313 THE α-ADRENERGIC RECEPTORS IN BROWN ADIPOCYTES OF DEVELOPING RATS. Josef P. Skala, Iqbal M. Shaikh & Wendy Cannon de Rodriguez. University of British Columbia, Department of Paediatrics, Vancouver, B.C., Canada, V5Z 1L7.

Specific binding of α_2-adrenergic ligands [^{3}H] clonidine and [^{3}H]RX 781097 was observed with plasma membrane fragments isolated from interscapular brown adipose tissue of 7-day-old rats. Scatchard and Woolf analyses of the data and results on specific displacement of [^{3}H] norepinephrine by low concentrations of epinephrine and yohimbine were consistent with the existence in brown adipocytes of a homogeneous class of the binding sites. Competition experiments on both α-agonists and α-antagonists binding were consistent with pharmacological definition of the α_2-adrenoreceptor subtype. Chemical denervation by 6-hydroxydopamine resulted in a more pronounced distinction of the two affinity states of the receptors. A two-slope Scatchard plot showed mean apparent K_D of 29 and 250 nM for the α_{2H} and α_{2L}, respectively. The maximum number of sites was somewhat higher (0.58 pmol/mg protein) than in control animals. Pre-treatment of infant rats with yohimbine (7 days of 10 mg/kg b.w. daily) has resulted in increased specific binding of [^{3}H] yohimbine. Comparison studies using the β_1-receptor ligand [^{3}H] dihydroalprenolol seem to indicate "hybrid" characteristics. Competition binding studies may be interpreted in terms that both the α- and β-adrenoreceptors in brown adipocytes of infant rats are "hybrid" species which may in fact reside within integrated protein molecules.

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= 314 INDUCTION OF THE MAJOR SURFACTANT APOPROTEIN DURING RABBIT FETAL LUNG DEVELOPMENT. Jeanne M. Snyder and Carole R. Mendelson (Spon. by C. R. Rosenfeld) Univ of Tex Southwestern Med Sch, Depts Cell Biol, Ob-Gyn, & Biochem, Cecil H. & Ida Green Ctr Reprod Biol Sci, Dallas, TX.

The major apoprotein associated with lung surfactant is a 35 kDa, sialoglycoprotein with an isoelectric point (pI) of 4.5. We used antibodies directed against the major rabbit lung surfactant apoprotein to characterize the induction of this protein during rabbit fetal lung development. Proteins from homogenates of fetal lung tissue were separated by two-dimensional (2D) gel electrophoresis, transferred to nitrocellulose paper and analyzed using an immunoblot technique. An immunoreactive apoprotein of 26 kDa, pI 4.5, was first detectable on day 24 of gestation. Based on its migration in 2D gels, the protein did not appear to contain sialic acid. The 35 kDa form of the protein was first detectable in lung homogenates from day 30 fetal rabbits. Lamellar bodies (LB), the storage form of surfactant in the type II cell, are first observed on day 26 of gestation. LB were purified from lung tissues of fetuses of 28 and 30 days gestation and their apoproteins were analyzed using immunoblot techniques. The apoprotein associated with the purified LB was the 35 kDa form of the protein. Therefore, a post-translational modification of the 26 kDa protein, possibly the addition of sialic acid residues, may be required for the association of the surfactant apoprotein with LB in the fetal lung type II cell.

315 MECHANISMS FOR SUPERIOR HYPEROXIC TOLERANCE IN NEWBORN STREPTOZOTOCIN OFFSPRING. Ilene Sosenko and Lee Frank, Univ. Miami Sch. Med., Pulmonary Research, Dept. of Medicine and Dept. of Pediatrics, Miami, FL.

Newborn offspring of streptozotocin-diabetic rats have surprisingly superior tolerance to hyperoxic exposure compared to control offspring (Ped. Res. 18:298A,1984). By day 13 of exposure to 95-100% O_2, 79% of streptozotocin offspring had survived, whereas only 20% of controls were alive, $p<0.01$. In order to determine the mechanism(s) of this improved survival, we examined baseline levels of whole lung disaturated phosphatidylcholine (DSPC) and antioxidant enzymes (AOE)(superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP)); and DSPC, AOE and lung microscopic morphometry following 4½ days of hyperoxic exposure in newborn streptozotocin (S) and control (C) offspring. There was a significant elevation in DSPC in S offspring pre-exposure (5.30±0.76 vs. 3.71±0.42mg/g lung, $p<0.05$), but not in DSPC post-exposure (2.36±0.45 vs. 2.28±0.22). Although no differences in AOE were found pre-exposure, elevations in AOE in response to O_2 were greater in S offspring. (Δ compared to pre-exposure values):

	SOD	CAT	GP(units/mg DNA)	
CONTROL	+43%	+80%	+195%	
STREPTOZOTOCIN	+46%	+102%	+236%*	*p<0.05

Morphometric examination revealed a significantly smaller mean linear intercept in the O_2-exposed S (1.46±.15) vs. C (1.87±.01mm X 10^{-2})($p<0.01$), indicating lesser O_2 inhibition of alveolar formation. These results suggest that newborn S offspring may manifest increased survival in high O_2 by increased ability to stimulate AOE and to preserve normal lung development.

= 316 FETAL RAT SURFACTANT AT THE ONSET OF AIR BREATHING C.I. Spain and S.L. Young (SPON A. Spock) Dept of Med, Duke University & V.A. Hospital, Durham, NC

Morphometric and biochemical measures of type II cell surfactant stores were combined to define late gestational maturation of the alveolar epithelium in its preparation for transition to air breathing. Fetal (G20,G22) and newborn (+10min) lung tissue was obtained from pathogen-free timed pregnant rats. Morphometric analyses of type II cells & their organelles was done using a stratified sample. Lamellar body (LB) fractions were obtained by sucrose density gradients & disaturated phosphatidylcholine (DSPC) was quantitated. The table gives the results of the volume fraction (Vv,%) of type II cell organelle content and the LB fraction and whole lung (WL) DSPC content. There was an increase in LB Vv from day 20 of gestation, with a parallel increase in LB DSPC. Within 10 min after birth, we found a significant increase in intracellular LB by morphologic and biochemical measures. This postnatal accumulation of intracellular surfactant could represent recycling of previously secreted phospholipid from the airspaces. Such an occurrence might supplement de novo pathways of surfactant production by newborn rat type II cells providing an additional source of surfactant flux.

AGE	CYTO	NUC	LB	MITO	LB	WL
day	%	%	%	%	mgDSPC/Gdry	
G20	60	31	2.9	3.3	1.0	1
G22	51	38	4.1	7.7	5.6	28
+10min	52	36	6.3	5.0	7.1	24

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317 EFFECT OF GLUCOSE ADMINISTRATION ON FETAL BREATHING ACTIVITY (FBA) IN THE BABOON - EPOCH ANALYSIS RESULTS. Raymond I. Stark, Henry R. Rey, College of P&S, Columbia U., Depts. of Pediatr. & Obstet.,Presb.Med.Ctr.NYC.

The relationship of breathing to other fetal activities was studied in 8 chronically catheterized pregnant baboons at 138-155 days of gestation following i.v. infusion of 25g of glucose to the mother. No significant changes in fetal heart rate (FHR), FHR variability and fetal or maternal blood pressure were observed during the infusion period.

A computer based epoch analysis method was used to automatically detect fetal breaths from a tracheal fluid pressure signal, distinguish episodes of breathing activity from apnea and generate parameters to characterize FBA. Although the number of epochs of FBA was essentially unchanged and breathing rate and variability increased slightly with glucose infusion, significant decreases in other FBA parameters were observed: mean epoch duration decreased from 13.7 to 12.4 sec; median number of breaths per epoch fell from 12 to 6 while the % of time spent in FBA dropped from 20.6% to 10.8%. Finally, mean breathing amplitude also decreased from -8.3 to -6.7 mmHg.

These results were unexpected. In human fetuses increases in plasma glucose have been associated with increased FBA. Our results may reflect variation among primate species. Nonetheless we noted that the direction of change in FBA parameters was consistent among all animals and suggests a need for study of the effect of maternal metabolic state on FBA.

● 318 HYDROXYACIDS AND ANGIOGENESIS: POTENTIAL ROLE IN THE RETINOPATHY OF PREMATURITY (ROP). Marie J. Stuart, Carolyn Ganley, Yamaja Setty. SUNY, Upstate Medical Center, Department of Pediatrics, Syracuse, New York.

Recent studies have suggested that hypoxemia may worsen ROP in humans and retinal neovascularization in the animal model. We have shown previously that the hydroxy acids (HETES) can modulate angiogenesis. 15-HETE was observed to be proangiogenic, while 12-HETE was inhibitory to this process. We report that 15 HETE is present in human vessels, and that this proangiogenic metabolite is increased following in vitro hypoxia. When human umbilical arterial microsomes were incubated with ^{14}C Arachidonic Acid (AA) 3 HETES were observed. Two of the HPLC purified metabolites were confirmed by GC-MS to be 11- and 15-HETE. In 7 further experiments, paired umbilical arterial segments were subjected to air bubbling (control) or to bubbling with a 95% N2-5% CO2 gas mixture (hypoxia) for 20'. Microsomes were prepared, incubated with 5μM^{14}C AA, extracted, plated and analyzed by TLC in a solvent system to separate HETES. Hypoxia enhanced the production of total vascular HETES (231±36, 1SE, pmol per mg protein) compared to control values of 168±31 ($p<0.01$). The increase in vascular 15-HETE under hypoxic conditions was even more significant (36±6 control vs 50±6 post hypoxia; $p<0.001$). Platelet production of antiangiogenic 12-HETE on the other hand was not affected by hypoxia (55.3±5.1 vs 52.1±5.7 pmol per 10^8 plts). These in vitro observations suggest a possible biochemical basis for the abnormal angigenic process that occurs during the proliferative phase of ROP i.e. an increase in the levels of proangiogenic 15-HETE.