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THE ROLE OF EDRF IN THE MAINTENANCE OF BASAL PULMONARY VASOMOTOR TONE IN THE NEONATAL PIGLET. Leif D. Nelin, Carol J. Thomas and Christopher A. Dawson. Departments of Pediatrics and Physiology, Medical College of Wisconsin, Milwaukee, WI, USA.

To begin to understand the role of EDRF in the control of pulmonary vascular tone in the neonate we studied isolated lungs from pigs (age 14.5±0.3 days, weight 3.2±0.3 kg) perfused at 100ml·min^{-1}·kg with autologous blood under zone 3 conditions. Pulmonary artery (Pa), left atrial (Pv) and airway pressures were continuously monitored. To determine the potential for stimulated release of EDRF we infused a 0.1 mg bolus of acetylcholine (ACH), and to block EDRF production we added N^G-nitro-L-arginine methylester (NAME) to the blood to achieve a concentration of 10^{-3}M.

	CONTROL	NAME
Pa (Torr)	16±3	24±4*
dPa (Torr)	-1.9±0.3	2.8±1.6*

Mean±SE, dPa change in Pa with ACH bolus, * different from control with $p < 0.05$.

ACH was given prior to and after NAME, prior to NAME ACH was a vasodilator and after NAME ACH was a vasoconstrictor. The 50% increase in Pa with the addition of NAME suggests that the basal production of EDRF from L-arginine has a modulating role in the control of pulmonary vascular tone in the isolated lungs from neonatal pigs. The change in response to ACH from a vasodilator to a vasoconstrictor following NAME suggests that the vasodilator action of ACH was mediated by EDRF production. Interference with EDRF synthesis may lead to the development of neonatal pulmonary hypertensive diseases.

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FETAL LUNG C-MYC EXPRESSION SUGGESTS A POSITIVE REGULATORY ROLE IN LUNG GROWTH. Cynthia K. Kellogg, Brent H. Cochran, Heber C. Nielsen. Boston Perinatal Center, Department of Pediatrics, New England Medical Center, and Department of Biology, Massachusetts Institute of Technology, Boston, MA, USA.

Oncogenes control cell proliferation and are proposed regulators of fetal development. C-myc codes for a nuclear protein that is necessary in the positive regulation of cell proliferation . When c-myc expression is blocked, cells cannot divide. When cell differentiation is induced, c-myc expression is dramatically reduced. In the developing lung, growth stops for differentiation to begin. Slowed growth and the start of differentiation occur sooner in females. We sought to begin to understand the regulatory role of c-myc in lung development by correlating its expression with known cellular events, proposing that c-myc expression is high during rapid fetal lung growth and is dramatically reduced when growth stops and differentiation begins. RNA was isolated from lungs of fetal rats and mice (days 17-22 in the rat and days 16-18 in the mouse). The presence of c-myc mRNA was determined by Northern blot analysis using a ^{32}P-labelled mouse c-myc cDNA probe. In both species, c-myc expression decreased substantially with increasing gestational age. In the fetal rat lung, c-myc expression was high in both sexes on day 17. C-myc expression decreased approximately 50% in the females by day 19, but not until day 20 in the males. By day 22, its expression decreased 74% in females and 76% in males. The timing of decreased c-myc expression corresponds to the onset of cellular differentiation and surfactant synthesis. Our findings suggest that c-myc has an important regulatory role in fetal lung growth and differentiation.

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DEVELOPMENT OF CELL SPECIFIC EPIDERMAL GROWTH FACTOR (EGF) BINDING IN FETAL RAT LUNG. Dennis A. Rosenblum, Heber C. Nielsen. Boston Perinatal Center, Department of Pediatrics, New England Medical Center, Boston, MA, USA.

EGF regulates fetal lung type II cell differentiation by advancing the developmental window under which fibroblast-type II cell communications occur. EGF acts by binding to its specific receptor, but the ontogeny of EGF receptor binding by specific lung cells is unknown. We hypothesized that EGF binding would exhibit developmental changes in the fetal lung fibroblast but not the type II cell, consistent with the primary role of the fibroblast in directing development of alveolar cells, and that these changes would occur earlier in the female fetal lung fibroblast, consistent with the earlier effect of EGF on female fibroblast-type II cell interactions. Day 17, 18, and 19 (term = 22 days) sex-specific fetal rat lung fibroblasts and type II cells were grown to confluence. Specific EGF binding was measured using 0.40 ng/ml ^{125}I EGF and a 500 fold excess of unlabelled EGF. Specific binding in day 17 female fibroblasts was twice that of day 17 male fibroblasts (102 ± 11 vs. 51 ± 10 cpm/nmol DNA; mean ± SEM, n=3). Specific binding doubled on day 18, maintaining the female-male difference. On day 19 binding levels were similar (females: 248 ± 69; males: 233 ± 78 cpm/nmol DNA, mean ± SEM, n=4). In contrast, specific binding in type II cells remained approximately 50 cpm/nmol DNA at all days, with no sex-specific differences. We conclude that cell-specific and sex-specific changes in EGF binding appear in the fetal lung at a developmental stage critical for type II cell differentiation. This is consistent with the effect that EGF has on the fibroblast in advancing fibroblast-type II cell communications.

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SENSITIVITY TO REACTIVE OXYGEN METABOLITES (ROM) OF HUMAN FIBROBLASTS (FB) COMPARED WITH ENDOTHELIAL CELLS (EC)
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In ischemia-reperfusion injury, ROM are produced and may cause tissue damage followed by fibrosis. Many cells types may be injured but ECs are the most vulnerable. We studied possible differences in the sensitivity to ROM between human fetal FBs and ECs.

Cells were labelled overnight with ^{14}C-adenine, washed and further incubated with either H_2O_2 or with xanthine oxidase (XO) and hypoxanthine (Hx). Nucleotides from cells and medium as well as catabolic products (Hx, xanthine and urate) from medium were separated and counted. Nucleotide depletion (or % of initial cpm remaining) is a sensitive index of cell damage.

FBs incubated with XO (80 mU/ml) and Hx (100 µM) for 4h retained 73±2% of cpm in cell nucleotides (control 85±4%), the rest appearing in catabolic products, whereas EC nucleotides were nearly totally depleted (11±2% remaining vs. 78±2% in controls). H_2O_2 at 20 µM for 10 or 30 min, or 100 µM for 10 min, did not deplete nucleotides from FBs, (92±2%, 92±5% and 88±5% remaining, respectively, vs. control 30 min 91±2%), while the corresponding figures for ECs were 74±9%, 55±9% and 48±7%, controls 80 ±3%. H_2O_2 at 100 µM for 30 min had a slight effect in FBs, (76±8%) but stronger in ECs (36±26%).

We conclude that FBs survive ROM-induced damage better than ECs and are thus able to proliferate in the reparative stage of tissue injury.

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INFLUENCE OF TYP-II CELL-CONDITONED MEDIUM ON THE PROLIFERATION OF FIBROBLAST CELL CULTURES. Paul Stevens, Martin Rey, Vera Meienreis-Sudau, Department of Neonatology, Free University Berlin, Berlin, Germany.

Lung fibroblast proliferation is an important component of diseases such as bronchopulmonary dysplasia. A possible mechanism contributing to this phenomenon could be the loss of inhibitory influences by the adjacent epithelial layer, which is damaged early in the course of the disease.

In the present study the influence of rat type-II cell-conditioned medium (CM) on the proliferation of human fetal fibroblasts was measured.

Methods: CM from type II cells cultured in 1 or 10% fetal calf serum for 1 day (1%1d; 10%1d) or 3 days (1%3d; 10%3d) were added (1:10) to fibroblast cultures. Fibroblast proliferation was measured by incorporation of bromodeoxyuridine (BrdU), as estimated by ELISA.

Results: CM 1%1d inhibited incorporation of BrdU by 35% ($p<0.05$), CM 10%1d by 24% ($p<0.05$) of control (no CM). CM 1%3d stimulated the incorporation of BrdU by 8% (n.s.), CM 10%3d by 13% ($p<0.05$) above control.

Conclusions: Freshly prepared type-II cells in culture inhibit the proliferation of human fetal fibroblasts. After 3 days in culture this property is lost, and may be replaced by stimulatory influences.

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TRANS-THORACIC SOUND SPEED IN VENTILATOR DEPENDENT INFANTS: A PILOT STUDY. Manuel Durand, Kevin Sullivan, Cindy McEvoy, H.K. Chang. Depts. of Biomedical Engineering and Pediatrics, Univ. of Southern California and LAC+USC Medical Center, Los Angeles, California, USA.

The sound speed through the lungs is influenced by the lungs' material properties and density. Probing the lungs of intubated infants with sound may provide a means to identify changes in lung structure and composition that occur as a result of prolonged ventilation therapy. To examine the feasibility of this approach we measured the sound transit times in 6 ventilator dependent infants (birth weight 640-4960g, age 12-81 days, study weight 990-5140g) suffering from early or advanced chronic lung disease. A piezoelectric ceramic disc mounted at the entrance of the endotracheal tube was used to produce brief 3 KHz pulses that propagate into the lungs via the tube. The onset, duration, and intensity of the pulse was registered with an electret microphone at the tube entrance. A lightweight quartz accelerometer secured on the chest at the mid-thoracic level registered sound transmission across the lungs and chest wall. Transit times for the pulse to propagate from the tube entrance to the chest surface was estimated with spectral averaging and cross-correlation. The estimated transit times varied between 1.47 and 2.27 ms with an average of 1.72 ms (0.28 SD). These times correspond roughly to sound speeds of 28 (9) meters/s. The transit times correlated poorly with the body weight or chest circumference but correlated well (r= +0.90) with patient age. These results suggest that changes in the lungs material properties that occur with prolonged ventilation therapy may be a predominant factor determining parenchymal sound speed in infants with chronic lung disease.