EDITORIAL

Strengthening Research in Departments of Obstetrics and Gynecology

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SUMMARY AND RECOMMENDATIONS:

1. *The success rate for members of departments of obstetrics and gynecology who apply to the National Institute of Child Health and Human Development is as good or better than for all applicants to that institute. The number of applications per faculty member, however, has declined by 54% for MD faculty and 48% for PhD faculty in the period 1993–1995 compared with 1984–1986.*
2. *The Society for the Advancement of Women's Health Research should be approached with a view to establishing national fellowships to support undergraduate and medical students during a research experience in laboratories in departments of obstetrics and gynecology.*
3. *A committee made up of members of the Society for Gynecologic Investigation Council and the American Board of Obstetrics and Gynecology should be constituted to restructure residency training in our specialty. Training should consist of 2–3 years of core obstetrics and gynecology, followed by clinical subspecialty training of 2–3 years. Individuals who wish an academic career will then be able to devote an additional 2–3 years full-time to laboratory, patient-oriented, or epidemiologic research.*
4. *Departments that strive for a research orientation should create a division of reproductive biology with a full voice in executive decisions within the department.*

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KEY WORDS: National Institute of Child Health and Human Development, residency training.

The primary focus of this report is to review strategies by which research in departments of obstetrics and gynecology may be strengthened. The status of research in obstetrics has been reported recently[1] and will not be reviewed extensively. We believe, nevertheless, that a brief overview of current performance by obstetric departments should serve as an introduction to recommendations as to how research can be strengthened. Although there is perhaps an emphasis on basic or fundamental research in this report, we believe that within certain limits, the recommendations also apply to human or clinical research as well as epidemiologic research. The definitions used are those of the Committee on Addressing Career Paths for Clinical Research of the Institute of Medicine.[2] Fundamental or basic research seeks to answer fundamental questions about the nature of biology through a broad range of basic and/or clinical research. Most of these studies involve nonhuman materials, although some may involve human materials such as cell lines in culture. These studies may eventually lead to improvements in the prevention or cure of disease. Human research is the portion of clinical research in which patients serve directly as the research subjects, often referred to as patient-oriented or patient-related research. This category of research includes activities such as the characterization of normal and diseased human function; evaluation of new diagnostic techniques, approaches, and practice; and phase I–IV drug trials. This category of research has direct application to the prevention, diagnosis, treatment, or cure of disease; rehabilitation of the patient; or study of human pathophysiology. Epidemiologic research investigates the circumstances under which disease occurs in populations. It seeks factors that cause disease, such as environmental exposures, personal habits, genes, viruses, and the like. This research is the source of many ideas about the causes of disease, factors that determine high risk for development of disease, and methods to promote the prevention of disease.

In the period 1984–1995, the number of medical school

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main largely unknown, but may involve tissue-specific changes in the bioavailability of glucocorticoids through altered local 11β-HSD expression and action. The present study was designed to examine this hypothesis using the fetal sheep model of 8 hours of sustained intrauterine hypoxemia, with the following two specific objectives. First, we sought to determine whether changes occur in the levels of 11β-HSD1 and 11β-HSD2 mRNA in the established fetal organs of major interest, as well as in the placenta. Second, we examined whether these changes also affect proteins, as reflected in the tissue level of their enzymatic activity.

MATERIALS AND METHODS

Surgical Procedures

Twelve fetal sheep of mixed breed were surgically prepared for study between days 107 and 110 of gestation (term = 145 days). The detailed surgical procedures and postoperative care of the animals have been described previously.[21] Polyvinyl catheters (V3; Bolab, Lake Havasu City, AZ) were placed in the fetal brachiocephalic artery for blood sampling, in a forelimb vein for injection of antibiotics, and in the amniotic cavity (V11; Bolab) for measurements of amniotic pressure. Postoperatively, the animals were maintained in individual cages suitable for continuous monitoring and were allowed at least 3 days to recover from surgery before the experiments began. Care of experimental animals followed the guidelines of the Canadian Council on Animal Care.

Experimental Protocol

Each animal was studied during a 2-hour normoxic control period and during an 8-hour experimental period of either sustained hypoxemia (n = 7) or continued normoxia (n = 5; controls). During the normoxic control period, a perspex chamber (volume 4.6 ft^3), designed to alter maternal inspired gas concentration while permitting the ewes to eat and drink, was placed in the cage and perfused with air at 40 L/minute, as described previously.[21] For the hypoxia group, sustained hypoxemia was then induced by lowering the oxygen concentration within the perspex chamber to 11–8% with 3% CO_2 added, as monitored by periodic blood gas measurements. For the control group, ewes continued to breathe room air in the perspex chamber at 40 L/minute throughout the 8-hour experimental period. To monitor fetal arterial blood gas status, we took blood samples from all fetuses during the normoxic control period and at 1, 4, and 8 hours during the experimental period. Blood gases and pH were measured using an ABL-3 blood gas analyzer (Radiometer, Copenhagen, Denmark) with temperature corrected to 39.5C.

Tissue Collections

At the end of the experimental period, the ewe and the fetus were killed with an overdose of Euthanyl (MTC Pharmaceuticals, Cambridge, Ontario, Canada). Fetal liver and kidney as well as placental tissues were dissected, frozen rapidly in liquid nitrogen, and stored at −80C until analysis.

RNA Extraction and Northern Blot Analysis

Total cellular RNA was extracted using lithium chloride/urea.[22] The size and the relative abundance of 11β-HSD type 1 and 2 mRNAs were assessed by Northern blot analysis, as described previously.[4] Briefly, denatured RNA samples (20 μg) were subjected to agarose gel (1%) electrophoresis in the presence of formaldehyde and were transferred overnight by capillary blotting to a Zeta-Probe membrane (Bio-Rad Canada Ltd., Mississauga, Ontario). The RNA was fixed by ultraviolet (UV) cross-linking (Gene Cross-Linker; Bio-Rad) to the membrane, which was then baked under vacuum at 80C for 60 minutes. The blot was hybridized at 42C for 16 hours in the presence of formamide (50%) and [^{32}P]-sheep 11β-HSD1[4] or 11β-HSD2[12] cDNA prepared by random priming[23] with [^{32}P]dCTP (3000 Ci/mmol; Du Pont Canada). We used a cDNA for mouse 18S rRNA as an internal control for gel loading and efficiency of RNA transfer, as described previously.[4]

Assay of 11β-HSD2 Activity

The unidirectional 11β-HSD2 dehydrogenase in the fetal kidney was determined by a radiometric conversion assay using cortisol as physiologic substrate, as described.[24] Briefly, fetal kidney tissues (0.4–0.5 g) were homogenized in 20 volumes of ice-cold 10 mmol/L sodium phosphate buffer, pH 7.0, containing 0.25 mol/L sucrose (buffer A). The homogenate was used immediately in the assay as described below.

The assay tubes contained approximately 100,000 cpm of the labeled cortisol, 100 nmol/L of nonradioactive cortisol, and 250 μmol/L of nicotinamide adenine dinucleotide. Buffer B (0.1 mol/L sodium phosphate buffer, pH 7.5) was added to bring the volume up to 0.4 mL. After 10 minutes of incubation at 37C, 100 μL of tissue homogenate containing 100–300 μg protein was added. Incubation was done for 15–30 minutes (preliminary studies indicated that the rate of reaction was linear with time from 15 to 120 minutes and with the amount of tissue homogenates containing 0.1–1.2 mg protein). The reaction then was arrested, and the steroids were extracted with 4 mL ethyl acetate containing 40 μg of a mixture of nonradioactive cortisol and cortisone as carrier steroids. The extracts were dried, and the residues were resuspended in 100 μL methanol. A fraction of the resuspension was spotted on a thin-layer chromatography plate, which was developed in chloroform/methanol (9:1, v/v). The bands containing the labeled cortisol and cortisone were identified by UV light of the cold carriers, cut out into scintillation vials, and counted in Scintisafe Econol 1 (Fisher Scientific, Toronto, Canada). The rate of cortisol to cortisone conversion was calculated from the specific activity of the labeled cortisol and the radioactivity of cortisone, and results were expressed as the amount of cortisone (picomoles) formed per minute per milligram protein.

Data Analysis

Results obtained from animals in the control and hypoxia groups are presented as group means ± standard error of the mean (SEM). Statistical significance for the blood gas and pH

Table 1. Fetal Arterial Blood Gas and pH Measurements

Measurement	Control period	Experimental period		
		1 h	4 h	8 h
Control group (n = 5)				
PO_2 (mmHg)	26.8 ± 1.1	27.9 ± 2.4	25.6 ± 0.7	26.0 ± 1.3
PCO_2 (mmHg)	49.5 ± 1.5	50.8 ± 0.8	49.1 ± 1.6	49.4 ± 1.3
pH	7.36 ± 0.01	7.36 ± 0.01	7.37 ± 0.01	7.37 ± 0.02
Base excess (mmol/L)	2.0 ± 0.8	2.2 ± 0.6	2.3 ± 0.8	2.3 ± 0.7
Hypoxia group (n = 7)				
PO_2 (mmHg)	25.7 ± 0.6	17.1 ± 1.0*	15.9 ± 0.7*	13.9 ± 0.8*
PCO_2 (mmHg)	47.6 ± 1.4	46.5 ± 1.2	43.6 ± 1.4	44.3 ± 1.4
pH	7.36 ± 0.01	7.36 ± 0.01	7.34 ± 0.02	7.26 ± 0.03
Base excess (mmol/L)	1.2 ± 0.5	0.8 ± 0.5	−2.4 ± 0.9*	−6.6 ± 1.6*

PO_2 = oxygen pressure; PCO_2 = carbon dioxide pressure.
Data are presented as group mean ± SEM.
*$P < .01$.

data was determined using analysis of variance for repeated measures, followed by Scheffe's test if a significant F ratio was obtained ($P < .05$). To determine the relative abundance of 11β-HSD type 1 and 2 mRNA as well as 18S rRNA, we measured the relative optical density of the autoradiographic films using a computerized densitometry analysis system (Image Quant; Molecular Dynamics Inc, Sunnyvale, CA). For each RNA sample, the ratio of 11β-HSD type 1 or 2 mRNA signal to 18S rRNA signal was calculated, and group means were obtained. Statistical significance for the 11β-HSD type 1 and 2 mRNA as well as for 11β-HSD2 activity data was determined using a t test for small samples with variances not assumed to be equal, as described by Bailey,[25] with confidence limits based on the Student t distribution.

RESULTS

Fetal Arterial Blood Gases and pH

Changes in fetal arterial blood oxygen pressure (PO_2), carbon dioxide pressure (PCO_2), pH, and base excess in both the control and hypoxic groups are shown in Table 1. Sustained hypoxemia resulted in a progressive decrease in fetal arterial PO_2 from 25.7 ± 0.6 mmHg during the control period to 13.9 ± 0.8 mmHg by 8 hours of study. There was a small but nonsignificant decline in fetal arterial PCO_2, likely because of maternal hyperventilation in spite of the added 3% inspired carbon dioxide. There was a variable decrease in fetal arterial pH to 7.26 ± 0.03 (range 7.41–7.10) by the end of study, which could be attributed to progressive metabolic acidosis, as indicated by the associated changes in base excess. In the control fetuses, no significant changes were observed in any of these blood measurements.

11β-HSD2 mRNA and Activity in the Fetal Kidney

As shown in Figure 1, there was a tendency for a decrease (P = .08) in the level of 11β-HSD2 mRNA in the fetal kidney after 8 hours of sustained hypoxemia. Moreover, this decrease in 11β-HSD2 mRNA levels for hypoxia-group fetuses correlated well with the degree of fetal acidosis as measured by arterial base excess at 8 hours of hypoxia (r = 0.92, $P < .01$) (Figure 2). However, there were no corresponding changes in the level of renal 11β-HSD2 enzyme activity as determined at this time (control, 2.4 ± 0.1 pmol/minute/mg protein; hypoxia, 2.1 ± 0.2 pmol/minute/mg protein). When the same blot was reprobed with the ovine 11β-HSD1 cDNA, the mRNA for 11β-HSD1 was undetectable (data not shown).

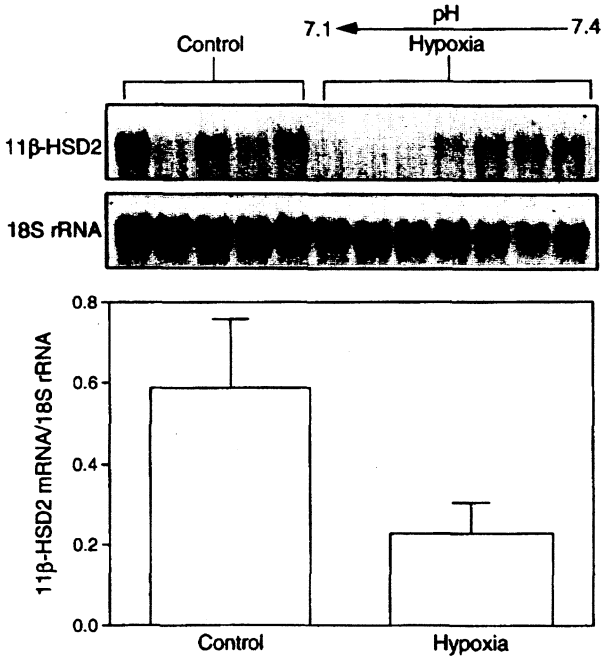

Figure 1. Top: Northern blot of total RNA (20 μg in each lane) from the kidneys of control and hypoxia-group fetuses hybridized sequentially with radiolabeled cDNAs encoding 11β-hydroxysteroid dehydrogenase (HSD)2 and 18S rRNA. Fetuses in the hypoxia group are shown according to fetal arterial pH measured at 8 hours of sustained hypoxemia (range 7.10–7.41). Bottom: quantification of mRNA signals by computerized densitometry with 11β-HSD2 mRNA level expressed as a ratio of 18S rRNA for control and hypoxia-group fetuses (mean ± SEM) (P = .08).

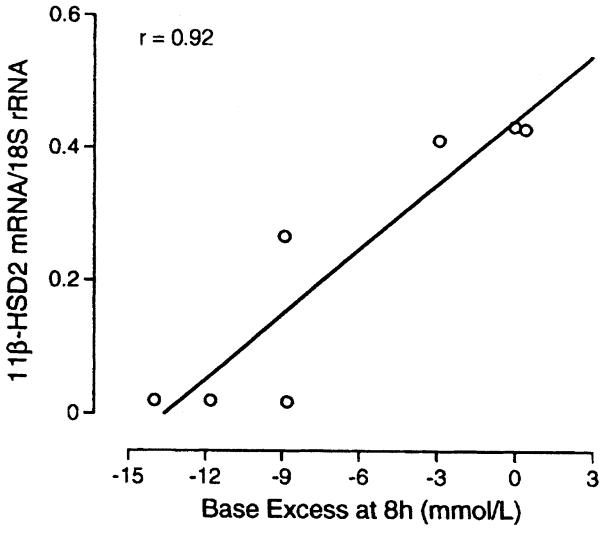

Figure 2. Correlation of 11β-hydroxysteroid dehydrogenase (HSD)2 mRNA levels (expressed as a ratio of 18S rRNA level) from the kidneys of hypoxia-group fetuses with the arterial base excess at 8 hours of hypoxia ($r = 0.92$, $P < .01$).

11β-HSD1 mRNA in the Fetal Liver and in the Placenta

Levels of 11β-HSD1 mRNA in the fetal liver and in the placenta are shown in Figures 3 and 4, respectively. Sustained fetal hypoxemia did not result in a significant change in 11β-HSD1 mRNA abundance in either of these tissues. When the same blots were reprobed with the ovine 11β-HSD2 cDNA, the mRNA for 11β-HSD2 was undetectable in both the fetal liver and the placenta (data not shown).

DISCUSSION

In our previous studies, we established the fetal sheep model by demonstrating that fetal liver and kidney are the major sites of 11β-HSD type 1 and 2 biosynthesis, respectively.[26] We have also shown that the expression levels of 11β-HSD1 gene in the liver and of 11β-HSD2 gene in the kidney are differentially regulated during fetal development.[24] Intrauterine hypoxemia is a potent stressor for the fetus inducing alterations in fetal growth and is associated with various metabolic and endocrine responses.[20,21] The purpose of the present study was to determine the effects of sustained hypoxemia on the expression of 11β-HSD1 gene in the fetal liver and of 11β-HSD2 gene in the fetal kidney. Because the ovine placenta predominantly expresses the 11β-HSD1 gene,[16] we also examined 11β-HSD1 gene expression in the placental tissues. To confirm that hypoxia did not alter the normal pattern of tissue-specific expression of 11β-HSD type 1 and 2 genes in the fetal liver and kidney as well as in the placenta, we also studied the expression of 11β-HSD1 mRNA in the fetal kidney and of 11β-HSD2 mRNA in the placenta and fetal liver and found these to be undetectable.

In the present study, 8 hours of induced hypoxemia resulted in a variable degree of fetal acidosis (pH 7.41–7.10), which was entirely metabolic in nature. Although there was a trend toward a decrease in the level of 11β-HSD2 mRNA in the kidneys of hypoxic fetuses, this was not significant. However, there was a highly significant correlation between the level of 11β-HSD2 mRNA in the kidneys of individual hypoxia-group animals and the degree of associated metabolic acidosis, such that with arterial base excess less than −6 mmol/L, 11β-HSD2 mRNA levels were markedly reduced. This finding suggests that the effect of hypoxia on the expression of the 11β-HSD2 gene is dependent on the severity of the hypoxic insult and/or associated acidosis, at least as measured with 8 hours of study, thus accounting for the variable responses in the hypoxia-group fetuses. Of note, the 11β-HSD2 mRNA levels in the kidneys of the control fetuses were also variable. This may be attributed to tissue heterogeneity; it has been well established that the expression of 11β-HSD2 gene within mammalian kidney is highly cell-specific, in that it occurs only in distal convoluted tubules and collecting ducts.[27–29]

There was no corresponding change in the tissue level of 11β-HSD2 enzyme activity in the kidneys of hypoxia-group

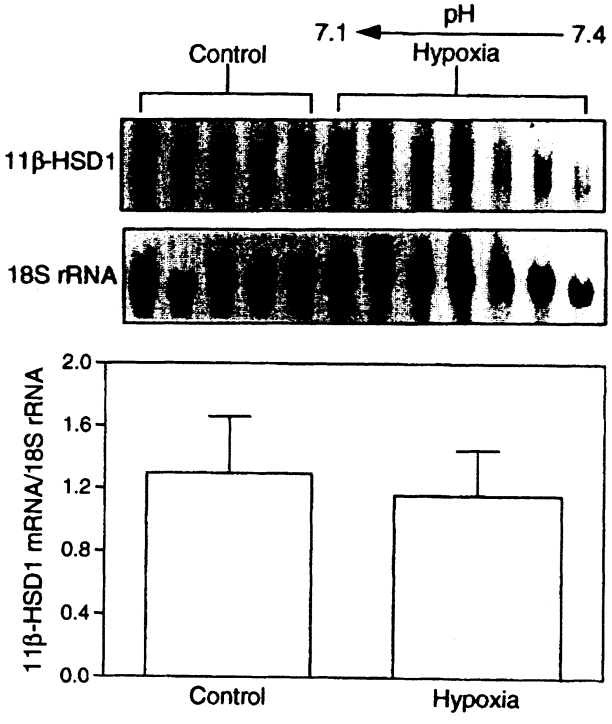

Figure 3. Top: Northern blot of total RNA (20 μg in each lane) from the livers of control and hypoxia-group fetuses hybridized sequentially with radiolabeled cDNAs encoding 11β-hydroxysteroid dehydrogenase (HSD)1 and 18S rRNA. Fetuses in the hypoxia group are shown according to fetal arterial pH measured at 8 hours of sustained hypoxemia (range 7.10–7.41). Bottom: quantification of mRNA signals by computerized densitometry with 11β-HSD1 mRNA level expressed as a ratio of 18S rRNA for control and hypoxia-group fetuses (mean ± SEM).

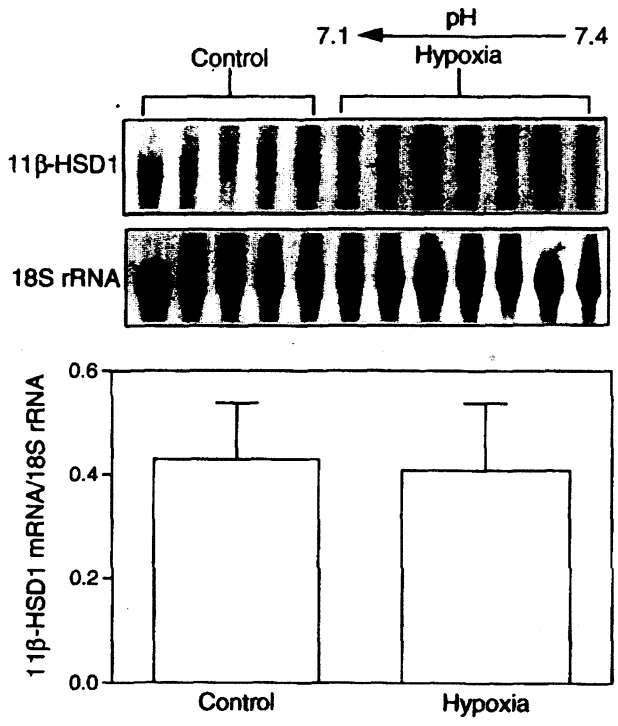

Figure 4. Top: Northern blot of total RNA (20 μg in each lane) from the placentas of control and hypoxia-group fetuses hybridized sequentially with radiolabeled cDNAs encoding 11β-hydroxysteroid dehydrogenase (HSD)1 and 18S rRNA. Fetuses in the hypoxia group are shown according to fetal arterial pH measured at 8 hours of sustained hypoxemia (range 7.10–7.41). Bottom: quantification of mRNA signals by computerized densitometry with 11β-HSD1 mRNA level expressed as a ratio of 18S rRNA for control and hypoxia-group fetuses (mean ± SEM).

fetuses, indicating that the tissue level of 11β-HSD2 protein was not altered. The apparent dissociation—whereby 11β-HSD2 mRNA is decreased while protein levels remain unchanged—suggests that there is a time lag between the two and that 8 hours of induced hypoxemia is not long enough for changes in protein levels to become evident. Alternatively, the lack of a corresponding change in 11β-HSD2 enzyme activity may be due to an inhibition of 11β-HSD2 mRNA translation.

In contrast, levels of 11β-HSD1 mRNA in both placental and fetal liver tissues were not altered by sustained fetal hypoxemia or associated acidosis. This suggests either that these two tissues are not readily responsive to hypoxemia and the resultant acidosis or that the expression of the 11β-HSD1 gene is regulated by factors other than fetal hypoxemia. Although the precise physiologic significance of this differential effect of acidosis on 11β-HSD type 1 and 2 gene expression in preterm fetal sheep is unknown, it may represent an adaptive response whereby stress-sensitive hormones such as cortisol can be increased by decreasing renal 11β-HSD2 enzyme expression and thus cortisol metabolism. Moreover, this may allow a targeted increase in cortisol within the kidney, which in turn would have a significant impact on electrolyte balance, with an increase in mineralocoriticoid activity as a result of cortisol's acting like aldosterone, leading to sodium retention and possibly hypertension.

In conclusion, we have demonstrated, for the first time, that fetal hypoxemia-induced acidosis selectively down-regulates the expression of 11β-HSD2 mRNA in the fetal kidney. This may provide a further adaptive mechanism whereby fetal acidosis alters developmental processes by regulating the bioavailability of glucocorticoids in specific fetal organs through alterations of local 11β-HSD activity.

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