Changes in Adrenocorticotropic and Cortisol Responsiveness after Repeated Partial Umbilical Cord Occlusions in the Late Gestation Ovine Fetus*

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ABSTRACT

Despite many studies reporting fetal ACTH and cortisol (F) responses to acute fetal hypoxemia induced by several methods, effects of repeated short-term fetal hypoxia produced by umbilical cord occlusion (UCO) on ACTH and F are unknown. We examined fetal ACTH and F responses to repeated, controlled, 50% reductions in umbilical arterial blood flow (CUBF) produced by an inflatable cord occluder. Ten sheep fetuses were instrumented at 123–128 days gestation (dGA) with arterial, venous, and amniotic catheters. A common umbilical artery transit-time ultrasound flow probe was implanted to measure CUBF. An inflatable occluder was placed around the proximal portion of the umbilicus. In five fetuses (group I) at 131 ± 1 dGA (mean ± SEM), 12 UCOs (CUBF reduced by 50%), each lasting 5 min separated by 15 min recovery, were performed. Changes in fetal arterial blood gases, pH and plasma ACTH, and F concentrations were determined before, during, and after the 1st, 6th, and 12th UCOs. Sham experiments were conducted on the other five fetuses at 130 ± 1 dGA (group II). In group I, CUBF decreased to 49 ± 1% (mean ± SEM of 12 UCOs). After each UCO, CUBF returned to baseline within 5 min. A modest fall in fetal arterial PO$_2$ and arterial pH (21.2 ± 0.2 to 16.8 ± 0.2 mmHg and 7.33 ± 0.1 to 7.29 ± 0.1, respectively) and a mild increase in fetal PaCO$_2$ (49.9 ± 0.5 to 54.9 ± 0.4 mmHg; mean ± SEM of 12 UCOs) occurred with each UCO. Whereas preocclusion fetal ACTH concentrations increased by the 12th UCO, F remained unchanged. Fetal ACTH increased after the 1st, 6th, and 12th UCOs. Fetal F increased after the 1st and 6th UCOs but not after the 12th UCO. Fetal plasma ACTH and F remained unchanged throughout the experiments in group II fetuses. We conclude that: 1) partial reductions in CUBF induce significant activation of the fetal anterior pituitary-adrenocortical axis in late-gestation fetal sheep; 2) after repeated UCOs, fetal ACTH responsiveness is maintained, but fetal F responses become attenuated. (Endocrinology 138: 259–263, 1997)

IT IS WIDELY accepted that umbilical cord compression is one of the most common causes of variable fetal heart rate decelerations in late gestation. Several conditions associated with oligohydramnions, such as postmaturity, intraterine growth retardation, and advanced labor with ruptured membranes, cause an increased incidence of cord compression. Recurrent variable decelerations are associated frequently with these states. When predisposing conditions such as those described above exist, it is likely that umbilical cord occlusion (UCO) occurs repetitively. This is especially likely during labor. Evaluation of the effects of repeated UCOs on changes in the gain and magnitude of fetal adaptive responses is critically important to understanding fetal physiological and/or pathophysiological responses during gestation and, in particular, during labor.

Several studies have addressed fetal adaptation to a single UCO characterizing changes in fetal oxygenation (1–3), fetal arterial blood pressure and heart rate (1, 4, 5), fetal regional blood flow (2, 3, 5), and fetal plasma catecholamine concentrations (5, 6). However, quite surprisingly, to our knowledge, no study has reported fetal ACTH and cortisol (F) responses to UCO. In contrast, responses in fetal plasma ACTH and F concentrations to challenges such as hypoxia (7, 8), hemorrhage (9), and hypotension (10, 11) are well established for late gestation fetal sheep. Furthermore, only a few studies have investigated fetal responses to repeated UCOs (6, 12–14), but none of these studies have addressed fetal endocrine adaptive responses to controlled partial UCO.

In the late gestation sheep fetus, a 50% reduction of umbilical blood flow by UCO produces modest, but significant, reductions in arterial PO$_2$ (PaO$_2$) by 4–6 mmHg (1, 2). This degree of fetal hypoxemia may stimulate fetal ACTH and F release (15–17). In addition, it has been reported that reductions in venous return produced by fetal inferior vena caval obstruction have stimulatory effects on fetal plasma ACTH concentrations without necessarily concomitant decreases in arterial oxygen saturation (18). It is thus reasonable to hypothesize that partial UCO, which produces both mild hypoxemia and reductions in venous return, will have more pronounced effects on fetal plasma ACTH and F concentrations, compared with similar degrees of hypoxemia induced by other methods such as maternal hypoxemia (16, 19, 20) or uterine blood flow restriction (21–23).

The aims of the present study were to study chronically instrumented fetal sheep at 130 days gestation (dGA): 1) to characterize responses in plasma ACTH and F concentra-
tions to a partial UCO that reduces common umbilical arterial blood flow (CUBF) by 50%; and 2) to examine changes in ACTH and F secretion in response to repeated partial UCOS.

Materials and Methods

Care of animals

Mature Rambouillet-Columbia cross-bred ewes (n = 10) bred on a single occasion only and carrying a fetus of known gestational age were used. All procedures were approved by the Cornell University Animal Care and Use Committee. All facilities were approved by the American Association for the Accreditation of Laboratory Animal Care.

Surgery was performed under halothane general anesthesia at 125 ± 1 (mean ± SEM) dGA (term = 148 days) using techniques that have been described in detail (24). Briefly, polyvinyl catheters were inserted into a maternal carotid artery and jugular vein and advanced into the arch of the aorta and superior vena cava, respectively. The uterus was exposed through a midline abdominal incision. Fetuses were instrumented with polyvinyl catheters inserted via the carotid artery and jugular vein. An amniotic cavity catheter was also placed. An ultrasonic transit-time flow probe (6r Transonic Systems, Inc., Ithaca, NY) was implanted around the common umbilical artery retroperitoneally via the lower flank. An inflatable occluder (OC20HD, In Vivo Metric, Healdsburg, CA) was placed around the proximal end of the umbilical cord and secured onto the fetal abdominal wall.

Post operatively, the ewes were caged individually and given ampicillin (AMP-EQUINE7, SmithKline Beecham, West Chester, PA; 1 g i.v. and 0.5 g intraamniotically) for 5 days. The ewes were fed daily, and water was available ad libitum. All the fetuses were allowed to recover for at least 5 days after surgery before being studied.

Experimental procedure

On the experimental day, the value of CUBF was read from the flow meter every 5 min and recorded from 0900–1000 h. Baseline CUBF was calculated by averaging these recorded values. In five animals (group I), from 1000 h on, the fetus was challenged with 12 UCOS, each lasting 5 min with a 15 min interval. At each occlusion, the umbilical cord was compressed by infusing sterile physiological saline into the occluder to achieve 50% reduction in baseline CUBF (Fig. 1). Fetal and maternal blood samples (0.5 ml) were drawn simultaneously from the carotid artery catheters 1 min before (t = −1) and at the end (t = +5) of each UCO to determine arterial blood gases and pH with a blood gas analyzer (ABL500, Radiometer, Copenhagen; measurements corrected to 39 C). Fetal and maternal arterial blood samples (5 ml) for ACTH and F measurements were also drawn 1 min before (t = −1), at the end of (t = +5), and at 5 (t = +10) and 14 (t = +19) min after the 1st, 6th, and 12th UCOS using aseptic techniques and transferred into chilled polypropylene collection tubes. Blood was centrifuged at 4 C at 1200 × g for 5 min. Plasma was removed, aliquoted, flash-frozen in liquid N2, and stored at −20 C until assayed. Fetal red blood cells from the centrifuged tubes were resuspended in sterile heparinized saline (25 IU/ml) and returned aseptically into the fetal arterial circulation. In the five control animals (group II), the exact same procedures were conducted excluding the saline infusion into the cord occluder.

Mean CUBF was recorded at 1-s intervals continuously throughout the study using a data acquisition system. Three to 4 days after the occlusion or sham protocol, the ewe was euthanized with an overdose of pentobarbital (Fatal-Plus®, Vortech Pharmaceuticals, Dearborn, MI), and the fetal body weight was determined.

RIAs for ACTH and F

Plasma ACTH concentrations were measured with a commercial RIA kit (INCStar Corporation, Stillwater, MN) validated for hormone measurements in sheep plasma (25). Assay sensitivity (90% B/B0) was 9 pg/ml. The intra- and interassay coefficients for variation (C.V.) for quality control samples containing 34.7 pg/ml (pool of the assay kit), 10.9 pg/ml (fetal pool), and 53.9 pg/ml (maternal pool) were 6.8% and 12.5%, 12.8% and 19.0%, and 6.5% and 10.7%, respectively.

Plasma F concentrations were measured with a commercial RIA kit (Diagnostic Products Corporation, Los Angeles, CA) after extraction with methylene dichloride (26). F recovery was determined by mixing sheep plasma pool 1:1 with F solution of known concentration in human plasma (5, 10, 50, 100, 200, and 500 ng/ml). All F-spiked samples were diluted 1:1 with kit zero calibrator to ensure measurements from the linear region of the standard curve. Recovery was 97.4 ± 5.7%. Parallelism was demonstrated by serial dilution of sheep plasma in kit zero calibrator. Intraassay C.V. was 8.8% for sample containing 36.1 ng/ml (n = 20). Interassay C.V. was 2.3% for a quality control sample containing 29.9 ng/ml (n = 20). Assay sensitivity (90% B/B0) was 4.9 ng/ml (n = 27). Both ACTH and F were measured on samples that had not been previously thawed.

Data analysis

CUBF is expressed as a % ± SEM changed from the baseline. Preocclusion, occlusion, and recovery CUBF values were calculated by averaging data between t = −5 and t = 0; t = 0 and t = +5, and t = +10 and t = +15, respectively. Data that were normally distributed were analyzed with parametric statistical comparisons. Data that were not normally distributed were analyzed with distribution-free statistics.

Changes in CUBF during the experimental periods (preocclusion, occlusion, and recovery) were assessed using one-way repeated-measures ANOVA for group I and group II animals.

Values for blood gases, pH and ACTH, and F hormone are presented as means ± SEM at the 1st, 6th, and 12th UCOS. Differences in blood gases and pH between group I and group II fetuses were analyzed using Student’s t test for unpaired data. Preocclusion values for fetal plasma ACTH and F concentrations were compared between group I and group II fetuses at the 1st, 6th, and 12th UCOS using the Mann-Whitney test. Changes in preocclusion values of plasma ACTH and F concentrations in each group were analyzed between the 1st, 6th, and 12th UCOS using one-way repeated-measures ANOVA followed by the Student-Newman-Keuls test. Changes in plasma ACTH concentrations during the 1st, 6th, and 12th UCOS within respective groups also were analyzed using one-way repeated-measures ANOVA followed by the Student-Newman-Keuls test. Changes in plasma F concentrations during UCO at the 1st, 6th, and 12th UCOS in each group were analyzed using the Friedman test followed by the Student-Newman-Keuls test. For all statistical comparisons, significance was set at P < 0.05.

Results

Changes in CUBF during repeated UCOS

The baseline values of CUBF in group I and group II fetuses were 560 ± 83 and 634 ± 122 ml/min, respectively, or 157 ± 23 and 163 ± 28 ml/min/kg, respectively, when standardized to fetal body weight determined at necropsy. In group I fetuses, each of the 12 UCOS produced similar 50%
reductions in CUBF (Fig. 1). For all 12 UCOs, there were no differences in preoclusion, occlusion, or recovery CUBF during the experimental protocol. On average, each UCO produced a fall in CUBF from 97.8 ± 1.1% of the overall baseline (preoclusion) to 48.8 ± 0.6% (occlusion), returning to 97.6 ± 0.9% (recovery). No significant changes in CUBF were detected in group II fetuses during the experimental protocol.

Arterial blood gases and pH

In group I, arterial pH and PaO₂ decreased from 7.33 ± 0.7 to 7.29 ± 0.7 and from 21.2 ± 0.2 to 16.8 ± 0.2 mmHg, respectively, whereas arterial PCO₂ (PaCO₂) increased from 49.9 ± 0.5 to 54.9 ± 0.4 mmHg. The average values for arterial pH and blood gases at the 1st, 6th, and 12th UCOs in group I and II are presented in Table 1. Significant differences in arterial pH and PaO₂ were found between group I and group II fetuses at t = +5 during the 1st, 6th, and 12th UCOs. Significant differences in PaCO₂ were found only between the 1st and 12th UCOs but not at the 6th UCO. No significant changes in arterial blood gases and pH occurred in group II fetuses. Similarly, no changes were observed either in group I or II in maternal arterial blood gases and pH throughout the experimental protocol.

Plasma ACTH and F concentrations

No significant changes were observed in maternal plasma ACTH and F concentrations throughout the experimental protocol either in group I or II (Fig. 2).

There were no differences either in plasma ACTH or F preoclusion concentrations at the 1st UCO between group I (30.5 ± 4.0 pg/ml and 10.9 ± 2.5 ng/ml, respectively) and group II (22.5 ± 2.5 pg/ml and 5.0 ± 1.3 ng/ml, respectively) fetuses. In group I fetuses, significant increases in the preoclusion plasma ACTH concentrations occurred by the 6th and 12th UCOs (P < 0.05). In contrast, no changes in preoclusion plasma F concentrations were determined (Fig. 3A). Fetal plasma ACTH concentrations increased at the 1st, 6th, and 12th UCOs (P < 0.05). In contrast, significant increases in fetal plasma F concentrations occurred only after the 1st and 6th UCOs (P < 0.05) but not after the 12th UCO (Fig. 3A). Plasma ACTH and F remained unchanged from baseline in group II fetuses throughout the experimental protocol.

**Discussion**

Availability of transit time transducers enables instantaneous and continuous measurements of CUBF during reproducible quantitative occlusions of the umbilical cord. In the present study, baseline CUBF did not alter after repeated UCOs in each of the 12 reductions in CUBF. Thus, the same degree of challenge to the fetus occurred with each UCO. Whereas each 5 min, 50% reduction in CUBF produced only mild changes in fetal arterial blood gases and pH, significant activation of the fetal anterior pituitary-adrenocortical axis was observed. Increased fetal plasma ACTH and F concentrations after 50% UCO were similar to changes in plasma...
ACTH and F concentrations measured in the late gestation sheep fetus during 1 h of acute isocapnic hypoxemia induced by lowering the maternal inspired oxygen fraction, which reduced fetal PaO₂ by approximately 10 mmHg (19). The pronounced increases in fetal plasma ACTH and F concentrations during 5-min 50% UCOs in the present study may represent the combined effect of UCO-induced decrease in venous return to the heart in addition to changes in arterial blood gases and pH. Wood and colleagues (18) reported that inferior vena cava occlusion was a powerful stimulus to ACTH secretion, a response that is thought to be mediated, at least in part, by cardiovascular mechanoreceptors.

Despite an elevated baseline in fetal plasma ACTH concentrations by the 12th UCO, significant increases in fetal plasma ACTH were observed at the 1st, 6th, and 12th UCOs. This suggests that the fetal anterior pituitary responsiveness to 50% reduction in CUBF was maintained after 12 repeated challenges. In contrast, significant increases in fetal plasma F concentrations were measured only at the 1st and 6th UCOs but not at the 12th UCO despite the absence of an increase in baseline plasma F concentrations by the end of the experimental protocol. This dissociation in responsiveness of the fetal anterior pituitary and fetal adrenal cortex indicates an attenuation of adrenocortical responsiveness to ACTH after repeated partial UCOs.

Although short-term control of adrenocortical activity is widely believed to be largely determined by ACTH both in the adult and the fetus, a number of situations have been described in which adrenal F output and plasma ACTH concentrations are poorly correlated (27). Thus, it has become clear that additional factors other than ACTH may play both stimulatory and inhibitory roles in the control of adrenocortical activity. One such factor is the splanchnic innervation of the adrenal gland, which exerts an important modulating influence (28–31). It has been suggested that F may be released, in the absence of an increase in plasma ACTH, via a reflex pathway. Two critical experiments support this view. First, Edwards & Jones (32) reported that electrical stimulation of the splanchnic nerve in hypophysectomized calves produced a small, but significant, increase in plasma F concentration. Second, electrical stimulation of the splanchnic nerves augments the F response to exogenous ACTH in calves (32).

Section of the carotid sinus nerves in fetal sheep attenuates the increase in fetal plasma F to acute hypoxemia, without affecting ACTH concentration (20). A similar dissociation in plasma ACTH and F, in response to hypotension, occurs in fetal sheep after section of the splanchnic nerves (33). These experiments, taken together, demonstrate the existence of a neural reflex, possibly initiated by the carotid chemoreceptors and mediated via splanchnic nerve efferents, which may induce F secretion independent of an increase in ACTH and/or sensitize the adrenal cortex to circulating ACTH. It is thus reasonable to hypothesize that repeated UCOs may blunt adrenocortical responsiveness to ACTH by attenuating the stimulating or sensitizing effects of this neural reflex. Similarly attenuated F responses to exogenous ACTH have been demonstrated in late gestation fetal sheep during pregnancy at high altitude (34). In addition, increased contractility, produced by pulsatile maternal iv OT administration throughout the last third of gestation, blunts the F response to acute hypoxemia without affecting fetal plasma ACTH responses (16). Taking past and present studies together, preexposure of the fetus to repeated or long-term stress may alter the responsiveness of the fetal anterior pituitary-adrenal axis to a subsequent acute stress. It is likely that blunted adrenocortical responses are caused by changes in responsiveness at the adrenal, and not at the pituitary, level. It also is possible that the pituitary responsiveness, in addition to adrenal responsiveness, will eventually become attenuated to more prolonged repeated stress than that used in the current experimental paradigm. The mechanism mediating an attenuated adrenocortical response to recurrent or sustained stress is currently unknown. However, recently, it has been reported that ACTH receptor mRNA abundance is significantly lower in fetal sheep with placental restriction than in control fetuses (35). Furthermore, high-altitude long-term hypoxemia induces suppression of in vitro production of cAMP production from adrenocortical tissues in fetal sheep (36). These studies suggest that the attenuation of fetal adrenocortical responsiveness to repeated UCOs observed in the present study could be caused by changes at the receptor and/or the postreceptor levels.
Alternatively, it is possible that attenuation of plasma F responses after repeated UCOs may result from exhaustion of the fetal adrenal cortex steroid synthesizing capacity after repeated stimuli. However, this is unlikely because in sheep, fetal adrenals at this stage of gestation have an ability to maintain elevated plasma F levels for up to 48 h when they are stimulated with sustained hypoxemia (21, 23). The attenuation of the F response to long-term stress or repeated, intermittent stress on sensitive maturing tissues may have considerable protective value. In the rat, exposure of the developing nervous system to inappropriately high doses of glucocorticoid at critical times of prenatal development results in permanent decrease in hippocampal glucocorticoid receptors and a resetting of the hypothalamic-pituitary-adrenal system throughout life (37).

In conclusion, partial UCO produces significant activation of fetal anterior pituitary-adrenocortical axis in fetal sheep at 130 dGA. Although fetal ACTH responsiveness is maintained, an attenuation of fetal plasma F responses occurs after 12 repeated UCOs. Adrenocortical blunting to brief repeated stress may be a classic example of physiological adaptation, which can be demonstrated in the late-gestation sheep fetus.

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