OBSTETRICS

Role of catecholamines in maternal-fetal stress transfer in sheep

Florian Rakers, MD; Sabine Bischoff, DVM; Rene Schiffner, MD; Michelle Haase, MD; Sven Rupprecht, MD; Michael Kiehntopf, MD, PhD; W. Nikolaus Kühn-Velten, MD, PhD; Harald Schubert, PhD; Otto W. Witte, MD, PhD; Mark J. Nijland, PhD; Peter W. Nathanielsz, MD, PhD; Matthias Schwab, MD, PhD

OBJECTIVE: We sought to evaluate whether in addition to cortisol, catecholamines also transfer psychosocial stress indirectly to the fetus by decreasing uterine blood flow (UBF) and increasing fetal anaerobic metabolism and stress hormones.

STUDY DESIGN: Seven pregnant sheep chronically instrumented with uterine ultrasound flow probes and catheters at 0.77 gestation underwent 2 hours of psychosocial stress by isolation. We used adrenergic blockade with labetalol to examine whether decreased UBF is catecholamine mediated and to determine to what extent stress transfer from mother to fetus is catecholamine dependent.

RESULTS: Stress induced transient increases in maternal cortisol and norepinephrine (NE). Maximum fetal plasma cortisol concentrations were 8.1 ± 2.1% of those in the mother suggesting its maternal origin. In parallel to the maternal NE increase, UBF decreased by maximum 22% for 30 minutes ($P < .05$). Fetal NE remained elevated for >2 hours accompanied by a prolonged blood pressure increase ($P < .05$). Fetuses developed a delayed and prolonged shift toward anaerobic metabolism in the presence of an unaltered oxygen supply. Adrenergic blockade prevented the stress-induced UBF decrease and, consequently, the fetal NE and blood pressure increase and the shift toward anaerobic metabolism.

CONCLUSION: We conclude that catecholamine-induced decrease of UBF is a mechanism of maternal-fetal stress transfer. It may explain the influence of maternal stress on fetal development and on programming of adverse health outcomes in later life especially during early pregnancy when fetal glucocorticoid receptor expression is limited.

Key words: catecholamines, fetal programming, fetus, placenta, stress transfer

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According to a recent survey commissioned by the American Psychological Association, chronic psychosocial stress is a growing problem that affects >23% of all women with increasing intensity and prevalence.[1] During pregnancy, maternal psychosocial stress is a major and harmful environmental influence for the fetus that increases the risk of developing neuropsychiatric, metabolic, and cardiovascular diseases in later life.[2-4] It is generally considered that maternal stress is transferred to the fetus by cortisol, the effector hormone of the hypothalamic–pituitary–adrenal axis (HPAA). Inappropriately high fetal plasma levels of cortisol desensitize glucocorticoid receptors (GRs) involved in the negative feedback regulation of the fetal HPAA by epigenetic mechanisms. The resulting decrease in negative feedback regulation leads to increased activity of the HPAA during later life.[5,6] Although the HPAA matures at the end of gestation and central GRs are not widely expressed before midgestation,[7-10] maternal psychosocial stress in early to midgestation has the most pronounced programming effects on HPAA activity[11] and neurobehavioral disturbances in later life.[12] Exactly how maternal cortisol affects activity of the fetal HPAA and programs adverse health outcomes when central GRs are not yet widely expressed remains unclear. Moreover, corticosteroid-binding globulin increases in response to physiological changes in plasma cortisol concentration even before activation of the HPAA suggesting that bioavailability of cortisol during early pregnancy is

From Hans Berger Department of Neurology (Drs Rakers, Schiffner, Haase, Rupprecht, Witte, and Schwab), Institute of Laboratory Animal Sciences and Welfare (Drs Bischoff and Schubert), Institute of Clinical Chemistry and Laboratory Medicine (Dr Kiehntopf), and Center for Sepsis Control and Care (Dr Witte), Jena University Hospital, Jena, and Medical Laboratory Bremen, Bremen (Dr Kühn-Velten), Germany, and Center for Pregnancy and Newborn Research, Department of Obstetrics and Gynecology, University of Texas, San Antonio, School of Medicine, San Antonio, TX (Drs Nijland and Nathanielsz).

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The first 2 authors contributed equally to the study.

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Corresponding author: Florian Rakers, MD. florian.rakers@med.uni-jena.de

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limited.[13] One hypothesis for the pronounced stress effects during early pregnancy suggests that maternal stress is transferred to the fetus by catecholamines as part of the second limb of the stress axis,[3,4] even though catecholamines cannot cross the placenta in sheep or human beings.[14-16] Maternal infusion of catecholamines decreases uterine blood flow (UBF) by increasing the tone of the uterine arteries.[17] A decrease in UBF induced by uterine artery occlusion leads to a sustained increase in fetal catecholamines accompanied by a shift of fetal metabolism toward an anaerobic state, indicating fetal stress.[18] However, the effects of psychological stress on UBF and the consecutive changes in fetal metabolism have not been studied to date.

We hypothesized that maternal psychosocial stress is transferred to the fetus not only by cortisol but also by catecholamine-dependent mechanisms. Although catecholamines do not cross the placenta in significant amounts under physiological conditions,[14-16] maternal stress-mediated endogenous release of catecholamines may reduce UBF. As a consequence, reduced uterine perfusion may induce fetal stress and a shift in fetal metabolism toward an anaerobic metabolic state.[18] Chronic instrumentation of the pregnant ewe and her fetus permits physiological studies without long-term exposure to anesthetics and other pharmacological agents.[19] We studied the maternal and fetal responses to isolation stress in sheep pregnancy following implantation of maternal and fetal vascular catheters and uterine artery flow probes. We used alpha- and beta-adrenergic blockade to determine whether the UBF decrease is catecholamine mediated and the extent to which stress transfer from mother to fetus is catecholamine dependent.

Materials and Methods

Animal care and surgical instrumentation

All procedures were approved by the Thuringia Animal Welfare Committee. Seven Merino Longwool sheep were bred on a single occasion and underwent surgery at 110 ± 1 days of gestation (term, 150 days). Anesthesia was induced by intramuscular injection of 10 mg/kg^{-1} ketamine (Pfizer, Berlin, Germany) and 0.2 mg/kg^{-1} midazolam (Hameln Pharmaceuticals, Hameln, Germany). After orotracheal intubation, anesthesia was maintained by inhalation of 1.5% isoflurane (Actavis, Langenfeld, Germany). Ewes were instrumented with polyvinyl catheters with 1 mm inner (PVC Glasklar; Rüsch, Kernen, Germany) inserted into the carotid artery for blood sampling and maternal blood pressure (MBP) and maternal heart rate (MHR) recordings, and into the jugular vein for intraoperative fluid infusion and postoperative administration of antibiotics and analgesics. After maternal midline abdominal incision and hysterotomy, fetuses were instrumented with polyvinyl catheters in the jugular vein and carotid artery for blood sampling and to record fetal blood pressure (FBP) and fetal heart rate (FHR). A third catheter was placed in the amniotic cavity for postoperative administration of antibiotics and to record amniotic pressure for correction of FBP to the hydrostatic pressure. To determine UBF, a uterine flow probe (Animal Blood Flowmeter T 206, Transonic, Ithaca, NY) was placed around the uterine artery that supplied the pregnant horn.

Postsurgery ewes and fetuses received 1.0 g of ampicillin (Ratiopharm, Ulm, Germany) twice daily for 3 days together with 30-50 mg/kg^{-1} metamizol (WDT, Garbsen, Germany) for analgesia. All catheters were kept patent by infusion of 0.5 mL/h^{-1} saline containing 12.5 IU heparin/mL (Rotexmedica, Trittau, Germany). Animals were allowed to recover from the surgical procedure for 5 days.

Experimental protocol

At 115 ± 1 days of gestation, ewes underwent isolation stress for 2 hours involving no visual, auditory, or sensory contact with any other animals.[11] At 20 minutes before and during the isolation procedure, MBP, MHR, FBP, FHR, amniotic pressure, and UBF were recorded continuously. All biophysical variables were amplified and sampled at 1000 Hz using an electronic data acquisition system (PowerLab/Labchart Pro7; ADInstruments, Spechbach, Germany). Fetal and maternal arterial blood samples were taken 20 minutes before, and 2, 15, 60, and 120 minutes after the start of the isolation period. Blood gases, oxygen saturation, glucose, and lactate were determined using a blood gas analyzer with measurements corrected 39°C (ABL600; Radiometer, Copenhagen, Denmark). In all, 1 mL of arterial blood samples were collected in chilled EDTA acid tubes (S-Monovette EDTA, Sarstedt; Nümbrecht, Germany). Plasma was separated by centrifugation at 4°C for 10 minutes at 3000*g*, flash-frozen, and stored at −80°C for norepinephrine (NE) and cortisol determination. At 24 hours after the first isolation procedure, ewes underwent the same stress protocol but under adrenergic blockade. Ewes received 1 mg/kg^{-1} of labetalol (Sigma-Aldrich; Taufkirchen, Germany) intravenously over 30 minutes. The isolation stress challenge was commenced after reaching a steady state of MBF, MHR, and UBF. In line with previous studies published by other investigators by stopping the infusion, we avoided cumulative effects of labetalol over time during the stress period.[20]

Hormone analysis

Fetal and maternal NE was quantified by stable-isotope liquid chromatography-tandem mass spectrometry. In brief, catecholamines were enriched from plasma specimens on boric acid gel (AffiGel601; BioRad, München, Germany) in the presence of deuterated NE-d6 as internal standard (LGC Promochem, Wesel, Germany) and eluted with 0.75 mol/L acetic acid. After centrifugation, supernatants were separated by isocratic high-performance liquid chromatography on a pentafluorphenyl column in an Agilent 1260 LC (Agilent, Santa Clara, CA) prior to electrospray ionization allowing detection and quantification of selected ion fragments in an API5500 triple-quadrupole mass spectrometer (Applied Biosystems/Sciex, Darmstadt, Germany). The method has a detection limit of 5 ng/L^{-1} NA (retention time of 0.7 minutes) with an intraassay coefficient of variation of 3.9%.

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