

Neuropeptide Y in the Sheep Fetus: Effects of Acute Hypoxemia and Dexamethasone During Late Gestation*

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ABSTRACT

Plasma concentrations of neuropeptide Y (NPY) were measured in pregnant ewes and their fetuses under basal conditions and in response to acute hypoxemia during late gestation. The effects of fetal treatment with dexamethasone on these NPY responses were also examined. Under general anesthesia, 10 Welsh Mountain ewes and their fetuses were chronically instrumented between 117–120 days gestation (dGA; term is approximately 145 dGA) with vascular and amniotic catheters, and an ultrasonic probe around a femoral artery of each fetus. At 124 dGA, five fetuses were continuously infused iv with dexamethasone for 48 h at a rate of $1.73 \pm 0.16 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ while the remaining five fetuses received vehicle at the same rate. At 126 dGA, 45 h from the onset of either infusion, 1 h of materno-fetal hypoxemia was induced by reducing maternal F_iO_2 . During normoxia,

maternal plasma NPY concentrations were three times those measured in fetal plasma in both groups. During hypoxemia, P_aO_2 fell to similar levels in the control and dexamethasone-treated groups in both mothers and fetuses. In control animals, there was a significant increase in the NPY concentration in fetal, but not maternal, plasma during hypoxemia. Fetal treatment with dexamethasone significantly enhanced the fetal NPY response to acute hypoxemia but had no effects on basal NPY levels in the fetal or maternal plasma or on the maternal response to acute hypoxemia. These data show: 1) differences between the maternal and fetal plasma NPY response to maternal inhalation hypoxia; 2) that NPY may play a role in mediating fetal defense responses to acute hypoxemia; and 3) that fetal exposure to glucocorticoids modifies the fetal plasma NPY response to acute hypoxemia. (*Endocrinology* 141: 3976–3982, 2000)

IN THE MAMMALIAN fetus, episodes of acute hypoxemia elicit integrated fetal cardiovascular and endocrine responses that facilitate fetal survival (1). Cardiovascular responses to acute hypoxemia have been well characterized in fetal sheep and include a progressive increase in arterial blood pressure (2, 3) and a redistribution of the fetal combined ventricular output in favor of the adrenal, myocardial, and cerebral circulations at the expense of blood flow to the peripheral vasculature (4). Control of the redistribution of blood flow during acute hypoxemia involves activation of the sympathetic nervous system that is initiated by a carotid chemoreflex (1). This results in femoral vasoconstriction that is mediated by α -adrenergic efferents (3, 5), and that is used as an index of the redistribution of blood flow away from the peripheral vasculature (1). Once initiated, neurally mediated fetal peripheral vasoconstriction is maintained during hypoxemia by increased release into the fetal circulation of vasoconstrictor agents such as catecholamines (6) and arginine vasopressin (7). However, the contribution made by more recently discovered vasoconstrictor agents, such as neuropeptides, to fetal cardiovascular function either during basal or hypoxemic conditions remains unclear.

Neuropeptide Y (NPY) is a 36 amino acid peptide that is colocalized with norepinephrine in sympathetic nerve terminals (8, 9) and is present in the adrenal medulla in mammals (10). NPY may be coreleased with norepinephrine from sympathetic varicosities depending on the frequency and pattern of nerve stimulation (11). NPY has potent and long-lasting vasoconstrictor activity on selective vascular beds, acting both directly via Y_1 receptors and indirectly by potentiating the effects of other vasoconstrictor agents on vascular tone (12). Although adult plasma NPY levels are known to increase in several species under various physiological challenges, including hypoglycemia (13) and hemorrhagic hypovolemia (14), no data are available on plasma NPY concentrations under basal or hypoxemic conditions in sheep during fetal or adult life.

In all species studied to date, including the sheep, fetal plasma glucocorticoid concentrations increase toward term (15). Although the magnitude and timing of this fetal plasma glucocorticoid surge varies between species, it is invariably responsible for parturition maturation of organs essential for neonatal survival, such as the lungs, liver, and gut (15). It may also play an important role in maturing fetal cardiovascular and endocrine functions in preparation for delivery and extrauterine life. Fetal treatment with cortisol or synthetic glucocorticoids, such as dexamethasone and betamethasone in the final third of gestation, is known to increase fetal baseline arterial blood pressure (16) and femoral vascular resistance (17), and to cause changes in peripheral vascular sensitivity to exogenous vasoactive agents (16, 18). Fetal treatment with glucocorticoids has also been shown to depress circulating levels of catecholamines under basal con-

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ditions (17) and reduce the catecholaminergic response to acute hypoxemia (19). However, nothing is known about the effects of fetal treatment with glucocorticoids on plasma NPY concentrations under normal or stressful conditions in fetal sheep.

Therefore, the aims of the present study were: 1) to determine maternal and fetal plasma NPY concentrations during baseline conditions in sheep; 2) to determine the effects of acute hypoxemia on NPY concentrations measured simultaneously in maternal and fetal plasma; and 3) to assess the effects of fetal treatment with dexamethasone on basal plasma NPY levels and on the NPY responses to an episode of acute hypoxemia occurring during the period of glucocorticoid exposure.

Materials and Methods

Surgical preparation

All surgical and experimental procedures were performed under the UK Animals (Scientific Procedures) Act 1986. Ten Welsh Mountain sheep fetuses and their mothers were chronically instrumented between 117 and 120 days gestational age (dGA; term is approximately 145 dGA) as described previously in detail (20). Food, but not water, was withheld from the ewes for 24 h before surgery. Following induction with 20 mg·kg⁻¹ iv sodium thiopentone (Intraval Sodium; Rhone Mérieux, Dublin, Ireland), general anesthesia (1.5–2.0% halothane in 50:50 O₂/N₂O) was maintained using positive pressure ventilation. A Teflon catheter was inserted into a maternal femoral artery and advanced into the maternal descending aorta. A lower abdominal midline incision was made and the gravid uterus exposed. Fetal instrumentation was achieved in two stages. The first uterine incision exposed the fetal head, and translucent PVC catheters (id = 0.58 or 0.86 mm; od = 0.96 or 1.52 mm, respectively; Critchly Electrical Products, NSW, Australia) were inserted into a fetal carotid artery and jugular vein. The second uterine incision exposed the fetal hindlimbs, and catheters were inserted into a femoral artery and a femoral vein. An ultrasonic flow transducer (2R or 3S; Transonic Systems Inc., Ithaca, NY) was positioned around the contra-lateral femoral artery. Another catheter was anchored to the hindlimb for measurement of amniotic cavity pressure. The uterine incisions were closed in layers and at the end of surgery, catheters were filled with heparinized saline, and all catheters and flow probe leads were exteriorized via an incision in the maternal flank.

Postoperative care

Ewes were housed in individual pens, had free access to water and hay, were fed concentrates twice daily (100 g; Sheep Nuts No. 6; H&C Beart Ltd., Kings Lynn, UK), and generally resumed normal feeding patterns within 24 h of surgery. The ewes received 2 days of postoperative analgesia (3 g daily oral phenylbutazone; Equipalozone Paste E-pp, Arnolds Veterinary Products Ltd., Shropshire, UK). Antibiotics were administered daily to the ewe (0.20–0.25 mg·kg⁻¹ im Depocillin; Mycofarm, Cambridge, UK), to the fetus (150 mg·kg⁻¹ iv ampicillin, Penbritin; SmithKline Beecham Animal Health, Surrey, UK) and into the amniotic cavity (300 mg Penbritin). Daily maternal descending aortic and fetal carotid blood samples (0.4 ml) were taken for analysis of blood gas and acid-base status. Vascular catheters were maintained patent by a continuous infusion of heparinized saline (80 IU heparin·ml⁻¹ in 0.9% NaCl) at 0.5 ml·h⁻¹.

Pressure transducers (COBE; Argon, TX) were attached to the fetal femoral artery and amniotic cavity catheters. Calibrated mean fetal arterial and amniotic pressures and mean fetal femoral blood flow were recorded continuously at 1 sec intervals using a Data Acquisition System (Cornell University; Ithaca, NY).

Experimental protocol

At 124 dGA, animals were randomly assigned to one of two experimental groups. Five fetuses were continuously infused iv with dexamethasone (dexamethasone sodium phosphate; Merck, Sharp & Dohme

Ltd., Herts, UK) in heparinized saline (80 IU heparin·ml⁻¹ in 0.9% NaCl) for 48 h at a rate of 4.28 ± 0.38 μg·h⁻¹ delivered at 0.5 ml·h⁻¹ (1.73 ± 0.16 μg·kg⁻¹·h⁻¹, corrected retrospectively for fetal weight measured at the end of the experimental protocol). This dose rate did not induce labor during the study period. The remaining five fetuses were infused with heparinized saline at the same rate (0.5 ml·h⁻¹) to act as age-matched controls. No significant difference in fetal body weight was found between saline-infused (2.35 ± 0.22 kg, mean ± SD, n = 5) and dexamethasone-treated (2.49 ± 0.17 kg, n = 5) fetuses at delivery.

At 126 dGA, 45 h from the onset of either infusion, all of the animals were subjected to a 3 h experiment consisting of 1 h of normoxia, 1 h of hypoxia, and 1 h of recovery as described previously (3, 20). During normoxia, a large polythene bag was placed over the ewe's head and air was passed through the bag at a rate of 40 l·min⁻¹. Following this baseline period, materno-fetal hypoxemia was induced for 1 h by switching the gas mixture breathed by the ewe to 9% O₂ in N₂ (18 l·min⁻¹ air; 22 l·min⁻¹ N₂) with small amounts of CO₂ (1–2 l·min⁻¹) added to the inspire. This mixture was designed to reduce maternal descending aortic P_aO₂ to 35–40 mmHg and fetal carotid P_aO₂ to 11–13 mmHg. At the end of the period of hypoxemia, the bag was removed, and the ewe was allowed to breathe room air for the recovery period. Paired maternal descending aortic and fetal carotid arterial blood samples (1.0 ml) were collected at 15 (N15) and 45 (N45) min of normoxia, at 15 (H15) and 45 (H45) min of hypoxemia, and at 45 (R45) min of recovery for measurement of blood gases and acid-base status and for collection of plasma for hormone analysis. In addition, maternal and fetal arterial blood gas samples (0.4 ml) were taken at 5 min after the onset of the hypoxemia, to confirm that a rapid and appropriate fall in maternal and fetal P_aO₂ had occurred, and at 15 min of recovery. Following collection and blood gas analysis, blood samples were immediately centrifuged at 4 C (4000 rpm for 5 min). Plasma aliquots were flash frozen in liquid nitrogen and stored at –20 C until required for analysis. All hormone analyses were performed within two months of collection.

At the end of the experimental protocol, the ewes and fetuses were killed using a lethal dose of sodium pentobarbitone (200 mg·kg⁻¹ Pentoject; Animal Ltd., York, UK). The positions of the catheters and flow probe were confirmed and the fetuses weighed.

Measurements and calculations

Maternal descending aortic and fetal carotid blood gas and acid/base status were determined using an ABL5 blood gas analyser (Radiometer, Copenhagen, Denmark) and OSM2 hemoximeter (Radiometer, Copenhagen, Denmark). Measurements in maternal blood were corrected to 38 C and those in fetal blood to 39.5 C.

The amniotic cavity pressure was used as the zero pressure reference level. Fetal femoral vascular resistance was calculated by dividing supraamniotic femoral arterial blood pressure by mean fetal femoral blood flow.

Dexamethasone assay. Maternal and fetal plasma dexamethasone concentrations were measured by RIA as previously described in detail (20). In brief, dexamethasone levels were measured after ether extraction using tritium-labeled dexamethasone as tracer. Duplicate 100 μl plasma samples were extracted with 2.5 ml of diethyl ether. After freezing, the ether was decanted, evaporated, and the residue reconstituted in 500 μl of PBS. Aliquots of varying volumes were removed (depending on expected levels determined in pilot studies) made up to 450 μl with PBS and incubated with 100 μl PBS containing 16,000 dpm (1,2,4,6,7-³H) dexamethasone (Amersham Pharmacia Biotech, Buckinghamshire, UK) and 100 μl of sheep antidexamethasone antiserum (Bioclinical Services International, Cardiff, UK). Bound and free dexamethasone were separated using dextran-coated charcoal. After centrifugation, a 500 μl aliquot was removed for measuring radioactive content. All values were corrected for recovery (86%). The interassay coefficients of variation for 3 control plasma pools (1.8, 5.4, and 26.7 nmol·liter⁻¹) were 14.6, 9.3 and 8.2%, respectively. The lower detection limit of the assay was 0.2 nmol·liter⁻¹. The anti-dexamethasone antiserum showed a 1.6% cross-reactivity against cortisol and cross-reactivities of less than 0.5% against 11-deoxycortisol, corticosterone, testosterone, progesterone and estriol.

NPY assay. Maternal and fetal plasma NPY concentrations were measured by RIA (21). All samples were assayed in duplicate at the same time. The assay used rabbit antiserum and ¹²⁵I-labeled porcine peptide.

Separation of free and bound fractions was performed with dextran-coated charcoal. The assay was validated for use in ovine plasma using stripped ovine plasma and could detect less than 1 pmol·liter⁻¹ (95% confidence interval). The interassay coefficient of variation was 6.8%.

Statistical analyses

Values for all variables are expressed as mean ± SEM unless otherwise indicated. Statistical significance of any changes in the measured variables within and between treatment groups during the protocol were assessed using a two-way ANOVA with one repeated measure and the Tukey *post hoc* test (Jandel SigmaStat statistical software version 2.0; Jandel Corp., San Rafael, CA). Statistical significance of correlations was tested using the Pearson product-moment correlation test for normally distributed data (Jandel SigmaStat statistical software version 2.0). Significance was accepted when $P < 0.05$.

Results

Basal arterial blood gas status and plasma NPY concentrations in saline-infused animals

In the saline-infused animals under baseline conditions, maternal and fetal arterial blood gases and acid-base status were within the normal range (Tables 1 and 2). Under normoxic baseline conditions, mean maternal plasma NPY levels were approximately three times those measured in fetal plasma (9.7 ± 1.2 vs. 3.3 ± 0.4 pmol·liter⁻¹; $P < 0.05$, maternal vs. fetal plasma; Fig. 1, A and B).

Arterial blood gas status and plasma NPY responses during hypoxemia

A fall in maternal and fetal P_aO₂ occurred throughout the 1 h of hypoxemia (Tables 1 and 2). While the fall in maternal P_aO₂ was accompanied by an increase in pH_a and a fall in P_aCO₂, a fall in fetal pH_a occurred during hypoxemia in saline-infused animals. Mild hypocapnia was observed in saline-infused fetuses during hypoxemia and early in recovery (Table 2). Maternal and fetal blood gas and acid-base status returned to basal conditions by the end of the recovery period. While there were no changes in maternal plasma NPY concentrations during the hypoxemic challenge, fetal plasma NPY concentrations increased during hypoxemia,

approaching values similar to those measured in the mothers (Fig. 1, A and B).

Effects of dexamethasone

Plasma dexamethasone concentrations

The infusion regimen produced a sustained increase in fetal plasma dexamethasone concentrations that averaged 3.9 ± 0.2 nmol·liter⁻¹ over the 48-h period (20). Maternal plasma dexamethasone concentrations in both groups and fetal plasma dexamethasone concentration in the control group remained below the lower limit of detection of the assay (20).

Basal blood gas status and plasma NPY concentrations

During normoxia, maternal and fetal arterial blood gases and acid-base status were similar in dexamethasone-treated animals compared with controls (Tables 1 and 2). Under baseline conditions, treatment with dexamethasone did not alter maternal (9.7 ± 1.2 vs. 10.7 ± 1.2 pmol·liter⁻¹; saline vs. dexamethasone treatment) or fetal (3.3 ± 0.4 vs. 3.8 ± 0.5 pmol·liter⁻¹; saline vs. dexamethasone treatment) plasma NPY concentrations compared with saline-infused animals.

Blood gas status and NPY responses during acute hypoxemia

During hypoxemia, maternal and fetal arterial blood gases and acid-base status in the dexamethasone-treated animals reached similar values to those measured in the saline-infused animals (Tables 1 and 2). In dexamethasone-treated fetuses, carotid P_aO₂ fell rapidly (from 20.0 ± 0.7 to 12.8 ± 0.3 mmHg; $P < 0.05$) and by a similar extent to that achieved in the saline-infused fetuses by 5 min after the onset of hypoxemia. The falls in maternal and fetal P_aO₂ were maintained throughout the 1 h of hypoxemia and were similar in both groups of animals (Tables 1 and 2). During hypoxemia, similar reductions in fetal pH_a and

TABLE 1. Maternal descending aortic blood gases and acid-base status

	Normoxia		Hypoxemia		Recovery	
	N15	N45	H15	H45	R15	R45
pH _a						
Saline	7.46 ± 0.01	7.46 ± 0.01	7.50 ± 0.02	7.51 ± 0.02 ^a	7.48 ± 0.01	7.47 ± 0.01
Dexamethasone	7.46 ± 0.01	7.47 ± 0.01	7.49 ± 0.01	7.50 ± 0.01 ^a	7.49 ± 0.01	7.48 ± 0.02
P _a CO ₂ (mmHg)						
Saline	38.0 ± 1.1	37.0 ± 1.7	33.0 ± 1.2 ^a	33.0 ± 1.1 ^a	34.0 ± 0.7 ^a	35.0 ± 0.6
Dexamethasone	37.0 ± 1.3	35.0 ± 1.4	33.0 ± 1.1	33.0 ± 0.4 ^a	33.0 ± 0.4 ^a	34.0 ± 0.4
P _a O ₂ (mmHg)						
Saline	100.0 ± 5.0	102.0 ± 4.5	36.0 ± 2.4 ^a	39.0 ± 2.8 ^a	102.0 ± 4.5	104.0 ± 3.0
Dexamethasone	103.0 ± 4.8	103.0 ± 4.5	37.0 ± 1.2 ^a	41.0 ± 1.8 ^a	101.0 ± 3.4	102.0 ± 2.5
ABE (meq·l ⁻¹)						
Saline	4.0 ± 0.6	3.0 ± 0.7	4.0 ± 0.8	4.0 ± 0.6	2.0 ± 0.6	3.0 ± 0.6
Dexamethasone	3.0 ± 0.5	3.0 ± 0.7	3.0 ± 0.8	3.0 ± 0.5	2.0 ± 0.8	3.0 ± 1.0
Sat-Hb (%)						
Saline	89.5 ± 4.3	89.1 ± 3.3	62.6 ± 5.8 ^a	62.7 ± 5.2 ^a	91.3 ± 2.7	88.8 ± 2.5
Dexamethasone	89.4 ± 4.3	89.9 ± 3.6	65.8 ± 6.0 ^a	66.0 ± 6.1 ^a	91.7 ± 3.0	91.3 ± 2.9

Values are means ± SEM at 15 (N15) and 45 (N45) min of normoxia, at 15 (H15) and 45 (H45) min of hypoxemia and at 15 (R15) and 45 (R45) min of recovery for mothers of saline-infused (n = 5) and dexamethasone-treated (n = 5) fetuses. pH_a, Arterial pH; P_aCO₂, arterial CO₂ partial pressure; P_aO₂, arterial O₂ partial pressure; ABE, base excess; Sat. Hb, percentage saturation of hemoglobin. Significant differences ($P < 0.05$): ^a, differences by *post hoc* analysis indicating a significant main effect of time (two-way repeated measures ANOVA + Tukey test).

TABLE 2. Fetal carotid blood gases and acid-base status

	Normoxia		Hypoxemia		Recovery	
	N15	N45	H15	H45	R15	R45
pH _a						
Saline	7.33 ± 0.01	7.34 ± 0.01	7.33 ± 0.02	7.31 ± 0.03	7.27 ± 0.02 ^a	7.30 ± 0.02
Dexamethasone	7.34 ± 0.02	7.35 ± 0.01	7.33 ± 0.02	7.28 ± 0.03 ^a	7.25 ± 0.04 ^a	7.29 ± 0.03
P _a CO ₂ (mmHg)						
Saline	55.0 ± 1.0	54.0 ± 1.7	50.0 ± 1.7 ^a	48.0 ± 1.7 ^a	50.0 ± 0.9 ^a	52.0 ± 0.7
Dexamethasone	53.0 ± 0.5	51.0 ± 1.7	50.0 ± 1.6	50.0 ± 0.8	50.0 ± 0.9	52.0 ± 0.4
P _a O ₂ (mmHg)						
Saline	23.0 ± 1.8	21.0 ± 0.9	13.0 ± 1.3 ^a	12.0 ± 0.2 ^a	23.0 ± 1.6	20.0 ± 1.6
Dexamethasone	21.0 ± 0.7	20.0 ± 0.7	12.0 ± 1.0 ^a	12.0 ± 0.6 ^a	21.0 ± 1.4	19.0 ± 1.1
ABE (meq·l ⁻¹)						
Saline	2.0 ± 0.8	2.0 ± 0.8	0 ± 1.2	-2.0 ± 1.1 ^a	-3.0 ± 2.1 ^a	-1.0 ± 1.4
Dexamethasone	2.0 ± 1.1	2.0 ± 1.0	0 ± 1.4	-4.0 ± 2.3 ^a	-5.0 ± 2.7 ^a	-3.0 ± 2.1
Sat-Hb (%)						
Saline	61.1 ± 3.3	59.7 ± 3.2	32.3 ± 5.7 ^a	32.6 ± 3.5 ^a	59.0 ± 5.7	55.7 ± 5.9
Dexamethasone	56.4 ± 1.5	58.1 ± 3.1	34.5 ± 5.8 ^a	27.7 ± 1.3 ^a	57.1 ± 1.2	49.9 ± 3.3

Values are means ± SEM at 15 (N15) and 45 (N45) min of normoxia, at 15 (H15) and 45 (H45) min of hypoxemia and at 15 (R15) and 45 (R45) min of recovery for saline-infused (n = 5) and dexamethasone-treated (n = 5) fetuses. pH_a, Arterial pH; P_aCO₂, arterial CO₂ partial pressure; P_aO₂, arterial O₂ partial pressure; ABE, base excess; Sat-Hb, percentage saturation of hemoglobin. Significant differences (P < 0.05): ^a, differences by *post hoc* analysis indicating a significant main effect of time (two-way repeated measures ANOVA + Tukey test).

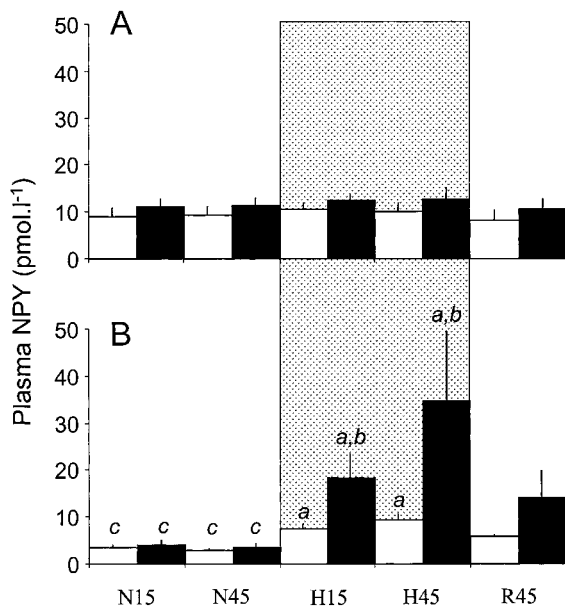


FIG. 1. Maternal (A) and fetal (B) plasma NPY concentrations during the experimental protocol. Values are mean ± SEM at 15 (N15) and 45 (N45) min of normoxia, at 15 (H15) and 45 (H45) min of hypoxemia, and at 45 (R45) min of recovery for saline-infused animals (n = 5; white bars) and dexamethasone-treated animals (n = 5; black bars). Significant differences (P < 0.05): a, differences by *post hoc* analysis indicating a significant main effect of time (two-way repeated measures ANOVA + Tukey test); b, differences by *post hoc* analysis indicating a significant main effect of treatment (two-way repeated measures ANOVA + Tukey test); c, fetal vs. maternal (Student's *t* test for unpaired data). Box represents the episode of acute hypoxemia.

base-excess occurred in dexamethasone-treated and saline-infused controls (Table 2). In dexamethasone-treated animals, the fetal plasma NPY response during acute hypoxemia was markedly enhanced compared with controls (Fig. 1B). In contrast, maternal plasma NPY concentrations were unaltered from baseline values during hypoxemia in the dexamethasone-treated group (Fig. 1A).

Cardiovascular variables

Cardiovascular variables were measured only in fetal animals (Table 3). While there was no significant difference in basal fetal arterial blood pressure between treatment groups, fetal basal femoral blood flow was reduced, and fetal basal femoral vascular resistance increased in dexamethasone-treated fetuses compared with controls (Table 3). In common with previous findings (3), an increase in arterial blood pressure and a fall in femoral blood flow occurred during acute hypoxemia in the saline-infused fetuses (Table 3). This elevation in fetal arterial blood pressure and fall in fetal femoral blood flow resulted in an increase in calculated fetal femoral vascular resistance during acute hypoxemia (Table 3). Despite depressed basal femoral blood flow and elevated baseline femoral vascular resistance values in dexamethasone-treated fetuses, a further sustained fall in femoral blood flow and a further sustained increase in femoral vascular resistance occurred in these fetuses during hypoxemia (Table 3). While there was a tendency for fetal arterial blood pressure to increase from baseline values during hypoxemia in dexamethasone-treated fetuses, this fell outside statistical significance (P > 0.05).

The relationship between plasma NPY concentration and calculated femoral vascular resistance for both groups of fetuses during normoxia and hypoxemia is shown in Fig. 2. There was a significant correlation between the natural log of the plasma NPY concentration and calculated femoral vascular resistance, irrespective of fetal treatment ($r^2=0.92$, P < 0.001).

Discussion

This study reports for the first time measurement of NPY concentrations in maternal and fetal ovine plasma under basal and hypoxemic conditions. Ovine maternal and fetal basal plasma NPY concentrations were found to be lower than those previously measured in rat (220 pmol·liter⁻¹), pig (30 pmol·liter⁻¹), and human (25 pmol·liter⁻¹) plasma (22). The maternal baseline plasma NPY concentration was ap-

TABLE 3. Fetal cardiovascular variables

	Normoxia		Hypoxemia		Recovery	
	N15	N45	H15	H45	R15	R45
FBP (mmHg)						
Saline	45.2 ± 5.2	46.1 ± 6.1	52.9 ± 7.5	59.5 ± 5.0 ^a	54.9 ± 6.4	50.4 ± 5.3
Dexamethasone	56.0 ± 3.4	56.9 ± 7.0	58.6 ± 7.3	64.3 ± 7.3	60.4 ± 6.5	58.2 ± 4.6
FBF (ml·min ⁻¹)						
Saline	31.4 ± 4.2	34.0 ± 3.9	15.7 ± 3.1 ^a	11.8 ± 2.2 ^a	22.9 ± 2.1	38.5 ± 3.6
Dexamethasone	19.5 ± 2.1 ^b	19.9 ± 2.8 ^b	14.2 ± 1.3 ^a	9.5 ± 3.5 ^a	19.1 ± 2.3	26.4 ± 3.4
FVR (mmHg·(ml·min ⁻¹) ⁻¹)						
Saline	1.84 ± 0.23	1.73 ± 0.30	4.87 ± 0.99 ^a	6.50 ± 1.60 ^a	2.91 ± 0.37	1.65 ± 0.18
Dexamethasone	2.83 ± 0.54 ^b	2.84 ± 0.46 ^b	5.60 ± 1.21	8.98 ± 2.50 ^a	3.34 ± 0.24	2.51 ± 0.33

Values are means ± SEM at 15 (N15) and 45 (N45) min of normoxia, at 15 (H15) and 45 (H45) min of hypoxemia and at 15 (R15) and 45 (R45) min of recovery for saline-infused (n = 5) and dexamethasone-treated (n = 5) fetuses. FBP, Fetal blood pressure; FBF, femoral blood flow; FVR, femoral vascular resistance. Significant differences ($P < 0.05$): ^a, differences by *post hoc* analysis indicating a significant main effect of time (two-way repeated measures ANOVA + Tukey test); ^b, differences by *post hoc* analysis indicating a significant main effect of treatment (two-way repeated measures ANOVA + Tukey test).

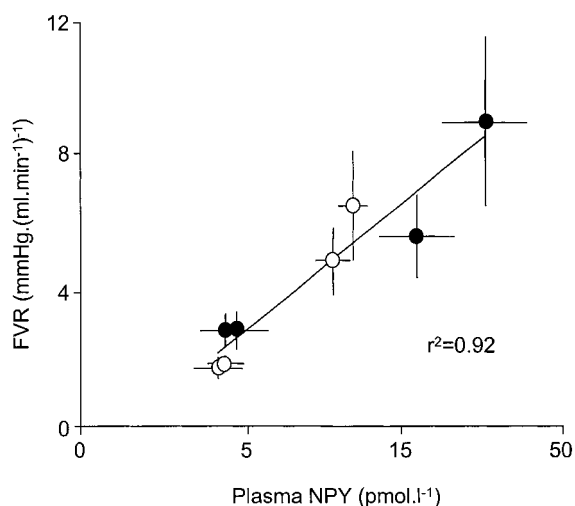


FIG. 2. Correlation between fetal plasma NPY concentration and femoral vascular resistance (FVR). Values are expressed as mean ± SEM for saline-infused (n = 5; open circles) and dexamethasone-treated (n = 5; filled circles) fetuses at 15 (N15) and 45 (N45) min of normoxia, and at 15 (H15) and 45 (H45) min of hypoxemia. r^2 , square of the Pearson product-moment correlation coefficient.

proximately three times that measured in the fetus under normoxic baseline conditions. While maternal NPY concentration remained unchanged from baseline, significant increments in fetal plasma NPY concentration occurred during hypoxemia, and these increments were greatly enhanced during fetal dexamethasone exposure.

The source of the circulating NPY measured in maternal and fetal plasma both under basal and hypoxemic conditions in the current study is unknown. NPY is known to be colocalized with norepinephrine in postganglionic sympathetic nerve terminals innervating the peripheral vasculature (8, 9) and to be present in high concentrations in the adult mammalian CNS (23) and adrenal medulla (10, 24). Despite the relatively high adrenal medullary NPY activity, adrenalectomy in adult rats had no effect on basal NPY or on the increment in plasma NPY in response to acute psychological stress (25). In addition, experiments in intact calves (11) and adrenalectomized, weaned lambs (26) have provided evidence for a negligible role of the adrenal medulla in contributing to the increase in circulating NPY concentrations

during splanchnic nerve stimulation. Taken together, these data suggest that adrenal medullary NPY contributes little to the increment in plasma NPY observed during splanchnic nerve stimulation and certain types of acute stress, and that overspill from perivascular sympathetic nerve terminals is the most likely source of plasma NPY levels under these conditions.

Little is known about the effects of hypoxemia on plasma NPY concentrations in the fetus, neonate, or adult in any species. In neonatal pigs, acute hypoxemia was found to increase circulating NPY and catecholamine concentrations, and these responses were enhanced by the nonselective adenosine receptor antagonist theophylline (27). In addition, prolonged exposure of adult rats to hypobaric hypoxia was found to elevate basal plasma NPY-like immunoreactivity, an effect that was attributed to increased adrenal medullary NPY output under these conditions (28). The present study reports measurements of fetal and maternal ovine plasma NPY concentrations during acute hypoxemia. In this study, the increase in fetal plasma NPY concentration in the absence of changes in maternal plasma NPY concentration suggests that the NPY released in response to the hypoxemic challenge was of fetal rather than maternal origin. Although placental expression of NPY has not yet been demonstrated in sheep, NPY is expressed in human decidual cells (29) and in human epithelial amnion cells and chorionic cytotrophoblast (30). Consequently, a component of the increase in fetal plasma NPY concentration in response to hypoxemia may have been contributed by placental release of NPY. The lack of maternal plasma NPY response in the presence of an increase in fetal plasma NPY levels indicates that the fetuses, but not the mothers, respond to their respective levels of hypoxemia induced in the current study. This is consistent with previous findings where similar levels of maternal and fetal hypoxemia as those achieved in the present study failed to elicit increases in maternal plasma ACTH and cortisol concentrations despite significant increments in the fetuses (20). While it is clear that there was no change from baseline in maternal plasma NPY concentrations during hypoxemia, it is unknown whether pregnancy alters basal plasma NPY levels as occurs in humans (31).

The functional significance of the increase in fetal plasma NPY during acute hypoxemia is unknown. The increment in

plasma NPY may represent increased overspill from perivascular sympathetic nerve terminals, reflecting increased sympathetic vasomotor tone, and/or may have an important endocrine role in the fetal defense response to acute hypoxemia. NPY has numerous cardiovascular effects (32) and is known to potentiate adrenergic transmission both *in vitro* (33) and *in vivo* (34). Indeed, exogenous NPY infusions potentiate the pressor and vasopressor responses to exercise in humans (35, 36) and to hemorrhage in adult rats (37). NPY is also known to have direct effects on cardiac function. For example, NPY administration has negative inotropic and coronary vasoconstrictor effects in canine (38) and porcine (39) hearts *in vivo* and in rabbit hearts (40) *in vitro*. NPY has also been shown to have a direct inhibitory effect on contractility in rat isolated ventricular myocytes (41). In this way, the increase in fetal plasma NPY may contribute to the increases in fetal femoral vascular resistance and may modify fetal myocardial contractility during acute hypoxemia.

In common with previous findings (42), fetal dexamethasone administration in the present study reduced baseline fetal femoral blood flow while increasing calculated femoral vascular resistance. However, dexamethasone treatment did not alter fetal basal plasma NPY concentration compared with control values. This demonstrates that increases in plasma NPY concentration do not contribute to the increment in fetal basal femoral vascular resistance observed with fetal dexamethasone treatment. In the present study, fetal treatment with dexamethasone greatly augmented the fetal NPY response to acute hypoxemia. In contrast to norepinephrine, NPY lacks synaptic reuptake mechanisms, and therefore changes in plasma NPY concentration may provide a better measure of sympathetic nervous system activity than changes in plasma catecholamine concentrations (43). Increases in plasma NPY concentration may also be an index of sympathetic nervous system discharge frequency since nerve stimulation at high frequencies results in NPY overspill that is proportionately greater than that of norepinephrine (43). Therefore, although the functional significance of the enhanced plasma NPY response to acute hypoxemia in dexamethasone-treated fetuses is unknown, it may indicate maturation of sympathetic nervous system activity and/or enhancement of discharge frequency. These findings are consistent with those of Segar *et al.* (44), who reported that maternal antenatal glucocorticoid administration increased renal sympathetic nerve activity postnatally in premature lambs. The augmented plasma NPY response in dexamethasone-treated fetuses may contribute to, or be an index of, enhancement of mechanisms that contribute to the maintenance of the magnitude of the femoral vasoconstriction observed during hypoxemia despite elevations in basal values of femoral vascular resistance in these fetuses. Accordingly, in this study plasma NPY concentrations were correlated to femoral vascular resistance values irrespective of fetal treatment, with the greatest levels of both variables being achieved during hypoxemia in the dexamethasone-treated fetuses.

Glucocorticoids induce parturition in the sheep (17), and consequently the dose of dexamethasone administered to the sheep fetus is critical in determining whether labor is initiated in this species. The present study used low doses of

dexamethasone that were below the threshold for inducing labor in Welsh Mountain ewes and produced step increases in fetal plasma dexamethasone concentration that averaged $3.2 \pm 0.8 \text{ nmol}\cdot\text{liter}^{-1}$ over the 48-h treatment period (20). This concentration is approximately one fifth of the mean value measured in umbilical arterial blood samples taken from human infants at Caesarian section 12 h following the completion of a course of maternal antenatal glucocorticoid treatment (5 mg dexamethasone im every 12 h for 48 h; 45) and is approximately one sixth of the mean concentration achieved by Derks *et al.* (17) during direct fetal infusion with dexamethasone.

The long-term implications of the findings of this study for normal fetal neural and cardiovascular development and for subsequent survival of the offspring in the perinatal period are unclear. NPY is the most abundant peptide in the brain and heart (46), and modulation of NPY activity by dexamethasone may have both central and cardiovascular effects. In fetal sheep, increases in immunoreactive NPY levels have been demonstrated in the median eminence and PVN of the hypothalamus with gestation, cortisol infusion and maternal undernutrition (47). In a similar way fetal treatment with dexamethasone may modify hypothalamic NPY content with possible sequelae for hypothalamo-pituitary function during prepartum maturation (15) and in response to perinatal stress. Because hypothalamic NPY is important in the regulation of hunger (48), there may also be implications for postnatal feeding behavior. NPY may also have important effects on cardiovascular development. These include promoting angiogenesis by activation of Y_2 receptors (49), and regulating neonatal myocyte proliferation and hypertrophy by Y_5 receptor-mediated activation of mitogen-activated protein kinases (50).

In conclusion, the data presented in the present study show significant differences in the basal concentrations of NPY between maternal and fetal ovine plasma. While maternal concentrations of NPY were unaltered from baseline, a significant increase in fetal plasma NPY occurred during acute hypoxemia. This increase in fetal plasma NPY was significantly enhanced during fetal exposure to dexamethasone. These data suggest that NPY may play a role in mediating the fetal cardiovascular response to acute hypoxemia and that fetal exposure to glucocorticoids modifies the fetal plasma NPY responses to acute hypoxemia.

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