

The Effect of Hypoxia on Neurohypophyseal Hormone Release in Fetal and Maternal Sheep

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Summary

The effect of hypoxemia on arginine vasopressin (AVP) and oxytocin (OT) release was investigated in the chronically catheterized fetus and ewe. During 30 min of 10% maternal oxygen delivery, mean (\pm SEM) arterial PO₂ decreased from 105 \pm 10.6 to 48 \pm 3.5 mm Hg in the ewe and from 21 \pm 1.3 to 12 \pm 0.8 mm Hg in the fetus (each $P < 0.001$). Arterial PCO₂ decreased from 35 \pm 4.4 to 29 \pm 1.0 mm Hg in the ewe, whereas fetal PCO₂ decreased from 43 \pm 2.3 to 35 \pm 3.5 mm Hg ($P < 0.05$). Blood pH increased from 7.44 \pm 0.03 to 7.56 \pm 0.04 in the ewe ($P < 0.01$) and from 7.36 \pm 0.004 to 7.40 \pm 0.006 in the fetuses ($P < 0.01$).

Baseline mean AVP levels were identical in ewes and fetuses (0.7 \pm 0.1 μ U/ml). After 30 min of hypoxia, plasma AVP levels remained unchanged in the ewes (0.9 \pm 0.1), but increased dramatically in the fetuses (47 \pm 21 μ U/ml) ($P < 0.001$). There was a highly significant correlation between the duration of hypoxia and log fetal AVP concentrations ($r = 0.85$). The log fetal plasma AVP also was inversely correlated to the log fetal PO₂ values ($r = 0.83$).

Mean baseline fetal and maternal plasma OT levels were 2.6 \pm 0.5 μ U/ml and 2.2 \pm 0.5 μ U/ml, respectively. After 30 min of hypoxia fetal and maternal OT values were 2.9 \pm 0.8 μ U/ml (not significant).

Abbreviations

AVP, arginine vasopressin
 OT, oxytocin

AVP and OT contents of the fetal pituitary and hypothalamus increase during gestation (18, 21). Fetal plasma AVP and OT levels are low during the last third of gestation and although the placenta is impermeable to AVP and probably OT, basal maternal and fetal blood concentrations of AVP and OT are similar (7, 12, 22, 23); however, it is clear that the ovine fetus is capable of responding to both osmotic and hypovolemic stimuli with AVP release early in the third trimester. After delivery, cord blood plasma AVP levels in newborn sheep and human infant are increased, more so after vaginal delivery than after Cesarean section delivery (1, 2, 3, 9, 10, 14, 15). This AVP elevation has been attributed to cerebral compression (6, 9) or stress and hypoxia (4, 5, 16).

Several studies using bioassay as well as radioimmunoassay methods for measuring AVP have shown that hypoxia is a potent stimulus for fetal AVP release (1, 17, 25). These studies often combined hypoxia with hypercarbia however, and none measured the OT response.

In fact, little is known concerning OT responsiveness in the

fetus. We and others have shown that cord blood plasma OT levels are elevated (2, 13), and there has been speculation that this elevation also may be due to hypoxia associated with delivery. The present study was undertaken to investigate the magnitude and timing of the AVP and OT responses to hypoxia, the latter as a single stimulus unassociated with hypercapnia and/or asphyxia.

MATERIALS AND METHODS

Animal preparation. Ketamine was administered to four date-bred ewes (Columbia-Suffolk), three with singleton fetuses and one with twin fetuses. The fetuses were 125–129 d gestational age. Hysterotomy was performed, the fetal leg was exteriorized, and a catheter was inserted into the fetal dorsal vein and artery and advanced to the inferior vena cava and dorsal aorta. The fetal leg was then replaced in the uterus, the fetal catheters were exteriorized to a pouch attached to the flank of the ewe, the uterine incision was sutured and maternal abdominal wall was closed. Femoral venous and arterial catheters were inserted into the ewe. Catheters were maintained patent with a dilute heparin solution (1 U/4 ml blood).

Chloramphenicol was given to the ewe (250 mg) and the fetus (25 mg), and instilled into amniotic fluid (250 mg) twice a day for five d postoperatively. Animals were maintained in indoor pens and received feedings of baled alfalfa and water *ad libitum*. Five d were allowed for recovery from surgery.

Study protocol. On the day of study, water was removed from the cage. A fetal blood sample was obtained and the study was undertaken only if the fetal pH exceeded 7.3. Thirty milliliters whole blood was removed from the maternal circulation into a heparinized syringe (1 U for 4 ml blood) for subsequent transfusions to the fetus.

After three (5 ml) baseline blood samples were obtained at 10-min intervals from the maternal femoral and fetal dorsal arterial lines for measurement of plasma AVP, OT, blood gases, and pH, the ewe breathed a gas mixture containing 10% oxygen and 90% nitrogen for 30 min. The gases were delivered to a bag placed over the ewe's head. A rapid flow rate was maintained so that CO₂ accumulation did not occur. Oxygen content of the inspired gases was monitored continuously using a Beckman oxygen analyzer calibrated to room air and 100% O₂. Three (5 ml) blood samples were obtained from the fetus and mother at 10, 20, and 30 min of 10% O₂ exposure. All samples were collected after twice the equivalent of the 1.5 ml dead space of the catheters was withdrawn. Fetal blood withdrawn was replaced immediately with an equal amount of maternal blood.

One milliliter of each blood sample was placed in an iced heparinized tuberculin syringe for immediate blood gas and pH measurements. Four milliliters of each blood sample was collected in iced tubes prepared with 15% EDTA, and spun at 2000

r/min in a refrigerated centrifuge. Plasma was separated within 1 h and divided into two 1-ml aliquots for storage at -20°C until measurement of AVP as described previously (20, 26). One milliliter of plasma was immediately extracted for OT by the addition of two volumes of spectral grade acetone; samples then were centrifuged at 2000 r/min for 20 min. The supernatant was decanted into the sample. An upper layer was allowed to form and the lower layer was aspirated into a 12 x 75-mm glass tube. The infranatant was then dried in a Savant condenser and stored at -20°C until radioimmunoassay. Details of the radioimmunoassay have been published previously (7, 26). Sensitivity of the AVP assay is $0.15 \mu\text{U/ml}$, sensitivity of the OT assay, $0.45 \mu\text{U/ml}$. Cross reactivities of arginine vasopressin, vasotocin, and mesotocin in the OT radioimmunoassay are 0.1%, 0.2%, and 0.7%, respectively. The OT radioimmunoassay within assay coefficient of variation is 6.7%. Mean (\pm SEM) OT extraction recovery was $72 \pm 3\%$.

Means and standard errors of the mean values were calculated for all data. Mean values for baseline and posthypoxia data as well as comparisons between fetal and maternal values were both determined by two-way analysis of variance. Correlation coefficients were calculated by linear regression analysis. All data are recorded as mean \pm SEM.

RESULTS

During the 30-min period of breathing 10% O_2 , the maternal PO_2 decreased from a baseline of 105 ± 10.6 (mean \pm SEM) to 48 ± 3.5 mm Hg ($P < 0.001$) whereas fetal PO_2 decreased from 21 ± 1.3 to 12 ± 0.8 mm Hg ($P < 0.001$). Maternal PCO_2 decreased from 35 ± 4.4 to 29 ± 1.0 mm Hg ($P < 0.05$) due to hyperventilation whereas fetal PCO_2 values decreased from 43 ± 2.3 to 35 ± 3.5 mm Hg ($P < 0.05$). Blood pH increased in the ewes from 7.44 ± 0.03 to 7.56 ± 0.04 and in the fetuses from 7.36 ± 0.004 to 7.40 ± 0.006 ($P < 0.01$) (Fig. 1).

Baseline plasma AVP levels were $0.7 \pm 0.1 \mu\text{U/ml}$ in the ewes and $0.7 \pm 0.1 \mu\text{U/ml}$ in their fetuses (not significant). After 30 min of 10% oxygen delivery to the ewes, the mean maternal plasma AVP level ($0.9 \pm 0.1 \mu\text{U/ml}$) was similar to the pre-study value. Fetal plasma AVP levels varied from 11–101 $\mu\text{U/ml}$ (mean, $47 \pm 21 \mu\text{U/ml}$; $P < 0.001$). Figure 2 shows the fetal and maternal plasma AVP concentrations before and during the hypoxic period. There was a highly significant correlation between the rise in log fetal AVP concentrations and duration of hypoxia (correlation coefficient, $r = 0.85$). Figure 3 shows a significant positive correlation between decreasing levels of (log) fetal PO_2 and (log) fetal plasma AVP values ($r = 0.83$).

Baseline fetal and maternal plasma OT levels were 2.6 ± 0.5 and $2.2 \pm 0.5 \mu\text{U/ml}$, respectively (not significant). After 30 min of breathing 10% O_2 , fetal and maternal plasma OT levels were 2.9 ± 0.8 and $2.0 \pm 0.7 \mu\text{U/ml}$, respectively.

DISCUSSION

Our study presents the first plasma OT measurements during fetal hypoxia. Our fetal animals had a vigorous plasma AVP response to hypoxia given as a single stimulus, but they manifest no plasma OT response. We have shown previously that increases in both plasma AVP and OT occur in response to hemorrhage or hypertonic saline infusion in adult dogs (26). Furthermore, increases in both AVP and OT levels have been described in cord blood by several investigators (2, 3, 9, 10, 13, 14, 15). The present results suggest that elevated cord blood OT levels are not due to fetal hypoxia. The precise stimulus remains to be clarified.

The timing of the elevations in cord blood AVP and OT levels (2, 3, 9, 10, 13, 14, 15) relative to labor and delivery is unclear, but the increase may occur before parturition (1). Stark *et al.* (22) measuring plasma AVP levels during adrenocorticotropin-induced labor in chronically catheterized fetal-maternal sheep preparations, found a significant increase during labor. This

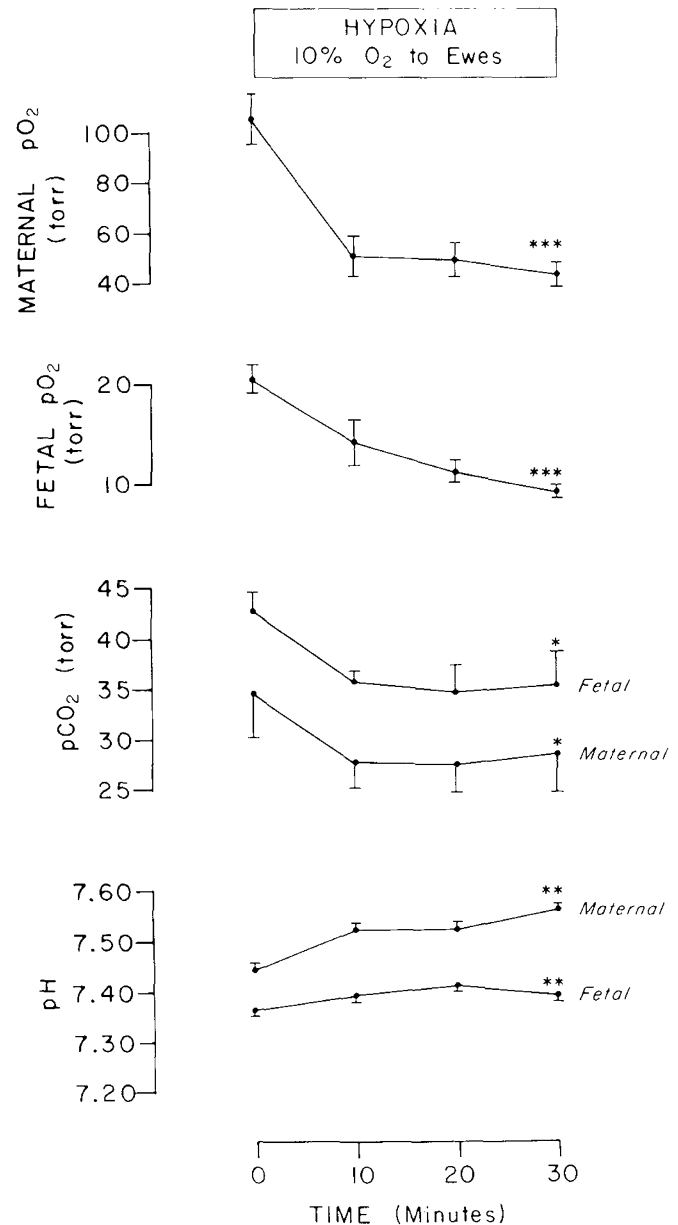


Fig. 1. Blood gas and pH values (mean \pm SEM) in maternal and fetal plasma during 30 min of 10% oxygen delivery to the ewe.

increase seemed to be inversely correlated to the level of fetal oxygenation. Other investigators (3, 9, 10) have reported a correlation between duration of labor and plasma AVP levels.

The mechanism responsible for AVP hypersecretion during delivery also is unclear. AVP concentrations in cord blood of infants delivered by Caesarian section usually are lower than values in infants born vaginally (19). One mechanism for fetal AVP release during labor and delivery might therefore be increased intracranial pressure (19), because in monkeys with chronically implanted intracerebral balloons, balloon inflation increases plasma AVP concentrations (6). Polin *et al.* (15) could not correlate increased plasma AVP levels after vaginal delivery with stress, cord blood pH, or 1-min Apgar scores, however.

Examining the role of hypoxia as a stimulus for AVP secretion, Rurak (17) found an increase in plasma AVP levels in fetuses of hypoxic ewes and observed that this increase was correlated with a fall in fetal blood pH. Our results do not confirm this correlation. Additionally, maternal plasma AVP levels did not increase in the study of Rurak or in the present study; however, the

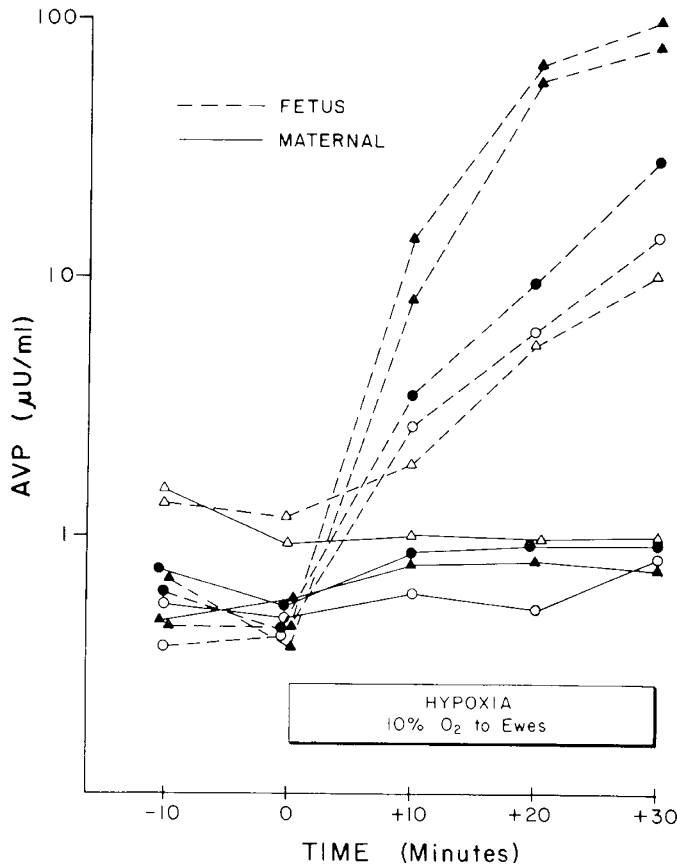


Fig. 2. Fetal and maternal plasma AVP levels during 10% oxygen breathing in four ewes and five fetuses.

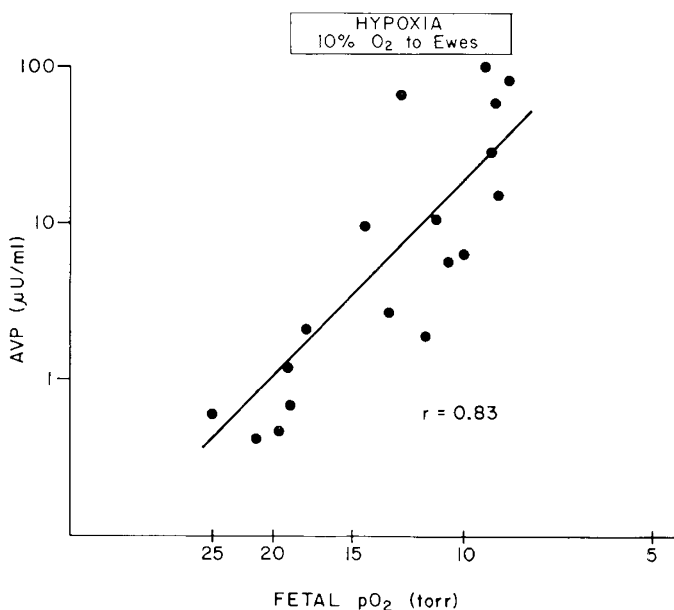


Fig. 3. Fetal plasma AVP and P_{O_2} values during 10% oxygen delivery to the ewe. Data are plotted on double logarithmic scales ($r = 0.83$).

sensitivity in the AVP bioassay employed by Rurak was $5.0 \mu\text{U/ml}$.

In a more recent study, Stark *et al.* (24) reported peak fetal plasma AVP values similar to ours after a 30-min period of 10% oxygen delivery to the ewe. The blood sampling schedule, however, failed to show the exponential increase in fetal plasma AVP levels which our data demonstrate. In addition these authors

reported a significant positive correlation between decreasing levels of fetal P_{O_2} and the logarithm of the fetal plasma AVP increment ($r = 0.75$). In our study a better correlation coefficient was observed using a logarithmic scale for both plasma AVP and P_{O_2} ($r = 0.832$, Fig. 3). In similar studies DeVane and colleagues (25) also studied fetal plasma AVP responses to maternal hypoxia in sheep, but the fetal AVP responses in that study were less than in the present report.

To study the mechanism of the AVP response to hypoxia, Rurak (17) severed the cervical vagosympathetic trunks in sheep fetuses and observed a somewhat reduced but intact increase in plasma AVP levels in response to hypoxia. In contrast, Share and Levy (19) demonstrated a role of carotid artery chemoreceptors in mediating the AVP response to hypoxia in adult dogs. This may represent species difference or differences between fetal and adult responses.

The observation in the present study that plasma AVP levels correlated with the duration of hypoxia suggests that hypoxia is a continuing stimulus to AVP secretion at least over a 30-min period. In addition this correlation is compatible with a progressive increase in AVP secretion with continuing duration of hypoxia and/or a decreased rate of AVP degradation in the hypoxic animals.

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Glucocorticoid-Thyroid Hormone Interactions in Fetal Rat Lung⁽¹³⁾

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Summary

Previous studies have shown that triiodothyronine (T_3) enhances the effect of dexamethasone on phosphatidylcholine (PC) synthesis in organ cultures of fetal rat lung. The aim of this study was to investigate whether similar interactions occurred *in vivo* and to explore possible mechanisms for this phenomenon.

Injection of 7.0 mg/kg T_3 into pregnant rats on d 18 and 19 of gestation resulted in a mean fetal serum T_3 level of 2380 ng/dl on d 20 (control, 84 ng/dl) and in maximal (34%) stimulation of choline incorporation into PC. Injection of 1.0 mg/kg betamethasone using the same protocol as for T_3 resulted in maximal stimulation of 33% and administration of both hormones together produced a 69% increase, an additive effect. The percentage of PC that was disaturated was increased with betamethasone, but decreased with T_3 . Betamethasone treatment resulted in an increase in the whole lung disaturated PC content, but treatment with T_3 did not. Betamethasone administration also increased fetal serum T_3 levels, but T_3 injection did not produce elevated fetal serum corticosterone levels.

Injection of T_3 *in vivo*, or exposure of explants of 18-d fetal lung to 100 nM T_3 for up to 48 h did not result in an increase in cytoplasmic glucocorticoid binding or nuclear translocation of the receptor steroid complex. Exposure of explants to glucocorticoid or T_3 *in vivo* or in culture (dexamethasone, 100 nM and T_3 , 100 nM; for 48 h) resulted in a significant increase in the activity of cholinephosphate cytidyltransferase, an enzyme in the choline incorporation pathway of PC synthesis. Exposure of explants to the combination of hormones resulted in stimulation that was equal to the sum of that produced by the single hormones but was not statistically significantly different from the glucocorticoid effect. The activities of other enzymes of phospholipid synthesis were not increased by exposure to either hormone, *in vivo* or *in vitro*.

The additive effects of T_3 and glucocorticoid with regard to choline incorporation into PC in fetal rat lung suggest that combined hormone therapy may be useful for the prevention of

respiratory distress syndrome in humans. Further animal studies are required, however, before clinical use can be considered.

Abbreviations

CDP, cytidine 5' diphospho
DIMIT, 3,5-dimethyl-3'-isopropyl-L-thyronine
PC, phosphatidylcholine
RDS, respiratory distress syndrome
 T_3 , triiodothyronine

Interactions between glucocorticoids and thyroid hormones occur in a number of tissues (31). A previous study from this laboratory showed that there are additive interactions between dexamethasone and T_3 with regard to phospholipid synthesis in explants of fetal rat lung in organ culture (11). Both dexamethasone and T_3 stimulated the rate of choline incorporation into PC in these cultures and the effect of the combination of these hormones was equal to or greater than the sum of their individual actions. Another of our previous studies indicated that glucocorticoids and thyroid hormones have different effects on fetal lung phospholipid metabolism, suggesting that these hormones act at different biochemical sites (10).

The aim of this study was 2-fold. First, we wanted to determine whether the interactions observed *in vitro* would occur *in vivo*. The second goal was to determine whether the interactions occur at the level of cytoplasmic glucocorticoid receptor, nuclear translocation of the receptor-steroid complex, or at the translational level. To explore this, we examined the effects of T_3 on dexamethasone binding, *in vivo* and *in vitro*, and the effects of glucocorticoids, T_3 , and a combination of these two hormones on the activity of a number of enzymes of phospholipid synthesis.

MATERIALS AND METHODS

In vivo studies. In order to avoid problems associated with direct fetal injection, all hormones were injected into the mother.