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Chemoreflex Contribution to Adrenocortical Function during Acute Hypoxemia in the Llama Fetus at 0.6 to 0.7 of Gestation*

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ABSTRACT

This study tested the hypothesis that the fetal llama, a species adapted to the chronic hypoxia of life at high altitude, demonstrates a potent carotid chemoreflex influence on adrenocortical responses during acute hypoxemia. Plasma ACTH and cortisol concentrations, and mesencephalic and adrenal blood flows were measured during a 1-h period of acute hypoxemia in six intact and four carotid sinus-denervated llama fetuses at 0.6–0.7 of gestation. Fetal PaO_2 was reduced from approximately 23 to about 14 mm Hg in both intact and carotid-denervated groups during acute hypoxemia. During hypoxemia, fetal plasma ACTH, adrenal blood flow, and, therefore, delivery of ACTH to the adrenals increased to similar extents in both intact and carotid-denervated fetal llamas. Despite this, the increase in plasma cortisol in hypoxemia in intact fetuses was absent in carotid-denervated fetuses. In addition, the increase in delivery of cortisol to the mesencephalon calculated in intact fetuses during hypoxemia did not occur in the carotid-denervated group. These data suggest that the integrity of the carotid chemoreceptors is indispensable to cortisol release during acute hypoxemia in the llama fetus, even at 0.6–0.7 of gestation. (*Endocrinology* **139:** 2564–2570, 1998)

THE CHRONIC hypobaric hypoxia of pregnancy at high altitude blunts fetal adrenocortical sensitivity, as sheep undergoing pregnancy at altitude have a markedly attenuated cortisol response to an ACTH challenge compared with lowland control animals (1).

In lowland fetal and newborn animals, a component of adrenocortical sensitivity is regulated via neural pathways. Stimulation of the preganglionic splanchnic sympathetic innervation to the adrenal glands markedly augments the adrenal cortisol response to exogenous ACTH in conscious hypophysectomized calves (2), and section of the splanchnic nerves attenuates the sensitivity of this steroidogenic response in both calves (3) and lambs (4). In late gestation fetal sheep, section of the carotid sinus nerves reduces the cortisol response to acute hypoxemia, without affecting the increase in plasma ACTH (5). Similarly, section of the splanchnic nerves attenuates the cortisol response to acute hypotension without affecting the increase in ACTH in fetal sheep (6). Therefore, Giussani *et al.* (5, 7) proposed that during acute stress in late gestation fetal sheep, a reflex arc initiated by the carotid chemoreceptors and mediated via splanchnic nerve efferents operates to increase cortisol secretion, either directly and/or by enhancing adrenocortical sensitivity to circulating ACTH.

In the fetus, blunting of adrenocortical sensitivity to circulating ACTH may be an appropriate adaptive response to prolonged episodes of hypoxemia, such as those associated with pregnancy at altitude, to protect sensitive maturing tissues from elevated plasma cortisol levels during fetal development. However, the question then arises as to how the altitude-adapted fetus can elicit an adequate cortisol response during a superimposed acute stress, such as may occur during labor and delivery. We propose that to elicit a cortisol response of appropriate magnitude in fetuses with blunted adrenocortical sensitivities, one strategy may be to increase acutely the gain of neural influences on adrenocortical function. Therefore, removal of neural influences on adrenocortical function may have more profound effects during acute stress in highland than in lowland species. The llama (*Lama glama*) is a species that has evolved under the influence of high altitude hypoxia and demonstrates genetic adaptations that persist even when animals are born and living at sea level (8, 9). This study tested the hypothesis that the llama fetus demonstrates a potent carotid chemoreflex influence on adrenocortical function during acute hypoxemia by investigating the effect on ACTH and cortisol responses to acute hypoxemia of carotid sinus nerve section during late gestation.

Some of the results reported in this study have been previously published as abstracts (10).

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Materials and Methods

Use of animals

We studied 10 pregnant llamas at 0.6–0.7 of gestation [gestational age estimated from fetal body weights, 2487 ± 196 g (mean ± SEM), where term is 7000–8000 g; ~350 days] (11) obtained from Lampa, a city 580 m above sea level. Upon arrival in Santiago (585 m above sea level), the llamas were housed in a open yard with access to food and water *ad libitum*, and they were familiarized with the study metabolic cage and the laboratory conditions for 1–2 weeks before surgical instrumentation.

Surgical preparation

Maternal and fetal surgeries were carried out on consecutive days using well established techniques previously described in detail (9, 12). In brief, after food and water deprivation for 24 h, the llamas were premedicated with atropine (1 mg, im; Atropina Sulfato, Laboratorio Chile, Santiago, Chile). Polyvinyl catheters (id, 1.3 mm) were placed in the maternal descending aorta and inferior vena cava via a hindlimb artery and vein under light general anesthesia (10 mg/kg ketamine, im; Ketostop, Drug Pharma-Invetec, Santiago, Chile) with additional local infiltration of lidocaine (2% lidocaine hydrochloride; Dimecaína, Laboratorio Beta, Santiago, Chile). The catheters were then tunnelled sc to exit at the maternal flank.

The following day the fetuses were instrumented under maternal general anesthesia [5–7 mg/kg sodium thiopentone (Tiopental Sódico, Laboratorio Astorga, Santiago, Chile) for induction and 1% halothane in 50:50 O_2 and N_2O for maintenance] according to one of two protocols chosen at random. In the intact group (n = 6) after a midline laparotomy, a fetal hind limb was withdrawn through a small hysterotomy. Polyvinyl catheters (id, 0.8 mm) were inserted into the fetal aorta via a hindlimb artery and into the inferior vena cava via a hindlimb vein. The fetal head was exposed through a second hysterotomy, and a catheter (id, 0.8 mm) was inserted into a carotid artery. Another catheter was placed in the amniotic cavity. The uterine incisions were closed in layers, and all vascular catheters were filled with heparinized saline (200 IU heparin in 0.9% NaCl), plugged with a copper pin, exteriorized through a maternal flank, and kept in a pouch sewn onto the maternal skin. In the denervated group (n = 4), the same surgical procedures were followed, but, in addition, the fetal carotid sinus nerves were cut bilaterally, as previously described (9).

During surgery, all animals were continuously hydrated with warm 0.9% NaCl solution (15–20 ml/kg·h) to compensate for any fluid loss. At the end of surgery and daily after surgery for 5 days, 1 million U penicillin (Penicilina G Sodica, Laboratorio Chile) and 500 mg kanamycin (Canamicina Sulfato, Laboratorio Chile) were administered in the amniotic fluid via the intraamniotic catheter. After surgery, the animals were returned to the yard, and at least 4 days of postoperative recovery were allowed before the beginning of the experiments. Vascular catheters were maintained patent by daily flushing with heparinized saline.

All animal care procedures and experimentation were conducted in accordance with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society and The British Animals (Scientific Procedures) Act, 1986.

Experimental procedure

The experiments were based on a 3-h protocol divided into three periods of 60 min: 1-h normoxia, 1-h hypoxemia, and 1-h recovery. A transparent polyethylene bag was placed over the llama's head into which known concentrations of O_2, N_2 and CO_2 were passed at a rate of about 35 liters/min. After 1 h of breathing air (normoxia), fetal hypoxemia was induced by reducing the maternal inspired fraction of O_2 to reduce fetal PaO_2 to between 12–15 mm Hg (hypoxemia). Maternal and fetal isocapnia were maintained by adding about 1% CO_2 to the maternal inspirate. After the hour of fetal hypoxemia, the llama was returned to breathing air for an additional 60 min (recovery).

Maternal and fetal arterial blood samples (0.5 ml) were taken after 15 and 45 min of normoxia, at 15-min intervals throughout the hypoxemic period, and after 15 and 45 min of recovery to measure arterial blood gases and pH (BMS 3 MKS Blood microsystem and PHM 73 Blood Gas monitor, Radiometer, Copenhagen, Denmark; measurements corrected to 39 C), percent saturation of hemoglobin, and hemoglobin concentration (OSMS hemoximeter, Radiometer). In addition, five 1.5-ml arterial blood samples were collected simultaneously from mother and fetus for measurement of maternal and fetal plasma concentrations of ACTH and cortisol. These blood samples were collected after 15 and 45 min of normoxia, after 15 (early) and 45 (late) min of hypoxemia, and after 45 min of recovery.

Blood samples for hormone analyses were collected under sterile conditions into syringes without anticoagulant and transferred into polypropylene tubes coated with EDTA (1 mg/ml) that were kept on ice. The samples were then centrifuged at 12,000 rpm for 1 min, and plasma was removed, aliquoted, and stored at −20 C until assayed. Hormone assays were performed within 2 months of plasma collection.

Hormone analyses

Maternal and fetal plasma ACTH and cortisol concentrations were measured by RIA, as previously described (5).

ACTH. Plasma ACTH was measured in duplicate using a double antibody ^{125}I RIA. All reagents were purchased in kit form from Diagnostics Products Corp. (Llambers, UK). Duplicate 100-μl plasma samples were incubated with 100 μl anti-ACTH antiserum and 100 μl [^{125}I]ACTH for 24 h. The bound and free hormone fractions were separated by mixing with 1.0 ml second antibody-polyethylene glycol solution. After centrifugation, the precipitate was retained for counting. The interassay coefficients of variation for three controls (26, 94, and 296 pg/ml) were 12.9%, 8.2%, and 6.7%, respectively. The lower limit of detection of the assay (90% bound/free ratio) was 8 pg/ml. The anti-ACTH antiserum showed 0.2% cross-reactivity against αMSH and no detectable cross-reactivity against β-endorphin, neurotensin, substance P, or somatostatin.

Cortisol. Plasma cortisol concentrations were measured in duplicate by RIA using tritium-labeled cortisol as tracer (13). Duplicate 50-μl plasma samples were mixed with an equal volume of sodium carbonate solution (1.7 M; pH 10.5) and extracted with 2 ml diethyl ether. After freezing, the ether was decanted and evaporated, and the residue was reconstituted in 500 μl PBS. Aliquots of varying volumes (depending on expected results from pilot studies) were removed, made up to 400 μl with PBS, and incubated with 16,000 dpm [1,2,6,7-^3H]cortisol (Amersham International, Aylesbury, UK) and 100 μl anticortisol antiserum (Steranti, St. Albans, UK). Bound and free cortisol were separated using dextran-coated charcoal, and after centrifugation, a 500-μl aliquot was removed for measuring the radioactivity content. Recoveries averaged 90%. The interassay coefficients of variation were 11.2%, 7.4%, and 6.8% for three control plasma pools (18.7, 46.5, and 88.6 nmol/liter). The lower detection limit of the assay (90% bound/free ratio) was 30 fmol/ml. The anticortisol antiserum showed the following cross-reactivities: 21-deoxycortisol, 50.8%; 11-deoxycortisol, 15.3%, corticosterone, 2.8%, cortisone, 2.0%; deoxycortisone, less than 0.6%, aldosterone, less than 0.6%, progesterone, 2.4%; 17β-estradiol, less than 0.6%; and estrone, less than 0.6%.

Fetal cardiovascular variables

Fetal heart rate, fetal arterial and venous blood pressures, and amniotic pressure were recorded continuously during the experimental protocol. Fetal combined ventricular output and adrenal blood flow were measured after 45 min of normoxia and after 15 (early) and 45 (late) min of hypoxemia by injection of radionuclide-labeled microspheres (^{57}Co, ^{113}Sn, and ^{46}Sc, respectively; New England Nuclear, Boston, MA) into the inferior vena cava. Reference samples were drawn from the fetal ascending and descending aorta at a rate of 3.24 ml/min for 1.5 min (14).

On completion of the experiments, the llama was anesthetized with sodium thiopental iv and killed with saturated potassium chloride.

Measurements and calculations

Fetal arterial and venous pressures were corrected for amniotic pressure. Fetal perfusion pressure was calculated by subtracting corrected fetal venous pressure from corrected fetal arterial pressure.

At postmortem, fetal tissues were dissected out. These tissues were carbonized, ground into a course powder, and placed in labeled vials,

and any radioactivity was counted with a multichannel γ pulse height analyzer (Mimaxi 5000, Packard, Downers Grove, IL).

Fetal combined ventricular output was calculated as the sum of blood flows to all organs. The distribution of blood flow during acute hypoxemia in the llama fetus was presented in detail previously (9). In the present study, only combined adrenal blood flow and mesencephalic blood flow are presented. Vascular resistance in the fetal adrenal and in the fetal mesencephalon were calculated by dividing fetal perfusion pressure at the times of microsphere injection by fetal adrenal and mesencephalic blood flows, respectively. In addition, fetal ACTH delivery to the adrenals and fetal cortisol delivery to mesencephalon were calculated at the times of microsphere injections by multiplying the measured hormone concentration by the respective organ blood flow.

Statistical analyses

Values for all variables are expressed as the mean ± SEM. All measured variables were compared between normoxia and hypoxemia/recovery using one way-ANOVA followed by the Student-Newman-Keuls test or using Student's *t* test for paired data with the Bonferroni correction. Comparisons between maternal and fetal plasma hormone concentrations or between intact and denervated fetuses were made using Student's *t* test for unpaired data. For all statistical comparisons, differences were considered significant at $P < 0.05$.

Results

Arterial blood gas status

Maternal. Changes in maternal arterial blood gas status are shown in Table 1. During normoxia, values for maternal pHa, PaO_2, $PaCO_2$, and hemoglobin saturation and concentration were similar for both groups, although the arterial O_2 content was lower in mothers of carotid-denervated fetuses. During hypoxemia, a similar decrease in maternal PaO_2 and hemoglobin saturation and a similar increase in hemoglobin concentration were measured for both groups of animals, although values for arterial O_2 content during hypoxemia were lower in mothers of carotid-denervated fetuses than in mothers of intact fetuses. These changes during hypoxemia occurred without alterations in maternal pHa or $PaCO_2$ from baseline. Any changes in maternal variables measured during hypoxemia returned to normoxic levels during recovery in both groups of animals.

Fetal. Changes in fetal arterial blood gas status are shown in Table 2. During normoxia, all variables were similar for intact and carotid-denervated fetuses. During hypoxemia, PaO_2 and hemoglobin saturation fell to similar levels in both groups; however, the fall in arterial O_2 content was larger in the carotid-denervated fetuses. An increase in hemoglobin concentration was only detected in the intact group of fetuses during hypoxemia. Although fetal $PaCO_2$ values were maintained during hypoxemia in both intact and carotid-denervated fetuses, a fall in pHa occurred in both groups by the end of the hypoxemic episode. Fetal pHa values remained depressed during the recovery period in both groups. All other measured variables returned to prehypoxemic levels in both intact and carotid-denervated fetuses.

Endocrine responses

Plasma ACTH concentrations. Plasma ACTH concentrations were similar in mothers of both intact and carotid-denervated fetuses and did not change from baseline throughout the experimental protocol (Fig. 1). In contrast, a pronounced increase in fetal plasma ACTH concentrations was measured in both intact and carotid-denervated fetuses during hypoxemia. The increase in fetal plasma ACTH during hypoxemia was similar in intact and carotid-denervated fetuses. However, although fetal plasma ACTH concentrations fell toward normoxic levels in intact fetuses, fetal plasma ACTH remained elevated in carotid-denervated fetuses during recovery.

Plasma cortisol concentrations. During hypoxemia, an increase in plasma cortisol concentrations was measured in mothers of intact and carotid-denervated fetuses. The magnitude of the increase in plasma cortisol during hypoxemia was greater in mothers of carotid-denervated fetuses, but this difference did not reach significance. Although maternal plasma cortisol concentrations returned toward normoxic values in the intact group, they remained elevated in the carotid-denervated group during recovery (Fig. 1).

TABLE 1. Maternal blood gases, pH, and hemoglobin in normoxia and hypoxemia in the llama

	Normoxia	Hypoxemia		Recovery
		Early	Late	
Intact				
pHa	7.41 ± 0.01	7.39 ± 0.02	7.39 ± 0.04	7.38 ± 0.02
$PaCO_2$ (mm Hg)	35.0 ± 1.0	36.0 ± 1.9	32.3 ± 1.9	32.0 ± 0.7
PaO_2 (mm Hg)	96.0 ± 2.5	31.0 ± 3.1[a]	31.0 ± 2.7[a]	90.0 ± 6.3
Hb saturation (%)	98.7 ± 1.0	63.0 ± 9.1[a]	62.1 ± 8.1[a]	99.7 ± 0.2
Hb concentration (g/dl)	11.2 ± 0.9	13.6 ± 1.3[a]	13.1 ± 1.1[a]	11.5 ± 0.7
O_2 content (ml O_2/dl)	14.6 ± 1.2	10.9 ± 1.2[a]	11.3 ± 1.1[a]	15.2 ± 0.6
Denervated				
pHa	7.42 ± 0.01	7.40 ± 0.05	7.36 ± 0.05	7.39 ± 0.02
$PaCO_2$ (mm Hg)	35.0 ± 1.5	34.0 ± 3.2	37.0 ± 3.5	33.0 ± 1.7
PaO_2 (mm Hg)	99.0 ± 2.5	29.0 ± 1.1[a]	29.0 ± 3.9[a]	93.0 ± 3.3
Hb saturation (%)	100.0 ± 0	45.0 ± 4.7[a]	50.0 ± 6.0[a]	100.0 ± 0.01
Hb concentration (g/dl)	8.8 ± 0.5	12.6 ± 0.2[a]	12.8 ± 0.4[a]	10.5 ± 0.3
O_2 content (ml O_2/dl)	11.8 ± 0.7[b]	7.5 ± 0.6[a,b]	8.2 ± 0.9[a,b]	14.0 ± 0.4

Values are the mean ± SEM during normoxia, at 15 (early) and 45 (late) min of hypoxemia, and during recovery. pHa, arterial pH; $PaCO_2$, arterial CO_2 partial pressure; PaO_2, arterial O_2 partial pressure; Hb, hemoglobin.

[a] Normoxia *vs.* hypoxemia/recovery.

[b] $P < 0.05$, intact *vs.* denervated.

TABLE 2. Fetal blood gases, pH, hemoglobin, blood pressure, and heart rate during the experiment in the llama

	Normoxia	Hypoxemia			Recovery
		5	Early	Late	
Intact					
pHa	7.35 ± 0.01		7.25 ± 0.02	7.24 ± 0.02[a]	7.18 ± 0.01[a]
$PaCO_2$ (mm Hg)	41.0 ± 1.2		43.0 ± 2.5	36.0 ± 1.9	37.0 ± 1.8
PaO_2 (mm Hg)	24.0 ± 0.9		14.0 ± 0.6[a]	13.0 ± 0.9[a]	23.0 ± 1.2
Hb saturation (%)	55.0 ± 3.3		18.0 ± 1.7	17.0 ± 1.7[a]	49.0 ± 2.6
Hb concentration (g/dl)	12.0 ± 0.3		14.0 ± 0.4	13.0 ± 0.5	11.0 ± 0.6
O_2 content (ml O_2/dl)	9.0 ± 0.4		3.4 ± 0.5[a]	3.5 ± 0.5[a]	7.3 ± 0.6
Fetal perfusion pressure (mm Hg)	37.8 ± 3.3	33.7 ± 5.8	45.2 ± 4.9[a]	39.7 ± 4.7	34.4 ± 0.9
Fetal heart rate (beats/min)	113 ± 6	88 ± 4[a]	116 ± 14	124 ± 9	135 ± 3[a]
Denervated					
pHa	7.36 ± 0.01		7.30 ± 0.01	7.17 ± 0.02[a,b]	7.11 ± 0.02[a,b]
$PaCO_2$ (mm Hg)	42.0 ± 1.3		38.0 ± 2.5	41.0 ± 3.1	41.0 ± 1.5
PaO_2 (mm Hg)	23.0 ± 0.7		13.0 ± 0.7[a]	15.0 ± 1.6[a]	27.0 ± 0.7[a,b]
Hb saturation (%)	60.0 ± 3.7		16.0 ± 2.5[a]	19.0 ± 4.2[a]	50.0 ± 5.6
Hb conc. (g/dl)	10.0 ± 0.4		11.0 ± 0.3[b]	11.0 ± 0.3[b]	11.0 ± 0.3
O_2 content (ml O_2/dl)	8.0 ± 0.3		2.3 ± 0.3[a,b]	3.0 ± 0.7[a,b]	8.3 ± 0.5
Fetal perfusion pressure (mm Hg)	39.3 ± 1.3	49.4 ± 4.1	57.9 ± 3.4[a,b]	45.6 ± 5.3	47.0 ± 3.3[b]
Fetal heart rate (beats/min)	116 ± 3	107 ± 5[b]	130 ± 8	121 ± 7	131 ± 6[a]

Values are the mean ± SEM during normoxia, at 15 (early) and 45 (late) min of hypoxemia, and during recovery. For cardiovascular variables an additional mean ± SEM value is presented calculated after 5 min of the onset of hypoxemia. pHa, Arterial pH; $PaCO_2$, arterial CO_2 partial pressure; PaO_2, arterial O_2 partial pressure; Hb, hemoglobin.

[a] $P < 0.05$ normoxia *vs.* hypoxemia/recovery.

[b] $P < 0.05$ intact *vs.* denervated.

Fetal plasma cortisol concentrations increased from baseline in early and late hypoxemia and remained elevated during the recovery period in intact fetuses. In contrast, despite elevated fetal plasma ACTH concentrations during both hypoxemia and recovery, fetal plasma cortisol concentrations remained unchanged from baseline throughout the experimental protocol in carotid-denervated fetuses.

Fetal cardiovascular changes

Perfusion pressure and heart rate. Detailed analyses of the fetal cardiovascular changes during acute hypoxemia have been previously reported (9). During normoxia, fetal perfusion pressure and fetal heart rate were similar in intact and carotid-denervated groups of fetuses. During acute hypoxemia, a rapid and transient fall in fetal heart rate occurred in intact fetuses, but not in carotid-denervated fetuses (Table 2). After the hypoxemic period, tachycardia was measured in both intact and carotid-denervated fetuses. A transient increase in fetal perfusion pressure was also measured during early hypoxemia in both intact and carotid-denervated fetuses. Although fetal perfusion pressure returned toward normoxic values in intact fetuses, it remained elevated in carotid-denervated fetuses during recovery (Table 2).

Combined ventricular output, adrenal blood flow, and adrenal ACTH delivery. During normoxia, values for fetal combined ventricular output, combined adrenal blood flow, adrenal vascular resistance, and delivery of ACTH to the fetal adrenals were similar in intact and carotid-denervated fetuses (Fig. 2). During hypoxemia, fetal combined ventricular output remained unchanged from baseline in both intact and carotid-denervated fetuses. In contrast, a pronounced increase in combined adrenal blood flow and a fall in combined adrenal vascular resistance occurred in both groups of fetuses during early and late hypoxemia. These changes were accompanied by an increase in ACTH delivery to the fetal adrenals during hypoxemia that was similar in intact and carotid-denervated fetuses (Fig. 2).

Mesencephalic blood flow and cortisol delivery. During normoxia, blood flow, vascular resistance, and cortisol delivery to the fetal mesencephalon were similar in intact and carotid-denervated fetuses (Fig. 3). During hypoxemia, mesencephalic blood flow and vascular resistance were maintained in both intact and carotid-denervated fetuses. In contrast, although a sustained increase in cortisol delivery to the mesencephalon was measured in early and late hypoxemia in intact fetuses, these increases did not occur in carotid-denervated fetuses (Fig. 3).

Discussion

The data reported in the present manuscript suggest that section of the carotid sinus nerves in the llama fetus completely prevented the cortisol response to acute hypoxemia, without affecting 1) the increase in fetal plasma ACTH concentrations, 2) the increase in fetal combined adrenal blood flow and the fall in combined adrenal vascular resistance, and, thus, 3) plasma ACTH delivery to the fetal adrenals. These results suggest that there is an important carotid chemoreflex component to cortisol release during acute hypoxemia in the llama fetus, which is well developed by 0.6–0.7 of gestation.

The situation of moderate sustained hypoxia, such as that associated with residence at high altitude, constitutes an environmental stress that at one time was considered harmless to the fetus. However, more recent observations indicate that, although it does not immediately threaten the life of the fetus, moderate hypoxia produces long term effects on development (for reviews, see Refs. 15–17). Although such alterations often do not become apparent until postnatal life,

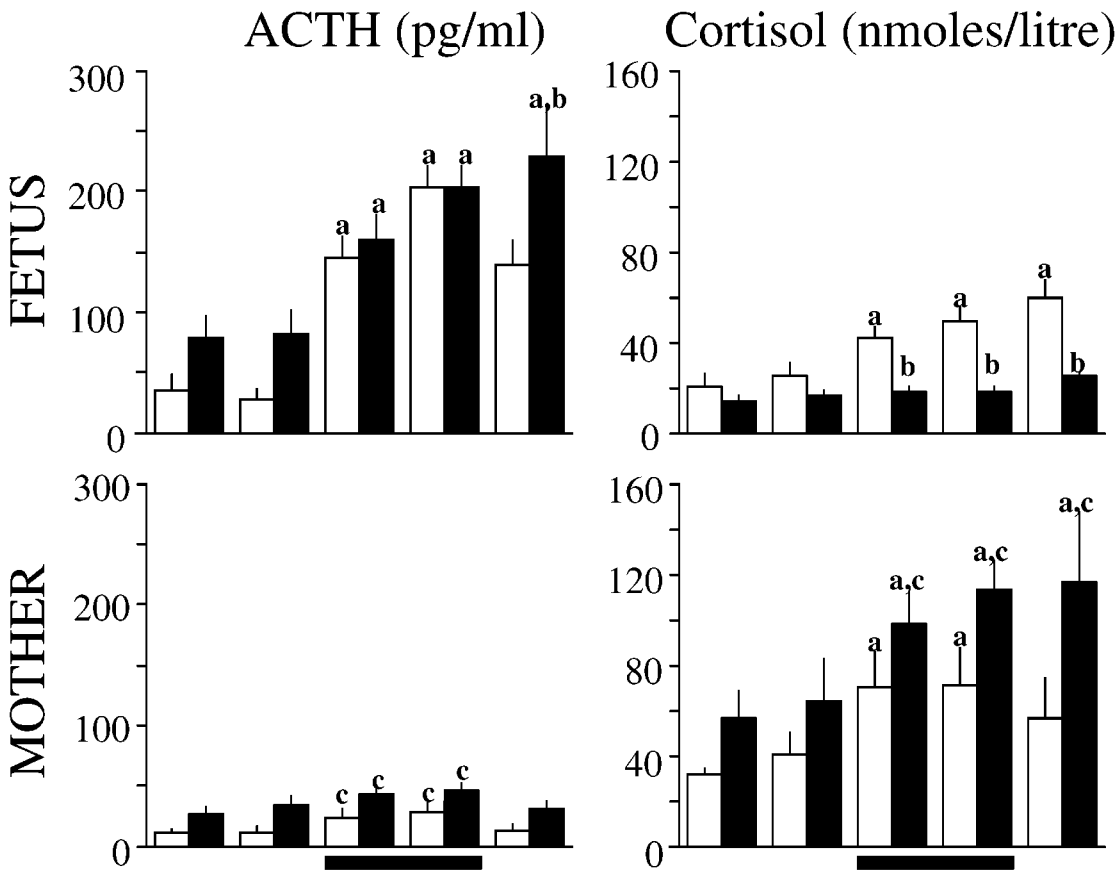

FIG. 1. Plasma concentrations of ACTH (picograms per ml) and cortisol (nanomoles per liter) in six intact llama fetuses and their mothers (*white bars*) and four denervated llama fetuses and their mothers (*black bars*). Plasma samples were taken after 45 min of normoxia, after 15 and 45 min of hypoxemia (*bar*), and after 45 min of recovery. Significant differences ($P < 0.05$) are: a, normoxia *vs.* hypoxemia/recovery; b, intact *vs.* denervated; and c, mother *vs.* fetus.

it has been suggested (15–17) that these disturbances may have their origin in subtle changes that occur during fetal life. For example, the human hypothalamo-pituitary-adrenal system demonstrates pronounced functional disturbances during postnatal growth at altitude; the adrenal gland undergoes hypertrophy within the first week after birth and is hyperactive from early postnatal life into adulthood (18). However, the effects of high altitude on fetal adrenocortical function have been little addressed.

Acute stress in the adult and the fetus is accompanied by increased cortisol release from the adrenal cortex. In the sheep fetus, this has been demonstrated using acute hypotension (19, 20), acute hemorrhage (21, 22), and acute hypoxemia (5, 23, 24). Although increased cortisol release is assumed to be largely determined by an increase in the plasma ACTH concentration, independent observations in both fetal and postnatal animals suggest an important neural component to cortisol release that may be initiated by a carotid chemoreflex under situations of acute hypoxemia (see introduction). Our data suggest that this chemoreflex modulation of cortisol release during acute hypoxemia is greater in the llama fetus than in the sheep fetus, as carotid denervation not only attenuated [as in the sheep fetus (5)] but completely prevented the increase in cortisol release during acute hypoxemia in the llama fetus, without affecting the ACTH response. This difference between the sheep (5) and llama (present study) fetus is further highlighted when considering that 1) the magnitude and duration of hypoxemia were similar in the two studies; 2) although the sheep study was carried out at 0.9 of gestation, the llama study was carried out at 0.6–0.7 of gestation; and 3) plasma ACTH and cortisol concentrations in both studies were determined by the same RIA techniques.

Blunting of basal fetal adrenocortical sensitivity to ACTH during pregnancy at high altitude (1) suggests that to elicit a cortisol response of appropriate magnitude to a superimposed acute stress, such as may occur during labor and delivery, greater amounts of ACTH, an increased bioactivity of ACTH, and/or an acute increase in the sensitivity of the adrenal cortex to circulating ACTH during the period of acute stress are required. A greater chemoreflex influence on cortisol release during acute hypoxemia in the llama fetus than in the sheep fetus provides support for one of these strategies. Additional evidence suggests that high altitude may modify neural, rather than endocrine, regulation of adrenocortical function. First, adrenal glands of life-long human high altitude residents are larger than those of age- and socially matched lowland controls (25). Neural regulation of adrenal growth is well established (26), and it may be that this plays a key role in this effect. Secondly, sustained hy-

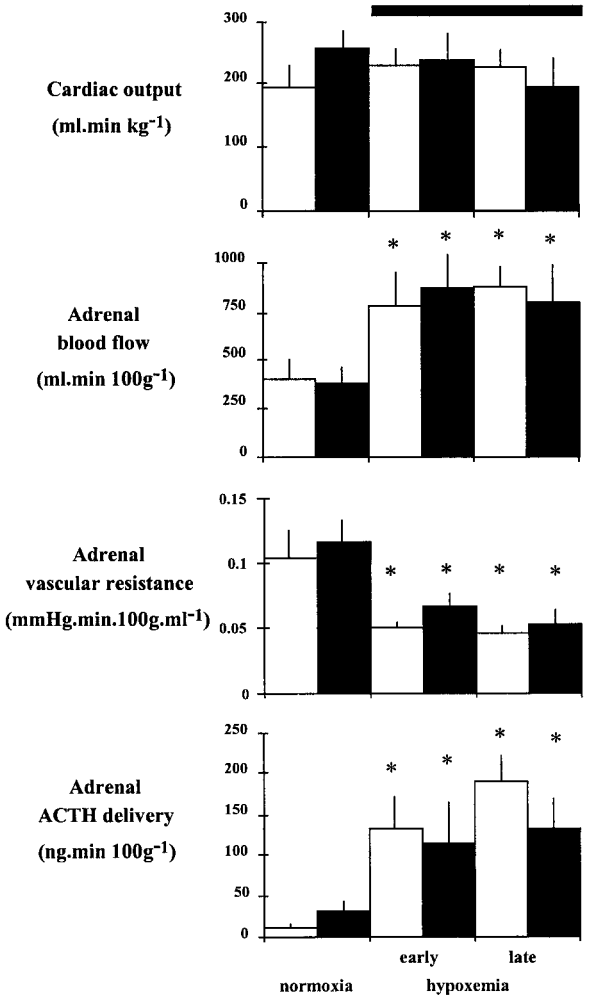

FIG. 2. Cardiac output, adrenal blood flow, adrenal vascular resistance, and ACTH delivery to the adrenals in intact (*white bars*) and denervated (*black bars*) llama fetuses during normoxia and after 15 (early) and 45 (late) min of hypoxemia (*bars*). Values are the mean ± SEM for each of the experimental periods. *, $P < 0.05$, normoxia *vs.* hypoxemia.

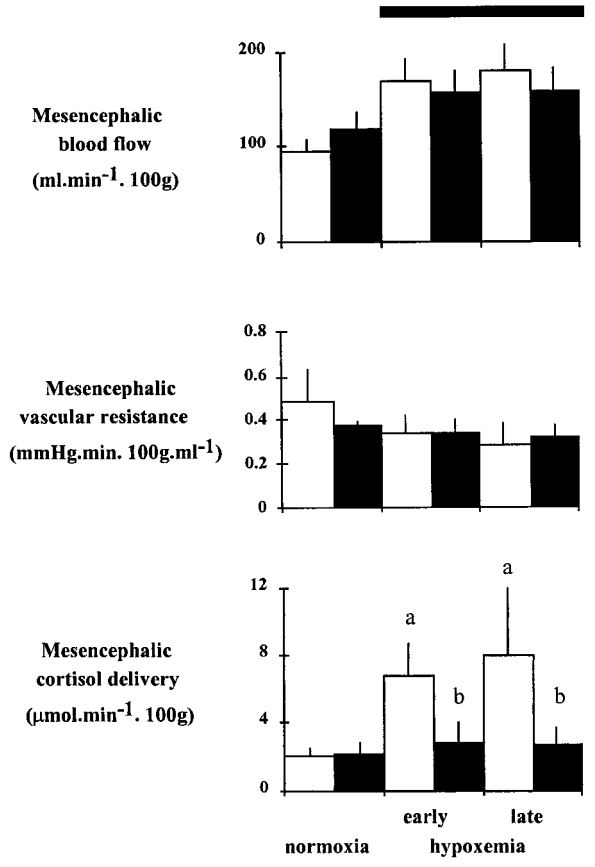

FIG. 3. Mesencephalic blood flow, vascular resistance, and cortisol delivery in intact (*white bars*) and denervated (*black bars*) llama fetuses during normoxia and after 15 (early) and 45 (late) min of hypoxemia (*bars*). Values are the mean ± SEM for each of the experimental periods. Significant differences ($P < 0.05$) are: a, normoxia *vs.* hypoxemia; and b, intact *vs.* denervated.

poxemia in the sheep fetus is associated with a return of plasma ACTH concentrations toward baseline and a persistence of elevated cortisol concentrations (27, 28), and the integrity of the splanchnic nerves is necessary to maintain this elevated cortisol level (29).

The mechanisms of neurally mediated influences on adrenocortical function remain unclear. Edwards and colleagues (30, 31) suggested that enhancement of the steroidogenic response to exogenous ACTH during stimulation of the splanchnic nerves may be accounted for in part by an increase in the rate at which ACTH is presented to the gland secondary to an increase in adrenal blood flow. However, the enhancement of the glucocorticoid output that occurs during splanchnic nerve stimulation exceeds that which may result from the rise in adrenal ACTH presentation (31). Furthermore, in the present study, section of the carotid sinus nerves in the llama fetus completely prevented the cortisol response to acute hypoxemia without affecting the increase in adrenal blood flow. Combined, these studies strongly support the idea that neural regulation of adrenal cortisol release can be mediated by mechanisms other than those purely acting via changes in adrenal blood flow. Several neuropeptides, including vasoactive intestinal polypeptide (32) and CRH (33), can stimulate an increase in adrenal glucocorticoid output, and intense CRH immunoreactivity has been demonstrated at the cortico-medullary interface of adrenal glands and the splanchnic nerves of fetal sheep in late gestation (34). However, as the administration of a CRH antagonist failed to reduce cortisol output during splanchnic nerve stimulation in hypophysectomized calves that were given ACTH, Jones and Edwards (33) did not favor a role for CRH in mediating the enhanced steroidogenic response during increased neural input to the adrenal cortex, at least in the calf.

Much of the ACTH in the fetal sheep pituitary and plasma is present in higher mol wt precursor forms of lower biological activity than ACTH, such as POMC (35). Processing of POMC to ACTH is gestation dependent (36) and is affected by situations of acute stress produced by hypoglycemia in man (37) and hemorrhage in adult dogs (38) or in fetal sheep in late gestation (39). Hence, the possibility exists that section of the carotid sinus nerves may affect the processing of POMC to ACTH and thus reduce the bioactivity of circulat-

ing ACTH in plasma during hypoxemia, but there have no studies to address this idea.

In the present study, section of the carotid sinus nerves prevented the increase in plasma cortisol but did not affect the increase in plasma ACTH concentrations or the fall in adrenal vascular resistance observed in intact llama fetuses during acute hypoxemia. These data suggest that there is no carotid chemoreflex component to ACTH release or increased adrenal blood flow during acute hypoxemia in the llama fetus. These results support the earlier observations of Itskovitz *et al.* (40) that sino-aortic denervation does not affect the increase in adrenal blood flow during hypoxemia in fetal sheep. These studies suggest that pituitary ACTH release and adrenal vascular responses during hypoxemia are mediated via local mechanisms rather than by chemoreflex responses.

Since carotid sinus nerve section in the llama fetus prevented an increase in plasma cortisol concentrations without affecting blood flow to the mesencephalon during acute hypoxemia, the increase in cortisol delivery to the mesencephalon calculated in intact fetuses during hypoxemia did not occur in carotid-denervated llama fetuses. This reveals a reduced negative feedback influence of cortisol at the hypothalamus and pituitary and may explain at least in part the persistence of elevated plasma ACTH concentrations in carotid-denervated llama fetuses during the recovery period.

In conclusion, carotid denervation in the llama fetus completely prevented a cortisol response to acute hypoxemia without affecting the increase in plasma ACTH concentrations or the fall in adrenal vascular resistance. These data suggest a potent chemoreflex influence on adrenal cortisol release during acute hypoxemia in the llama fetus. The llama fetus offers fruitful avenues for future research on the effects of high altitude on hypothalamo-pituitary-adrenal function.

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