marked metabolic acidemia, plasma concentrations of AVP increased gradually from 2.85 ± 0.23 to 5.26 ± 1.11 μU/ml at 120 min. After the NH_4Cl infusion plasma concentrations of AVP fell rapidly even though significant metabolic acidemia persisted, reaching a value of 3.16 ± 0.93 μU/ml within 60 min, a value not different from control ($p > 0.05$). Nevertheless, the relationship between the fall in pHa and rise in plasma AVP during the NH_4Cl infusion was highly significant ($r = -0.671$, $p = 0.001$, $n = 38$). Because the rise in plasma AVP may have been reflective of the osmolality of the infusate (2000 mosmol/kg H_2O), we performed control studies, infusing sodium chloride ($n = 4$) or glucose ($n = 5$) at the same rate, volume, and molar concentrations. In these studies, plasma concentrations of AVP were unchanged with both infusates: at control, AVP was 1.61 ± 0.16 and 1.49 ± 0.17 μU/ml for sodium chloride and glucose, respectively, and at 120 min of infusion values were 1.99 ± 0.51 and 1.42 ± 0.12 μU/ml ($p > 0.1$), respectively. As with NH_4Cl, there was no significant change in plasma osmolality.

The patterns of change in plasma concentrations of catecholamines before, during, and after the infusion of NH_4Cl are presented in Figure 3. Although plasma concentrations of DA, NE, and E gradually rose during the development of metabolic acidosis, these changes were not statistically significant (ANOVA) because of the large variability in the response of different animals. When this variability was taken into account by log transformation of the plasma concentrations, significant increases in only E were observed at 60, 80, and 120 min after beginning the NH_4Cl infusion. There were no significant correlations between the changes in pHa or plasma levels of AVP with either DA, NE, or E during the NH_4Cl infusion.

The fetal cardiovascular responses to metabolic acidemia are

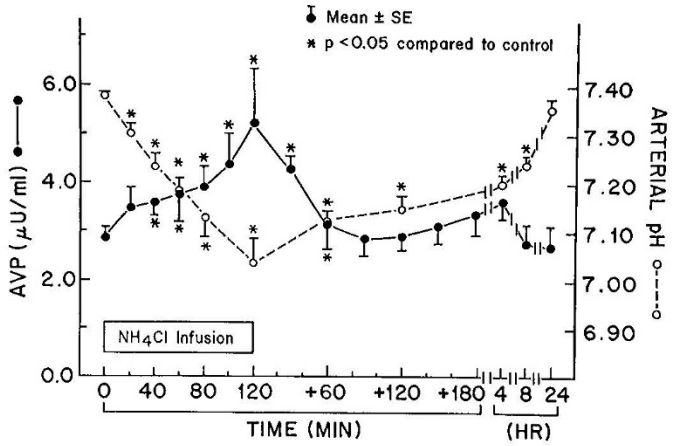

Fig. 1. The effect of NH_4Cl infusion (120 min) on fetal arterial pH and plasma concentrations of AVP.

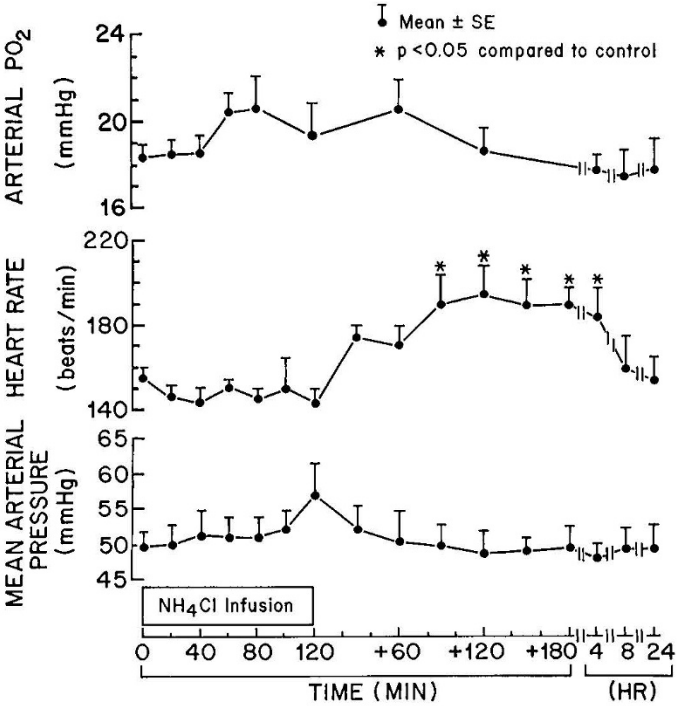

Fig. 2. The effect of NH_4Cl infusion (120 min) on fetal arterial PO_2, HR, and MAP.

shown in Figure 2. Fetal MAP was not significantly changed during or after the NH_4Cl infusion. There was a slight fall in fetal HR of approximately 10 beats/min during the NH_4Cl infusion; however, this was not significant. Following the NH_4Cl infusion, a profound increase in HR was observed within 30 min after completion of the infusion, and HR remained significantly elevated until 4 h postinfusion. During this prolonged period of fetal tachycardia, metabolic acidemia persisted, plasma AVP and E concentrations returned to values not different from control, and PaO_2 remained elevated. Although the plasma level of NE was not significantly elevated in the postinfusion period, the mean values remained above the preinfusion mean value. At 8 h postinfusion the HR had fallen to 162 ± 15 beat/min, a value not different from the control.

Amniotic fluid concentrations of AVP and catecholamines were measured serially in five experiments (Table 2). There were no significant changes in the concentrations of these hormones either during or after the NH_4Cl infusion. Furthermore, there was no evidence of meconium release by the fetus into the

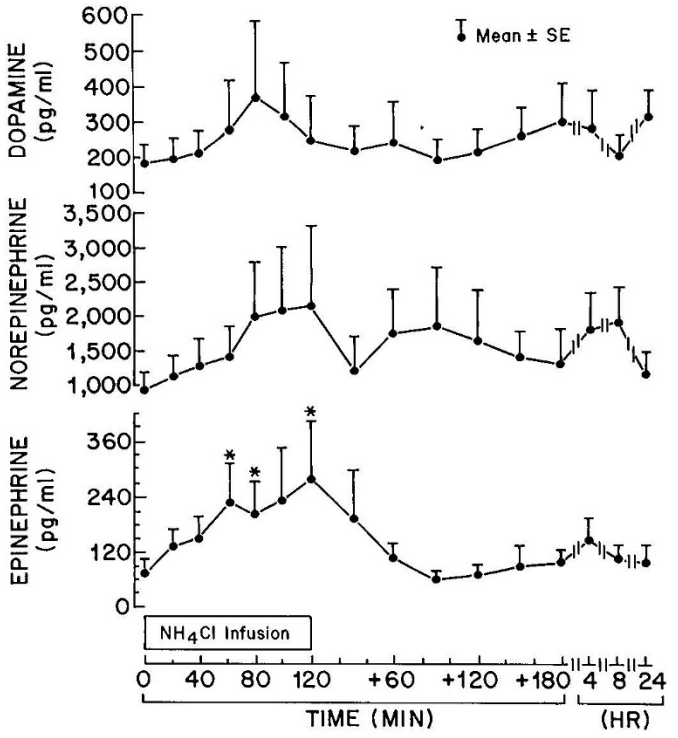

Fig. 3. The effects of NH_4Cl-induced acidemia on plasma concentrations of DA, NE, and E. Methods of statistical analysis are described in the text.

Table 2. *Amniotic fluid concentrations of AVP, DA, NE, and E before, during, and after the infusion of NH_4Cl into fetal sheep (n = 5) (mean ± SE)*

Time (min)	AVP (μU/ml)	DA (pg/ml)	NE (pg/ml)	E (pg/ml)
0	3.30 ± 0.61	498 ± 155	392 ± 165	77 ± 27
20	4.61 ± 0.77	329 ± 135	376 ± 199	28 ± 10
60	3.11 ± 0.71	352 ± 170	403 ± 173	33 ± 6
+60	3.24 ± 0.36	592 ± 314	522 ± 268	51 ± 19
+120	3.23 ± 0.47	323 ± 67	558 ± 181	35 ± 6
+180	3.56 ± 0.22	489 ± 195	2238 ± 1985	45 ± 15
+240	3.40 ± 0.26	828 ± 294	1251 ± 856	62 ± 33
+480	3.73 ± 0.55	943 ± 337	568 ± 307	39 ± 21
+1440	4.16 ± 0.67	675 ± 248	418 ± 154	37 ± 12

amniotic sac either during or after infusion of NH_4Cl. This was not unexpected, since plasma AVP values did not exceed 9 μU/ml and is consistent with our prior observations (25).

DISCUSSION

The fetus responds to various forms of intrauterine stimuli by releasing AVP from the neurosecretory granules of nerve endings located in the posterior pituitary gland as early as the middle third of pregnancy (23). Among the numerous nonosmotic stimuli that result in increased fetal AVP secretion are hemorrhage (7–9), parturition (3, 4, 14), vaginal delivery (2, 5), and episodes of hypoxemia/asphyxia (10–14, 26). Whereas these nonosmotic stimuli have been examined extensively in studies of fetal AVP secretion, the mechanism(s) actually responsible for the observed rise in neurohypophyseal AVP secretion is unknown. For example, during parturition it remains unclear if the stimulus for increased AVP secretion is reflective of the variable degrees of hypoxia, hypercapnia, and acidosis, the occurrence of physical phenomenon such as head compression, or the interaction between the numerous hormones secreted during parturition. Furthermore, since different methods have been used to alter fetal oxygenation, *e.g.* reduced maternal inspired oxygen (15) and cord compression (11), reported concentrations of fetal plasma AVP have differed substantially, thereby making it difficult to ascertain whether the fall in fetal PO_2 or pH or the rise in PCO_2 is primarily responsible for the observed increases in plasma AVP, or whether this reflects changes in cardiac return due to cord compression. We further demonstrated the complexity of this problem when we observed a 200-fold rise in plasma AVP levels during the simultaneous occurrence of hypoxemia, metabolic acidosis, and hypercapnia (*i.e.* asphyxia), but only a modest increase in the presence of hypoxemia (arterial oxygen tensions as low as 9 mm Hg) independent of changes in either pHa or $PaCO_2$ (12). Since this was suggestive that hypoxemia alone had only modest effects on fetal neurohypophyseal release of AVP, and since the effects of metabolic acidosis alone were not known, we sought to ascertain in the present studies the role of metabolic acidemia in the release of this peptide hormone. Furthermore, since the magnocellular system undergoes important developmental changes during the perinatal period in several species (27), it seemed reasonable that similar maturational changes might occur in fetal sheep. Therefore, we have studied a small window of development, 130 to 140 days, in order to reduce the potential significance of this and other maturational variables.

In the present studies, the NH_4Cl-induced metabolic acidemia had only a very slight effect on fetal AVP release, a 2-fold rise, when compared to that seen in animals with either hypoxemia (4-fold) or asphyxia (~200-fold) studied at a similar time in gestation (12). Although the NH_4Cl solution infused was of relatively high osmolality, 2000 mosmol/kg H_2O, we were able to demonstrate in control studies that neither the osmolar load nor the infused volume were responsible for the increase in plasma AVP levels. Furthermore, in none of these studies did we observe a change in plasma osmolality, sodium concentration, or hematocrit during the infusion of any of these substances. Thus, it is more likely that the rise in plasma AVP concentration may have resulted from the fall in arterial pH or was a direct effect of NH_4Cl on the magnocellular system and/or the posterior pituitary gland. Of interest, there was a significant rise and fall in the plasma levels of potassium that were mirror images of the changes in arterial pH. It is likely that this is reflective of the shift of potassium from the intracellular to the extracellular pools due to the severity of the metabolic acidemia. However, it is not known if changes in intracellular potassium have any effect on the fetal release of AVP.

Although the rise in plasma concentrations of AVP correlated with the decrease in arterial pH and changes in plasma osmolality did not occur or had no apparent effect, the levels of plasma AVP fell immediately after stopping the NH_4Cl infusion, returning to preinfusion values at a time when significant metabolic acidemia persisted, *i.e.* a pHa of 7.12 ± 0.03. Moreover, although the acidosis persisted for up to 24 h, plasma AVP concentrations remained at preinfusion levels. This would infer that either the threshold for AVP release by metabolic acidosis is around pH 7.00 or that the relationship of plasma AVP to arterial pH is superfluous. It is unlikely to be the former since plasma AVP concentrations gradually rose during the NH_4Cl infusions, with significant increases occurring when the pHa was ~7.20 (Fig. 1), and there was a positive correlation between the rise in AVP and the fall in pHa. Thus, it appears that the increase in fetal AVP secretion is probably reflective of the exposure of the fetus to NH_4Cl and that metabolic acidosis, as induced in the present paradigm, actually had little or no effect. Our results are in sharp contrast with those of Wang *et al.* (28) who observed in anesthetized adult dogs a 20-fold rise in plasma AVP concentrations following hydrochloric acid infusions that decreased the arterial pH from 7.38 to 7.05. Nevertheless, since these changes in arterial pH were comparable to those obtained during induced hypercapnia, where AVP release was considered mild (~3-fold), these investigators suggested that factors other than the simple increase

in the concentration of hydrogen ion in blood influenced the magnitude of the increase in plasma AVP seen with HCl (28). Our findings in unanesthetized fetal sheep support this hypothesis. However, the differences in AVP secretion in the adult and fetus could also reflect developmental (or species) differences, suggesting that the adult animal may be more vulnerable to metabolic acidosis.

As observed by Sklar and Schrier (1), a pitfall in the study of AVP release is the use of peripheral routes to administer agents, *i.e.* the agent, even if capable of crossing the blood-brain barrier, might exert its effect on AVP release via some alteration in the cardiovascular system and/or baroreceptor activity rather than by any direct central action. In our studies of fetal sheep there is nothing to suggest a peripheral effect through alterations in either baroreceptor or chemoreceptor activity, or changes in the cardiovascular system. There was no change in arterial PCO_2, and PO_2 was observed to rise. This tendency for arterial PO_2 to increase is probably reflective of the effects of the metabolic acidemia on the oxygen dissociation curve, *i.e.* the Bohr effect (29).

Although levels of plasma catecholamines tended to rise during the infusion of NH_4Cl, we found no statistically significant increase in either D or NE. However, plasma concentrations of E did increase significantly near the end of the NH_4Cl infusions. Moreover, there were no significant correlations between the fall in fetal arterial pH and the rise in plasma concentrations of these catecholamines. Thus, it appears that, as with AVP, pure metabolic acidosis induced by NH_4Cl is not a potent stimulus to the release of catecholamines from fetal chromaffin tissue in near-term fetal sheep and that the response of the fetal sheep is quite variable. This contrasts sharply with that seen in fetal sheep during significant hypoxemic-asphyxic episodes (17). However, as recently discussed (30), measurements of plasma catecholamine concentrations have many shortcomings. There are not only rapid changes in influx, efflux, or both, but also potential differences which are dependent upon the sampling site. In our study, the descending aorta was sampled, permitting us to compare our results with those of Cohen *et al.* (17), but not with Lewis *et al.* (31) or Jones and Robinson (32), who obtained their blood samples from the fetal carotid artery. We did not address the balance between the influx and efflux of these hormones; thus, the high variability observed in this study and that of others (17, 31, 32) may represent the important variability between the secretion and metabolism of these neurohormones. We also were unable to discern any clear relationship between the release of catecholamines in the fetus and the release of AVP in response to metabolic acidemia. Thus, the potential of this important relationship remains to be determined.

The delayed, yet consistent increase in fetal HR seen from 90 min to 4 h following the NH_4Cl infusion is strikingly similar to the delay of NH_4Cl-induced increases in fetal breathing previously reported by Molteni *et al.* (33). In those studies, vigorous fetal respiratory activity was consistently observed 60 to 90 min after completion of the infusion of NH_4Cl. They suggested that the delay was an effect mediated by central pH receptors. They studied fetal sheep at the same period of gestation as we did, infused NH_4Cl over 2–3 h at the same dose, and observed the arterial pH to decrease to a mean of 6.82, a value similar to the 7.03 reported herein. We did not measure the fetal breathing pattern or the cerebrospinal fluid pH; however, because of the similarity of our findings and the experimental paradigm with that of Molteni *et al.* (33), we suggest that there may be a relationship between the induction of fetal breathing and the increase in fetal HR that follows NH_4Cl-induced metabolic acidemia. The possibility of a central chemoreceptor component is of interest and warrants further investigation, especially since this could be important in the pathogenesis of intrauterine aspiration of meconium (25).

Thus, it appears from the results of our studies that metabolic acidosis independent from changes in PCO_2 and PO_2 has at best only a mild effect on the fetal secretion of AVP. Furthermore, it is possible that there is actually no direct effect of the rise in hydrogen ion concentration since plasma AVP concentrations were observed to fall to levels not different from control by 60 min after the NH_4Cl infusion, while arterial pH remained substantially depressed. This, however, does not negate the possibility of important interactions between changes in pH and PO_2 in the fetal release of AVP.

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