

- **289** INSULIN INHIBITS THE SYNTHESIS OF THE MAJOR SURFACTANT APOPROTEIN IN HUMAN FETAL LUNG IN VITRO. Carole R. Mendelson and Jeanne M. Snyder (Spon. by C. R. Rosenfeld). Univ Texas Southwestern Med Sch, Cecil H & Ida Green Ctr Reprod Biol Sci, Depts Biochem, Cell Biol, & Ob-Gyn, Dallas, TX.
- Fetal hyperinsulinemia may be a causative factor in the increased incidence of respiratory distress syndrome (RDS) in newborn infants of diabetic mothers. We previously observed, however, that insulin acted synergistically with cortisol to increase disaturated phosphatidylcholine synthesis in human fetal lung explants in vitro. Surfactant apoproteins, which comprise ~10% of surfactant composition, may serve an important role in surfactant function. In the present study, we utilized antibodies directed against the major human surfactant apoprotein, a 35 kDa glycoprotein, and immunoblot analysis to evaluate the effect of insulin on the specific content of this protein in human fetal lung explants in organ culture. The 35 kDa protein, which was not observed in the human fetal lung tissue prior to culture, was detectable in control explants after 2 days of incubation and was increased in concentration with increasing time in culture. Insulin caused a marked inhibition of surfactant apoprotein accumulation at concentrations as low as 0.5 nM. This inhibitory effect of insulin was dose-dependent and was apparent as early as day 2 of incubation. These findings are suggestive that fetal hyperinsulinemia may cause the production of surfactant deficient in the major apoprotein; this may provide an explanation for the increased incidence of RDS in infants of diabetic mothers.

- 290** DENERVATION OF PERIPHERAL CHEMORECEPTORS AND LUNG RECEPTORS DOES NOT AFFECT PRODUCTION OF TRACHEAL FLUID IN FETAL SHEEP. Daniel T. Murai, Linda D. Wallen, Ching H. Lee, Joseph A. Kitterman, Univ of Texas Health Sci Ctr, San Antonio & Univ of Calif, San Francisco and Cardiovasc Res Inst, Dept of Pediatrics, San Antonio & San Francisco.
- The factors that affect production of fetal tracheal fluid have not been completely elucidated. We studied 14 chronically catheterized fetal sheep (120-130 days) to determine if bilateral sections of the carotid sinus and vagus nerves affect production of tracheal fluid. Each fetus had ligation of the trachea and cannulation with a catheter (100 cm long, 1.5 mm I.D.) that diverted all tracheal fluid into a soft intrauterine bag. A loop of catheter was exteriorized to permit collection of fluid from the lungs and bag. Fluid was collected once daily for 6 days after surgery. Seven fetuses had bilateral sections of the carotid sinus and vagus nerves; 7 had sham operations. All fetuses had similar arterial blood gas tensions, pH and mean blood pressures and low voltage electrocortical activity. The incidence of fetal breathing movements was lower in the denervated fetuses; however the production of tracheal fluid was not significantly different except for POD# 1 (table). Volume of Tracheal Fluid (ml/h)
- | POD# | 1 | 2 | 3 | 4 | 5 | 6 |
|--------|----------|---------|----------|---------|----------|----------|
| DENERV | 5.1±1.5* | 7.0±0.6 | 7.2±1.7 | 8.0±2.3 | 8.1±2.8 | 8.3±2.2 |
| SHAM | 7.6±1.7 | 9.3±4.5 | 10.6±6.7 | 9.8±5.6 | 10.3±5.5 | 10.6±5.9 |
- (Values are mean±SD; POD=post operative day; *p<0.05)
- We conclude that denervation of peripheral chemoreceptors and lung receptors with afferent nerves in the vagus nerve does not affect production of tracheal fluid in fetal sheep. (USPHSHL27356)

- = 291** ROLE OF β -ADRENERGIC RECEPTORS (BAR) AND ENDOGENOUS CATECHOLAMINES IN SURFACTANT RELEASE BUT NOT LUNG WATER ABSORPTION IN FETAL RABBITS. John V. McDonald, Jr., Linda K. Gonzales, Philip L. Ballard, and James M. Roberts. Univ Calif San Francisco, Dept Peds and Cardiovasc Res Inst, San Francisco, CA; Mt. Zion Hospital, Dept Peds, San Francisco, CA
- Exogenous β -agonists stimulate release of surfactant (SAM) and absorption of lung water, presumably through interaction with BAR. To evaluate the role of endogenous catecholamines in these processes, we injected fetuses (28-d gestation) with an irreversible BAR antagonist, bromacetyl-(1-8) diamino- β -menthone alprenolol (B, 30-40 mg/kg) or vehicle. BAR concentration was reduced from 215 ± 11 to 46 ± 16 fmol/mg protein, measured by [125 I]cyanopindolol binding to lung particulate, with no change in K_D or adenylate cyclase activity not mediated by BAR. SAM in lavage was decreased ~30% in 2 nonbreathing groups (Table).
- | Age(d) | Delivery | Breathe | Lavage Phospholipid-P (μ g/mg DNA) | P |
|--------|----------|---------|---|------------|
| | | | Control | Treated |
| 30 | c/s | — | 2.41 ± .22 mean | 1.66 ± .17 |
| 31 | c/s | — | 3.23 ± .42 ± SE | 2.71 ± .92 |
| 31 | vag | — | 4.75 ± .32 | 3.21 ± .23 |
| 31 | vag | + | 4.99 ± .53 | 6.08 ± .45 |
- B appears to inhibit SAM release per se rather than synthesis since treatment did not affect tissue saturated phosphatidylcholine, [3 H]choline incorporation by lung minces, or phospholipid composition of lavage. In studies of lung water, B did not affect the decreases observed after vaginal vs c/s (no labor) delivery or after birth. We conclude that endogenous catecholamines participate in SAM but not water flux in the fetus.

- 292** RELATIONSHIP BETWEEN FETAL BREATHING MOVEMENTS (FBM) AND CIRCULATING PROSTAGLANDIN E_2 CONCENTRATIONS ([PGE $_2$]) IN SHEEP. Daniel Murai, Linda Wallen, Chu-Ching Lee, Francoise Mauray, Ronald Clyman, Joseph Kitterman, Univ of Texas Health Sci Ctr, San Antonio & Univ of Calif, San Francisco and Cardiovasc Res Inst, Dept of Ped, San Antonio & San Francisco.
- In sheep, PGE $_2$ inhibits FBM and the prostaglandin synthetase inhibitor, meclofenamate, stimulates FBM. Infusions of PGE $_2$ increase [PGE $_2$] and meclofenamate decrease [PGE $_2$]; however the relationship between [PGE $_2$] and FBM has not been reported. We studied 9 chronically catheterized fetal sheep to determine this relationship. FBM (by tracheal catheter) and low voltage electrocortical activity (LVECoA) were recorded continuously after surgery. Plasma [PGE $_2$] was measured by RIA; samples were obtained at the end of 24 h periods. On separate occasions, we infused PGE $_2$ (n=6, 0.8 μ g/kg/min), meclofenamate (n=8, 0.5 mg/kg/h) or glucose (n=5, 22 mg/kg/min). Infusions were separated by ≥ 48 h. All studies were ≥ 6 days after surgery. Arterial blood gas tensions, pH and mean blood pressures during infusions were similar to controls. During control and early labor periods the daily incidence of FBM (15.5-56.7%, range) and of LVECoA (48.7-66.1%) was not dependent upon [PGE $_2$] (55.5-1074 pg/ml) (n=22, r=.27). PGE $_2$ infusions decreased FBM (0-41.3%), did not alter LVECoA (43.3-66.3%) and increased [PGE $_2$] (249.5-611.5 pg/ml). Meclofenamate infusions increased FBM (72.9-99.8%), did not alter LVECoA (47.3-66.8%) and decreased [PGE $_2$] (3-36.5 pg/ml). Glucose did not affect FBM (15.6-42.7%), LVECoA (48.7-55.3%) nor [PGE $_2$] (51.0-1265 pg/ml). Over the range of [PGE $_2$] only the relationship with FBM was significant (n=41, r=.53). Our results suggest that FBM may be dependent upon circulating [PGE $_2$] in fetal sheep (USPHS HL-27356).

- 293** DEXAMETHASONE ACCELERATES SMALL INTESTINAL ENTEROCYTE MEMBRANE MATURATION. Josef Neu, Charles K. Ozaki, Wendell N. Crim, Kimon J. Angelides. (Spon. by Donald V. Eitzman) U. of Fla. College of Medicine, Dept of Pediatrics, Gainesville.
- Spectral characteristics of the fluorescent probe diphenyl-hexatriene (DPH) were studied in enterocyte brush border membranes (BBM) of rats ranging from fetal life to adulthood. Postnatal effects of glucocorticoids were studied by administering 50 mg/kg hydrocortisone (H) t.i.d. x 3 d with sacrifice at 14 days. Antenatal effects were determined by the administration of 0.4 mg/kg dexamethasone (D) b.i.d. to 20 day old pregnant rats for 2 days with sacrifice and analysis at 22 days gestation. Probe characteristics were studied between 40°C and 12°C in each preparation. Enrichment in maltase activity from homogenate to final brush border pellet was used to determine purity of membranes. Microviscosity curves were shifted upward in the more mature animals and in those administered glucocorticoids, indicating a more "rigid" membrane in these animals, as follows:
- | Treatment Group | Slopes | Y-intercept | R | P-value |
|-------------------|--------|-------------|-------|---------|
| 10 d old | -76 | 6733 | -0.96 | .0012 |
| 19 d old | -51 | 6839 | -0.77 | .025 |
| 14 d old (Saline) | -67 | 6509 | -0.76 | .030 |
| 14 d old (H) | -77.6 | 7525 | -0.82 | .013 |
| Fetal (Saline) | -34.6 | 5291 | -0.62 | .099 |
| Fetal (D) | -66.7 | 6353 | -0.87 | .005 |
- These suggest a glucocorticoid mediated acceleration in small intestinal enterocyte maturation in the infant and fetus and may be related to a lower incidence of necrotizing enterocolitis in infants whose mothers have been administered glucocorticoids.

- = 294** SEX DIFFERENCES IN SURFACTANT SYNTHESIS IN FETAL RABBIT LUNG ORGAN CULTURE. Heber C. Nielsen and Cynthia Gebhardt (spon. by J.B. Warshaw). Department of Pediatrics, Southwestern Medical School, Dallas, Texas.
- A basic question in understanding the mechanism of sex differences of fetal surfactant production is whether surfactant synthesis is involved. We studied whether the female fetus synthesizes more surfactant phosphatidylcholine (PC) and saturated phosphatidylcholine (SPC) than the male at the time when the fetal lung is responsive to glucocorticoid. Organ cultures were made of sex specific fetal rabbit lungs of 21 days gestation in Waymouths media +10% charcoal-stripped fetal calf serum + 10^{-8} M dihydrotestosterone (DHT). After 5 days 3 C-glycerol + 10^{-8} M dexamethasone (dex) was added. After 24 hours lamellar bodies (LB) were isolated by centrifugation in discontinuous sucrose density gradients. Average LB recovery was 33-36%. 3 C-PC and 3 C-SPC were quantitated in the LBs. 3 C-SPC was significantly higher in female lungs with or without DHT and was enhanced by dex (No dex: females 198±71, males 130±55; CPM/mg prot, mean±SD, p<.005. Dex: females 389±65, males 160±17; p<.02). 3 C-PC with DHT or dex had similar results. Dex stimulated females but not males. These data indicate that surfactant sex differences result at least in part from differences in synthesis. The cellular mechanisms causing the sex difference appear to be already operative by 21 days gestation in the rabbit and are not further affected by androgen or glucocorticoid. This is important for ultimate elucidation of the mechanism of sex differences in normal fetal cellular development.