Effects of Acute Hypercapnia on Maternal and Fetal Vasopressin and Catecholamine Release¹

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ABSTRACT. Although fetal asphyxia, *i.e.* hypoxemia, acidosis, and hypercapnia, increases plasma arginine vasopressin (AVP) >40-fold, hypoxemia and metabolic acidosis occurring independently cause only 5-fold and 2-fold increases, respectively. To determine the effects of hypercapnia on AVP release, we examined the effects of acute hypercapnia on AVP secretion in six pregnant sheep and their fetuses at 135 ± 4 d ($x \pm$ SD), exposing the ewe successively to room air, 30% O₂, 30% O₂ plus 10% CO₂, 30% O₂, and room air, and monitoring uterine blood flow, as well as maternal and fetal mean arterial pressure, heart rate, arterial blood gases, and plasma AVP and catecholamines. Oxygen exposure had no effect on the ewe or fetus. During O₂ plus CO₂ exposure, the ewes and fetuses developed hypercapnia in the absence of hypoxia, arterial CO₂ tension increasing to 8.38 ± 0.87 kPa (62.9 ± 6.5 mm Hg) and 10.0 ± 0.61 kPa (75.2 ± 4.6 mm Hg) ($p < 0.001$), respectively, at 30 min of exposure. Although fetal heart rate and mean arterial pressure were unchanged, maternal values rose 61 and 30% ($p < 0.001$), respectively. At 30 min of O₂ + CO₂ exposure, maternal norepinephrine increased from 2.23 ± 0.74 to 8.52 ± 3.97 nmol/L ($p = 0.15$) and fetal epinephrine increased from 0.27 ± 0.10 to 2.271 ± 0.90 nmol/L ($p = 0.01$); plasma AVP was not significantly increased in the ewe or fetus, although levels rose from ~ 45 to 127 ± 48 and 137 ± 64 pmol/L ($p = 0.10$), respectively. Hypercapnia alone is not a major determinant of AVP secretion in the mother or fetus; thus, the marked rise in fetal AVP secretion observed during asphyxial insults may reflect interaction between the effects of hypercapnia and hypoxemia. (*Pediatr Res* 30: 368–374, 1991)

Abbreviations

MAP, mean arterial pressure
E, epinephrine
NE, norepinephrine
PaO₂, arterial O₂ tension
PaCO₂, arterial CO₂ tension
ANOVA, analysis of variance
pH_a, arterial pH

Alterations in the homeostatic milieu trigger a complex series of events that serve to protect the individual organism against environmental insults. Asphyxia, *i.e.* the simultaneous occurrence of hypoxemia, acidosis, and hypercapnia, is a striking illustration of an intense "stress," and subsequent adaptive responses reflect a complex interaction of factors related to the metabolic, neuroendocrine, and cardiovascular systems. Although these responses have been examined in adult animals of several species (1–6), the ontogeny of these responses during fetal development is not fully understood.

In fetal sheep the response to stress has been characterized by increased secretion of AVP and adrenal catecholamines (7). By using the fetal sheep as a model, we and others (5, 8–12) have observed that fetal asphyxia, regardless of method of induction, results in substantial increases in plasma AVP concentrations, *i.e.* >40-fold rises over baseline levels. In contrast, we have shown (11) that during moderate fetal hypoxemia, *i.e.* a 45% decrease in PaO₂ without alterations in either arterial pH or PCO₂, plasma levels of AVP rose only modestly (5-fold over baseline values). Furthermore, induction of marked metabolic acidemia had little effect on fetal AVP secretion, *i.e.* only a 2-fold rise over baseline levels (13). Because neither hypoxemia alone nor pure metabolic acidosis resulted in the substantial increase in plasma AVP seen during fetal asphyxia, the role of hypercapnia in fetal secretion of AVP, as well as the interaction of these variables, remained to be elucidated.

Catecholamines are also released during asphyxia. Rose *et al.* (14) demonstrated that acute hypercapnic acidosis resulted in increased circulating levels of both E and NE in adult dogs. However, NE was the predominant catecholamine released, especially when hypercapnia was combined with hypoxemia. They suggested that the major source of NE was the sympathetic nerve terminals. They also observed an increase in MAP and heart rate in response to hypercapnia. No one, to our knowledge, has addressed the effects of acute hypercapnia on circulating catecholamines in a fetal animal. Moreover, the relationship between plasma levels of these hormones and cardiovascular responses during hypercapnia has not been examined in the fetus.

Therefore, the purpose of the study presented here was to determine the effects of acute hypercapnia on fetal secretion of AVP, dopamine, NE, and E, to examine the concomitant fetal hemodynamic responses, and to compare these alterations with those occurring simultaneously in the pregnant ewe.

MATERIALS AND METHODS

Animal preparation. Pregnant sheep ($n = 6$) of mixed Western breed bearing singleton fetuses were used in these experiments. Studies ($n = 8$) were performed at 135 ± 4 ($x \pm$ SD) d of gestation (term, ~ 145 d). The surgical preparation has been described previously (11, 15). In brief, animals were fasted 24 h before surgery, which was performed at 124 to 126 d of gestation. After spinal anesthesia with hyperbaric lidocaine (12 mg), *i.v.* pentobarbital (~ 15 mg/kg) was administered as needed. While em-

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ploying sterile procedures, the maternal abdomen was opened, the pregnant uterus was delivered, and a fetal hindlimb was brought out through a uterine incision. Polyvinyl catheters were implanted into a fetal femoral artery (7.5 cm) and vein (15 cm). The fetal hindlimb was returned to the amniotic sac, an amniotic catheter was inserted, and the uterus was closed with a double layer of sutures. The uterus was then returned to the maternal abdomen, and electromagnetic flow probes (Micron Instruments Inc., Los Angeles, CA) were implanted around both main uterine arteries proximal to their bifurcation. The fetal catheters and flow probe leads were brought out of the maternal abdomen through a stab wound in the fascia, and the abdominal incision was closed. Polyvinyl catheters were implanted in both maternal femoral arteries (15 cm) and veins (20 to 25 cm). All catheters were filled with heparinized saline, closed with sterile pins, and brought out to the maternal flank with the flow probe leads via a s.c. tunnel, where they were maintained in a canvas pouch attached to the skin with steel pins. Pregnant animals received intramuscular mezlocillin (0.5 g) and penicillin (600 000 U) on the day of surgery and the 2 following days. Mezlocillin (50 mg) was also instilled in the amniotic sac at surgery and every other day thereafter. After surgery, all animals were maintained in individual stalls in the laboratory and had free access to water and food (Purina Commercial Creep Chow II, G; Ralston-Purina Co., St. Louis, MO). Studies were performed at least 5 d post-operatively. Fetal sheep with abnormal blood gases or pH were not studied.

Experimental protocol. Acute hypercapnia was induced in pregnant ewes and their fetuses by sequentially exposing the ewe to the following gas mixtures: room air for 30 min, 30% O₂ for 30 min, 30% O₂ plus 10% CO₂ for 30 min, 30% O₂ for 30 min, and room air for 60 min. To obtain changes in inspired O₂ and CO₂, a plastic bag connected to a gas blender was loosely placed over the head of the ewe at the end of the initial room air period and the appropriate gas mixture was administered at ~10 L/min. As previously reported, this procedure was well tolerated in all animals (11). In preliminary experiments, we observed that some ewes developed acute hypoxemia and metabolic acidosis in conjunction with respiratory acidosis; therefore, to prevent fetal and/or maternal hypoxemia as a confounding variable, we administered 30% O₂ to the ewe, establishing control room air and hyperoxia periods both before and after each exposure to CO₂. This protocol was approved by the Institutional Review Board for Animal Research. Two animals were studied twice and were allowed 2 to 4 d between experiments. There was no gestational age effect noted in these experiments.

MAP in the lower abdominal aorta of the ewe and fetus was continuously monitored with pressure transducers (model 4-327-1019; 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). Intraamniotic pressure was recorded with another recorder electronically integrated with the first; the reported values for MAP are corrected for amniotic pressure. Heart rates were obtained at intervals from a direct recording of the phasic signal. Maternal respiratory rates were also obtained at the same intervals from direct observation. Uterine blood flow was monitored continuously with square-wave electromagnetic flowmeters (model RC1000; Micron Instruments, Los Angeles, CA) and was recorded by means of another two-channel recorder, which also was electronically integrated. Results are presented as total uterine blood flow.

Heparinized blood was obtained from the fetal and maternal femoral artery catheters for measurements of blood gases and pH (0.5 mL), hematocrit (0.2 mL), AVP (2.0 mL), and catecholamines (1.0 mL). Samples were collected at 15 min in room air, at 10 and 20 min in 30% O₂, at 10-min intervals in 30% O₂ plus CO₂, at 15 and 30 min in 30% O₂ after the end of CO₂ exposure, and then at 30 min, 60 min, and 20 h in room air. Additional blood for measurement of AVP also was obtained

7.5 min after the end of CO₂ exposure. Each sample of fetal blood collected for AVP and catecholamine measurements was centrifuged immediately, the plasma was removed, and the erythrocytes were resuspended in a volume of sterile isotonic saline equal to the plasma volume and reinfused into the fetus in the manner previously reported (15). This procedure has been shown to have no effect on AVP secretion (13). Maternal blood was handled similarly, but red cells were not reinfused. Fetal (0.5 mL) and maternal (0.5 mL) plasma osmolalities were measured at each of these times in the first four experiments. Fetal plasma lactate levels (0.4 mL) were determined in two initial experiments.

Samples of amniotic fluid (2 mL) were collected at 15 min in room air, at 20 min in O₂, at 10-min intervals in CO₂ plus O₂, at 15 and 30 min in O₂ after ending CO₂, and at 30 min, 60 min, and 20 h in room air for measurement of AVP.

Assays. Arterial blood samples for blood gases, pH, and hematocrit were collected in 1.0-mL heparinized syringes and were kept on ice until analyzed. The blood gases and pH were measured with a pH/gas analyzer (model 113; Instrumentation Laboratory, Lexington, MA). The hematocrit was measured on these samples by the micro-method of Wintrobe. Plasma osmolality was measured by freezing-point depression with an automatic osmometer. Lactic acid concentrations were determined by using a lactate dehydrogenase enzymatic assay on plasma (200 μ L) after deproteinization with 8% perchloric acid.

Blood samples for measurement of AVP were collected in chilled heparinized syringes and were immediately transferred to sterile plastic centrifuge tubes. After centrifugation at 10 000 $\times$ g for 60 s in a Beckman microfuge, the plasma was removed, frozen immediately on dry ice, and stored at -20°C until the time of assay. Plasma AVP was measured by RIA with rabbit antiserum R-71. The production of antiserum R-71 and the validation of this RIA have been described by Skowsky *et al.* (16). At the time of assay, the samples were thawed and acidified (pH 2.0) by the addition of 0.1 mL of 1 N HCl and AVP was extracted from the plasma by the methods of Crofton *et al.* (17) with C₁₈ Sep Pak cartridges (Waters Associates, Milford, MA). The recovery of AVP is 97 $\pm$ 2.0%. The extract was evaporated to dryness, and the residue was resuspended in 400 μ L of assay buffer. The RIA for AVP in our laboratory has been described (11). The sensitivity of the assay is 18 pmol/L.

Blood samples for measurements of catecholamines were centrifuged at 10 000 $\times$ g for 1 min, and the plasma was mixed with an equal volume of 0.6 M perchloric acid containing 0.1% EDTA. The supernatant was stored at -20°C until the time of assay for measurement of dopamine, NE, and E. Catecholamines were quantified by the radioenzymatic assay of Ben-Jonathan and Porter (18). This assay is linear from 0 to 5 μ g for each catecholamine and has a sensitivity of 10 to 30 pg.

Data analysis. Repeated measures ANOVA was used to analyze changes over time; when significance was observed with an *F* value corresponding with *p* < 0.05, the data were further analyzed by Duncan's multiple range test for each variable and/or paired *t* test. Regression lines were obtained by the least-squares method. These analyses were performed by using the SAS system (SAS Institute Inc., Cary, NC) on a VAX computer. The data are presented as means and one SEM unless otherwise specified.

RESULTS

Respiratory and metabolic alterations. Baseline data for maternal and fetal (Table 1) arterial blood gases, pH, and hematocrit are similar to data previously reported from our laboratory (11, 13, 19). Maternal PaO₂ increased significantly at 10 min of O₂ exposure (Fig. 1) and remained elevated throughout CO₂ exposure, returning to control levels after the animals were allowed to breathe room air. By 10 min of CO₂ inhalation, the ewes developed significant respiratory acidosis [PaCO₂ = 7.06 $\pm$ 1.47

Table 1. Baseline maternal and fetal data ($n = 8$)*

	Maternal	Fetal
Heart rate (beats/min)	94 ± 6.0	157 ± 5.4
MAP (kPa)	10.8 ± 0.32 (81 ± 2.4)	6.13 ± 0.17 (46 ± 1.3)
Respiratory rate (breaths/min)	82 ± 5.9	
Uterine blood flow (mL/min)	1049 ± 226	
Arterial blood gases		
PaO ₂ (kPa)	13.6 ± 0.48 (102 ± 3.6)	2.27 ± 0.12 (17 ± 0.9)
Paco ₂ (kPa)	4.13 ± 0.08 (31 ± 0.6)	5.60 ± 0.15 (42 ± 1.1)
pH	7.44 ± 0.02	7.36 ± 0.02
Hematocrit	27.6 ± 0.8	31.0 ± 0.55

* Values presented are means ± SEM. Values in parentheses are mm Hg.

Fig. 1. Changes in maternal arterial blood gases and pH during successive gas exposures ($n = 8$). Analysis is by ANOVA.

kPa (53 ± 11 mm Hg); $p < 0.001$], which persisted throughout CO₂ exposure; within 15 min after ending CO₂ inhalation, both Paco₂ and pH_a rapidly returned to preexposure levels. At 30 min of CO₂ exposure, animals were breathing slowly (57 ± 1.7 breaths/min) and deeply, demonstrating moderate respiratory distress; however, maternal respiratory rate was not significantly changed until the recovery period, at which time it increased 2- to 3-fold to rates of 125 to 150 breaths/min ($p < 0.001$). Maternal respiratory rate returned to preexposure values within 90 min after ending CO₂ exposure. Finally, maternal hematocrit increased significantly during CO₂ exposure to 0.33 ± 0.1 (33 ± 1.3 %; $p < 0.001$) but quickly returned to basal values during recovery.

Fetal arterial blood gas changes are illustrated in Figure 2. The alterations in fetal arterial blood gases followed a pattern similar to that seen in the ewe. That is, fetal PaO₂ gradually rose during maternal oxygen exposure and was significantly elevated throughout the period of O₂ plus CO₂ exposure. As in the ewe,

Fig. 2. Changes in fetal arterial blood gases and pH during successive gas exposures ($n = 8$). Analysis is by ANOVA.

respiratory acidosis rapidly developed during CO₂ exposure, Paco₂ increasing to 10.0 ± 0.61 kPa (75 ± 4.6 mm Hg) by 30 min of CO₂ ($p < 0.001$) and pH_a decreasing to 7.16 ± 0.04 ($p < 0.001$); values during CO₂ exposure were not different. After the CO₂ was removed, Paco₂ and pH_a rapidly returned to levels not different from control and remained unchanged thereafter. All blood gases were normal 20 h later. Fetal hematocrit was unchanged during and after CO₂ exposure.

To further examine changes in the metabolic milieu, we measured plasma osmolality in maternal and fetal blood in four experiments. There were no alterations in either maternal or fetal plasma osmolality during the successive administration of these gas mixtures. Moreover, fetal plasma lactate was unchanged in two of these experiments, providing additional evidence that only respiratory acidosis had occurred.

Maternal and fetal cardiovascular responses. Control values for maternal and fetal cardiovascular measurements (Table 1) are similar to those previously reported (11, 13, 19). Oxygen exposure had no significant effects on any variable (Figs. 3 and 4). However, during CO₂ exposure, there was significant maternal tachycardia, which gradually resolved after ending CO₂ exposure ($p < 0.05$), returning to baseline values 60 min later. MAP also

Fig. 3. Maternal cardiovascular changes during successive gas exposures ($n = 8$). Analysis is by ANOVA.

Fig. 4. Fetal cardiovascular changes during successive gas exposures ($n = 8$). Analysis is by ANOVA.

increased and remained elevated during hypercapnia but returned to control levels more rapidly than heart rate, decreasing to basal values within 30 min after the exposure to CO_2 was stopped. Despite these maternal hemodynamic responses, there were no significant changes in uterine blood flow in response to hypercapnia.

The fetal cardiovascular responses to hypercapnia are shown in Figure 4. In contrast to that observed in the ewe, fetal heart rate was unchanged during hypercapnia. However, toward the end of CO_2 exposure, heart rate began to rise and, during the recovery period, there was development of significant tachycardia, which persisted during the second period of O_2 exposure, gradually returning to control levels by 20 h. The response in corrected fetal MAP was quite variable (Fig. 4); although there was a 20% increase in fetal MAP during and immediately after CO_2 exposure, this was not statistically significant ($p > 0.05$; ANOVA). Of note, amniotic fluid pressure increased from 0.80 ± 0.33 to 1.47 ± 0.44 kPa (6.0 ± 2.5 to 11 ± 3.3 mm Hg; $p < 0.001$) by 30 min of CO_2 exposure and fell to 1.08 ± 0.33 kPa (8.1 ± 2.5 mm Hg) at 7.5 min postexposure, a value not different from control. This likely reflects the substantial maternal respiratory distress observed and increases in intraabdominal pressure rather than uterine contractions because uteroplacental blood flow was unaffected.

Plasma AVP. Plasma concentrations of maternal and fetal AVP before, during, and after CO_2 exposure are presented in Table 2 and illustrated in Figure 5. Control values are similar to those previously reported by us for ewes or fetal sheep studied at a similar gestational age (13). Despite the presence of moderately severe hypercapnia, there were only modest and, interestingly, parallel alterations in maternal and fetal plasma AVP concentrations (Fig. 5), neither of which reached significance at any time point ($p = 0.1$), even by using a log transformation of the data to account for the variability seen.

Amniotic fluid levels of AVP were 48.5 ± 7.7 pmol/L in the control period and were unchanged during the course of these studies ($p > 0.05$).

Plasma catecholamines. The pattern of change in plasma catecholamine concentrations during successive gas exposures is presented in Table 2. Maternal plasma concentrations of catecholamines were highly variable; therefore, we used the log values to analyze these data. Although there was a 3- to 4-fold increase in maternal NE and E levels between exposure to O_2 alone and O_2 plus CO_2 exposure and a gradual return to baseline thereafter, these changes were not statistically significant ($p > 0.1$; ANOVA). Nonetheless, a significant, albeit a weak, relationship was observed between maternal PaCO_2 and NE ($r = 0.37$; $p = 0.006$) and PaCO_2 and E ($r = 0.53$; $p < 0.001$). There were also no significant changes in plasma dopamine.

The pattern of change in fetal plasma catecholamine levels was similar to that seen in the ewe. Again, there was marked variability and data were analyzed as noted above. Fetal plasma concentrations of dopamine and NE were not significantly changed. On the other hand, fetal plasma concentrations of E increased 4-fold at 30 min of CO_2 exposure ($p = 0.01$) compared with O_2 alone; as in the ewe, levels gradually returned to values not different from control after the end of hypercapnia. When we examined the relationship between fetal PaCO_2 and plasma catecholamines, there was a significant correlation only between PaCO_2 and E ($r = 0.58$; $p < 0.001$).

DISCUSSION

Among mammals, different forms of stress result in increased secretion of AVP and catecholamines. In the fetus, hypoxemia (11), asphyxia (8, 10–12, 15), hypotension (20), hemorrhage (21, 22), parturition (12, 23), cord occlusion (9), and increases in plasma osmolality (24) are potent stimuli resulting in substantial increases in plasma AVP concentrations. Although the effects of hypovolemia and hyperosmolality on hormone secretion are better understood, it is unclear how those stresses associated with changes in arterial blood gases and pH result in increased fetal AVP or catecholamine release. Nonetheless, "hypoxia" has been suggested as the primary stimulant. In view of this, we have attempted to elucidate the mechanisms responsible for AVP

Table 2. Hormonal changes in pregnant ewes and their fetuses in response to oxygen and/or carbon dioxide inhalation ($n = 8$)*

	Inspired gas				
	RA† (15 min)	O ₂ † (20 min)	O ₂ + CO ₂ † (30 min)	O ₂ (30 min)	RA (60 min)
Maternal					
AVP (pmol/L)	55.1 ± 1.1	48.2 ± 8.2	129 ± 47	63.0 ± 18	43.7 ± 5.5
DA (nmol/L)	0.37 ± 0.16	0.67 ± 0.31	0.49 ± 0.08	0.39 ± 0.14	0.67 ± 0.16
NE (nmol/L)	2.23 ± 0.74	2.77 ± 0.82	8.52 ± 3.97	6.88 ± 2.28	3.42 ± 0.69
E (nmol/L)	0.56 ± 0.15	0.88 ± 0.43	3.36 ± 2.45	0.51 ± 0.28	0.55 ± 0.10
Fetal					
AVP (pmol/L)	41.9 ± 7.1	46.2 ± 7.7	137 ± 63	101 ± 35	46.9 ± 6.4
DA (nmol/L)	0.37 ± 0.14	0.53 ± 0.18	0.91 ± 0.14	0.67 ± 0.14	0.75 ± 0.22
NE (nmol/L)	3.23 ± 0.33	5.12 ± 2.61	12.4 ± 3.89	4.36 ± 1.07	7.96 ± 3.19
E (nmol/L)	0.27 ± 0.10	0.55 ± 0.16	2.27 ± 0.90‡	0.62 ± 0.17	0.32 ± 0.60

* Values presented are means ± SEM.

† RA represents room air or fraction of inspired oxygen = 0.21; O₂ represents fraction of inspired oxygen = 0.30; CO₂ represents fraction of inspired carbon dioxide = 0.10; DA, dopamine.

‡ $p = 0.01$ compared with control.

Fig. 5. The effects of hyperoxic hypercapnia on maternal and fetal plasma concentrations of AVP ($n = 8$).

release in response to asphyxia/hypoxemia by examining each of the components of an asphyxial episode (11, 13).

During acute asphyxia, *i.e.* the simultaneous occurrence of hypoxemia, acidosis, and hypercapnia, we and others have reported a 40- to 240-fold rise in ovine fetal plasma AVP (5, 8–12). In contrast, exposure to hypoxemia [PaO_2 as low as 1.47 kPa (11 mm Hg)] in the absence of either metabolic acidosis or hypercapnia (*i.e.* respiratory acidosis) results in relatively small increases in plasma AVP (11). Because this suggested that hypoxemia alone may not be the major determinant of AVP release during asphyxia, we examined the role of acute metabolic acidemia in the release of this peptide hormone in fetal sheep of similar gestational ages (13). In those studies, NH_4Cl infusion resulted in a fall in fetal arterial pH to 7.04 ± 0.05 , but only a 2-fold rise in plasma AVP concentration, which was not related to the fall in pH. Thus, it was evident from these studies that neither severe metabolic acidosis nor a 45% fall in PaO_2 was solely responsible for the marked increases in plasma AVP reported to occur during acute fetal asphyxia.

In the studies presented here, we sought to determine the role of acute hypercapnia and respiratory acidosis in the fetal secretion of AVP. Although this has been examined in adult animals (4, 25, 26), it has not been studied in the fetus. In preliminary studies, ewes frequently developed hypoxemia and metabolic acidosis during acute hypercapnia; therefore, we chose to maintain oxygenation by providing 30% inspired O₂. In these studies, oxygen exposure had no effect on maternal or fetal cardiovascular or hormonal parameters. Maternal and fetal hypercapnia occurred simultaneously and within 10 min of maternal exposure to 30% O₂ plus 10% CO₂, resulting in the development of

persistent and significant respiratory acidosis in the absence of either hypoxia or metabolic acidosis. However, the gradual rise in maternal and fetal plasma concentrations of AVP did reach significance over a broad range of hypercapnia, *i.e.* up to 10.7 kPa (80 mm Hg). Our observations in the ewe are consistent with those of Raff *et al.* (25), who, in studies of normoxic hypercapnia in anesthetized adult dogs, achieved values for PaCO_2 and pH_a similar to those observed by us in the unanesthetized pregnant ewe. Thus, similar mechanisms for AVP secretion may be at work in the mother and fetus. It is noteworthy that, in the adult, Raff *et al.* (3) also observed no change in plasma AVP during normocapnic hypoxia [$\text{PaO}_2 \approx 5.33$ kPa (40 mm Hg)], an observation consistent with our studies in the unrestrained, unanesthetized ewe and fetus (11).

In the studies presented here, we examined other variables that might affect AVP secretion, including uteroplacental blood flow, and none of these factors was significantly altered. For example, decreases in O₂, alterations in plasma osmolality, and decreases in MAP did not occur. Stimulation of the peripheral chemoreceptors has also been shown to increase AVP secretion in the adult (26). Although maternal carotid chemoreceptors were obviously stimulated, as evidenced by the 50% rise in heart rate, the modest rise in plasma AVP was not significant. It may be that the simultaneous rise in maternal MAP acted to attenuate maternal AVP secretion, as suggested by several investigators (2, 3, 26), thereby resulting in nonsignificant increases. This is supported by the achievement of maximum maternal AVP levels after CO₂ exposure when MAP had returned to control levels. A similar series of events may have occurred in the fetus, because plasma AVP also did not attain maximum values until the end of CO₂ exposure, when fetal heart rate was significantly elevated. In contrast to that in the mother, the rise in fetal MAP was modest (~20%) and was not significant after correction for changes in amniotic sac pressure. Thus, the potential inhibitory effect of fetal MAP on AVP secretion is unclear. Moreover, the effects of increases in fetal MAP and chemoreceptor stimulation on AVP secretion have not been studied. Atrial natriuretic peptide has also been shown to attenuate or inhibit AVP secretion in the adult (27–29). We recently reported that increases in fetal MAP will cause substantial increases in right atrial pressure and the secretion of atrial natriuretic peptide (30). Therefore, it is possible that this series of events would inhibit or attenuate AVP secretion in both the ewe and fetus. Further studies are necessary to prove this.

Catecholamines are also released during asphyxia (2, 14). In the studies presented here, both NE and E were secreted by the pregnant ewe during hypercapnia, although this was quite variable. Furthermore, the relative increases in both catecholamines were similar, 2- to 3-fold, suggesting that the primary source of

Fig. 6. The effect of marked hypercapnia on fetal secretion of AVP during a fall in arterial oxygen content.

maternal catecholamines might be the sympathetic nerve terminals as well as the adrenal medulla—conclusions similar to those obtained in adult dogs (14). In the fetus, NE and E also increased during hypercapnia, but only the latter reached significance, and both gradually fell after ending CO_2 exposure. In contrast to that seen in the ewe, the relative rise in E was more than 2-fold greater than that of NE, 316 versus 142%, suggesting that during fetal hypercapnia the predominant source of catecholamines is the adrenal medulla, a conclusion consistent with the observations of others (7). The differences between the adult and fetal animals in their levels and distribution of catecholamines may thus be explained on the basis of the relative contribution to the circulating pool of catecholamines from these two sources. The observations presented here suggest that the primary fetal and maternal hormonal response to the degree of hypercapnia experienced is the increased release of catecholamines, particularly E, rather than AVP.

It is noteworthy that fetal and maternal plasma levels of catecholamines increased maximally during CO_2 exposure, whereas the maximum rise in plasma concentrations of AVP was not attained until the end, or soon after the end, of CO_2 exposure. Thus, the observed rise in MAP likely reflects the effects of the catecholamines or another pressor agent rather than those of AVP. This would also explain the cardiovascular responses previously seen during acute fetal hypoxemia (11), where the increase in fetal MAP also occurred before the rise in plasma levels of AVP. That more than one agent is associated with the fetal cardiovascular responses to hypoxemia or stresses has been suggested by others (31, 32). It also is possible that the earlier increase in catecholamine secretion might be responsible in part for the subsequent rise in plasma AVP levels. This is supported by *in vivo* and *in vitro* studies of the adult, in which catecholamines have been shown to modulate AVP secretion (33). Moreover, neurohistochemical studies of adult tissues have clearly established that the magnocellular neurons are surrounded by catecholamine-containing varicosities, especially NE (34). Thus, the relatively "late" and possibly attenuated rise in plasma AVP in the mother and fetus, although nonsignificant, might reflect the effect of catecholamine stimulation rather than that of hypercapnia. In contrast, Block *et al.* (35) have suggested that in fetal sheep α_1 -adrenergic stimulation may serve to inhibit AVP secretion. Alternatively, both fetal and maternal AVP responses to hypercapnia may be slow as compared with catecholamine secretion.

In the adult, Raff *et al.* (25) have reported that synergism may

exist between the different components of asphyxia in the secretion of AVP. It is noteworthy that, in a preliminary experiment (Fig. 6) in which PaCO_2 increased from 5.33 to 12.0 kPa (40 to 90 mm Hg) and O_2 content fell from 9.8 to 5.7 vol%, plasma AVP levels rose to 4550 pmol/L, a value 40-fold greater than that seen in this study, where oxygenation was maintained. This observation can be extrapolated to suggest that oxygen delivery to the neurohypophysis, measured as the product of blood flow times O_2 content, determines if there is substantial tissue hypoxia/asphyxia and thus AVP release. Although PaO_2 was maintained, this probably reflects a shift in the oxygen dissociation curve due to the changes in pH_a and PaCO_2 . This animal, therefore, demonstrates that asphyxia, *i.e.* simultaneous alterations in CO_2 , O_2 , and pH , may be important in fetal AVP release and that synergism likely occurs *in vivo*. The minimal effects of each asphyxial component seen in this and earlier studies (11, 13) suggest that responses to asphyxia reflect a complex phenomenon, resulting in the release of local factors that act on the magnocellular neurons, potentially releasing other yet unidentified circulating vasopressinergic substances or stimulating the CNS directly.

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