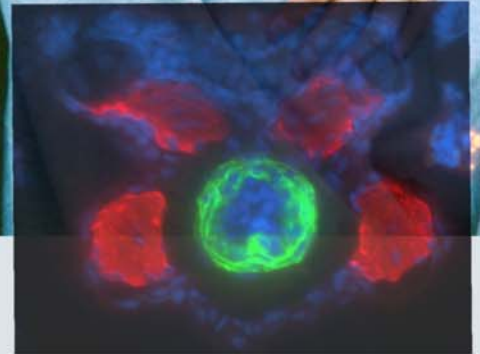
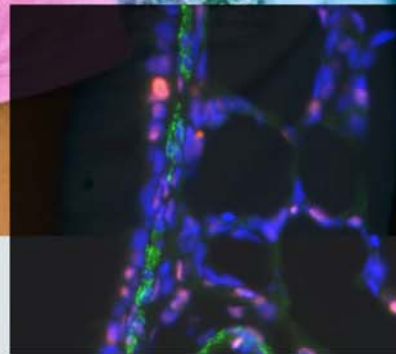




Fifth Annual NPRI Retreat



Thomas Conference Center
February 10, 2006

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Special Guest: Gail Martin, PhD
2006 Brumley Lecturer

9:00 Group Photo

9:15 Welcome, Ron Goldberg, MD

9:20 Comments from Joseph St. Geme, MD, Chairman of Pediatrics

9:30 Keynote Address: Geoff Ginsburg, MD, PhD, Professor of Medicine, Director, Center for Genomic Medicine:

“Personalizing Medicine: The Impact of Genomics”

10:30 Coffee Break

10:45 Silvia Rho (Meyers lab): “Hedghog signaling required for A-V cushion formation”

11:05 John Klingensmith, PhD: “Organizer genes and holoprosencephaly”

11:25 Bavia Trivedi, MD (Creazzo lab):

11:45 Leo DiNapoli (Capel lab): “Fgf9 is required for germ cell survival in the XY gonad”

12:05 Page Anderson, MD: “Calcium-mediated transcription of a cardiac specifying gene program and acquisition of a cardiac phenotype in liver stem cells”

12:30 Lunch

1:30 Neonatal Networks: Michael Cotton, MD; Jennifer Li, MD

2:30 James Reynolds, MD: "Studies on fetal physiology during maternal manipulation"

2:50 Katherine Kevill, MD: “Role for macrophage migration inhibitory factor in neonatal respiratory distress syndrome.”

3:10 Jennifer Turi, MD: "Mechanisms of iron accumulation in airway epithelial cells in cystic fibrosis"

3:30 Tea Break

3:50 Richard Auten, MD: “Inhaled nitrosoethanol prevents hyperoxia impaired post-natal lung development”

4:10 Eric Schultz, MD: “CXCR2 receptor and mast cell recruitment in newborn lung”

4:30 Lu Wang, MD: “Mechanical adaptation of maturing airway smooth muscle”

4:50 Kalid Ashour, MD (Sunday lab): Bombesin inhibits alveolarization and promotes pulmonary fibrosis in newborn mice: Diverse roles for GRP receptors

5:10 Wine and cheese social

HEDGEHOG SIGNALING REQUIRED FOR A-V CUSHION FORMATION

Silvia Rho and Erik Meyers, Departments of Cell Biology and Pediatrics (Neonatology)

Endocardial cushions of the developing heart contribute to formation of the atrio-ventricular (AV) valves and septation of the adult heart into four chambers. Cushions form from specific-spatiotemporal signaling between endothelial and myocardial cells causing an endothelial-to-mesenchymal transformation (EMT) at the AV canal at ED 9.5 in mice. To understand the pathogenesis of congenital heart defects such as AV valve abnormalities and AV canal defects, it is important to elucidate the molecular cues that guide EMT and endocardial cushion growth and remodeling. This study examines the role of Sonic hedgehog (Shh) signaling in AV cushion development, which has been suggested by recent studies (Meyers and Goddeeris, unpublished data) utilizing a *Cre/loxP* approach to inactivate either *Shh* or *Smo* in the heart, pharyngeal endoderm, and core pharyngeal arch mesenchyme via expression of *Nkx2.5Cre*. These mutant mice had AV canal defects. Thus, we are currently investigating which tissues in the AV canal, myocardium and/or endocardium, are an important source of *Shh* expression and which tissues need Shh signaling in order for AV cushions to form and grow properly. Initial data suggest that SHH ligand travels from outside the heart to the AV canal to affect EMT. Whole-mount mRNA *in situ* hybridization has shown no *Shh* expression in the heart of wild-type mouse embryos at ED 10.5. However, we have localized SHH protein in ED 10.5 hearts via section immunofluorescence. SHH protein appears to accumulate within the ventricular and atrial wall. We are also using an *in vitro* model of EMT to assess the role of SHH in EMT. More definitive information is expected to come from inactivating Shh signaling in either the myocardium or endocardium via the *Cre/loxP* recombinase system and examining this effect on heart morphology and cushion development. To locate the source of the Shh signal, we have eliminated *Shh* specifically in myocardial cells using *TntCre* and in endocardial endothelial cells using *Tie2Cre*. To determine the critical tissue for receiving the Shh signal, we have eliminated *Smo* expression using *TntCre* and *Tie2Cre*. Analysis of mice with *TntCre*-mediated deletion of *Shh* and *Smo* in myocardial cells shows no AV valve or septal defects. This suggests that it may be endocardial cells that require Shh signaling to ensure successful cushion development. The mechanism of any observed abnormalities will be studied through comparison of EMT, cellular proliferation, and apoptosis in mutant vs. control embryos.

DIGENIC MOUSE MODELS FOR HOLOPROSENCEPHALY AND LATERALITY DEFECTS

John Klingensmith and Ryan Anderson
Dept. of Cell Biology

We have previously demonstrated a role for the Bone Morphogenetic Protein (BMP) antagonists Chordin and Noggin in local function of the tissues that organize the ventral midline during mouse head development. Functional defects in these tissues lead to holoprosencephaly (HPE), a common and often lethal syndrome of forebrain and craniofacial midline tissue deficits. In humans, several HPE loci involve heterozygous mutations in genes of the Shh or Nodal signaling pathways. However, in general, the etiology of human HPE is heterogeneous; most likely, it typically results from multiple disruptions in convergent developmental processes and signaling pathways. Here, we explore the influence of abnormally elevated BMP activity upon compromised Shh or Nodal signaling in the gastrula organizer and its derivatives during early head development and left-right axis formation in the mouse. We find that two mutations in key organizer genes often lead to HPE and other midline defects, when either mutation alone does not. Analysis of these mutants suggests that the fundamental defects leading to HPE occur early in gastrulation. Our results offer novel insights into the molecular and cellular basis of HPE in humans, and provide testable models for the genetics of HPE in humans.

FUNCTIONAL TRPC CHANNELS IN EARLY CHICK HEART DEVELOPMENT.

Bhavya Trivedi, Victoria Graham, R. Neal Shepherd, and Tony L. Creazzo

Introduction: The mechanisms for cardiac excitation-contraction coupling (ECC) and receptor mediated Ca^{2+} responses in the early developing heart are poorly understood and it is unlikely that all proteins responsible for Ca^{2+} handling have been identified. Since the early embryonic heart expresses fewer junctional ryanodine receptors than at later stages, IP_3 -sensitive Ca^{2+} stores may play a greater role in early Ca^{2+} homeostasis. In smooth and skeletal muscle cells, canonical transient receptor potential channels (TRPC) function to refill Ca^{2+} stores after IP_3 induced release. Embryonic chick cardiomyocytes also have IP_3 - Ca^{2+} signaling pathways initiated by surface agonists such as acetylcholine and angiotensin II. TRPC channels have been identified in the adult heart, but their expression during embryogenesis has not been examined. **Hypothesis:** TRPC channel proteins are expressed in the embryonic chick heart and these channels can result in significant Ca^{2+} and Ba^{2+} influx after Ca^{2+} store depletion. **Methods:** RT-PCR and fluorescent antibody labeling was used to determine expression patterns of TRPC channels in embryonic day (ED) 3 and ED11 chick hearts. Hamburger-Hamilton (HH) stage 14 heart tubes and ED11 ventricle were enzymatically dissociated and cultured overnight to study TRPC function in single, isolated cardiomyocytes with Fura-2 fluorescent $\text{Ca}^{2+}/\text{Ba}^{2+}$ imaging and whole-cell patch clamp recording. Fluorescent Ca^{2+} imaging was also used to study spontaneously beating heart tubes dissected from ED3 chick embryos. **Results:** RT-PCR demonstrates that TRPC4 is equally expressed in the ED3 heart tube and in the ED11 ventricle. TRPC1 and TRPC4 fluorescent antibody labeling reveals signal throughout the atrial and ventricular myocardium at both developmental stages. Most TRPC channels are activated by Ca^{2+} -store depletion. After exposure to a 0 Ca^{2+} external solution for several minutes, single cultured cardiomyocytes have no response to 10mM caffeine, but had large Fura-2 signals upon exposure to a bath solution containing 2mM Ba^{2+} (ED2: n=25, ED11: n=19). This response is blocked by 100 μM SKF-96365, a TRPC channel inhibitor (ED2: n=5, ED11: n=4). Ca^{2+} influx is also seen but only in the presence of 10 μM thapsigargin (ED2: n=2, ED11: n=2). The whole-cell patch clamp technique was employed to record inward Ca^{2+} and Ba^{2+} currents in Ca^{2+} -depleted cells. With no IP_3 in the patch pipette, application of 2mM Ba^{2+} or 2mM Ca^{2+} results in no detectable inward current at negative holding potentials (ED2: n=4; ED11: n=9). However, with 10 μM intracellular IP_3 , an inward current is seen at -80mV upon exposure to Ca^{2+} (ED2: -3.0 pA/pF, n=1; ED11: -2.4 \pm 0.8 pA/pF, n=6) or Ba^{2+} (ED11: -4.1 \pm 0.6 pA/pF, n=4). In ED11 cells, this inward current is completely blocked by 50 μM 2-aminoethyl diphenylborinate (2-APB; n=2) which blocks the IP_3 receptor/TRPC pathway either by direct action on IP_3 receptors or TRPC channels. Remarkably, 2-APB also abruptly inhibits spontaneous beating of freshly dissected ED3 heart tubes (n=4), clusters of ED2 cultured cells (n=3), and clusters of ED11 atrial cells (n=3). **Conclusions:** Our results clearly demonstrate that TRPC1 and TRPC4 channels are present in the ED3 and ED11 chick heart and in cultured cardiomyocytes from these stages. In addition, our $\text{Ca}^{2+}/\text{Ba}^{2+}$ imaging and patch-clamp experiments reveal a Ca^{2+} and Ba^{2+} influx that is consistent with TRPC channel activity. This current is seen only in the presence of intracellular IP_3 which is in agreement with other studies that showed that IP_3 is necessary for activation of some TRPC channels. Inhibition of spontaneous beating in the heart tube and cultured cells by 2-APB suggests an important functional role for the IP_3 receptor/TRPC channel Ca^{2+} handling system.

FGF9 PROMOTES SURVIVAL OF GERM CELLS IN THE FETAL TESTIS

Leo DiNapoli, Jordan Batchvarov, and Blanche Capel
Department of Cell Biology

In addition to its role in somatic cell development in the testis, our data have revealed a role for *Fgf9* in XY germ cell survival. In *Fgf9* null mice, germ cells in the XY gonad decline in numbers after 11.5 days post coitum (dpc), while germ cell numbers in XX gonads are unaffected. We present evidence that germ cells resident in the XY gonad become dependent on FGF9 signaling between 10.5 dpc and 11.5 dpc and that FGF9 directly promotes XY gonocyte survival after 11.5 dpc, independently from Sertoli cell differentiation. Furthermore, XY *Fgf9* null gonads undergo true male-to-female sex reversal as they initiate but fail to maintain the male pathway and subsequently express markers of ovarian differentiation. By 14.5 dpc these gonads contain germ cells that enter meiosis synchronously with ovarian gonocytes. FGF9 is necessary and sufficient for 11.5 dpc XY gonocyte survival and is the earliest reported factor with a sex-specific role in regulating germ cell survival.

CALCIUM-MEDIATED TRANSCRIPTION OF A CARDIAC SPECIFYING GENE PROGRAM AND ACQUISITION OF A CARDIAC PHENOTYPE IN LIVER STEM CELLS

Page Anderson
Department of Pediatrics (Cardiology)

The mechanisms underlying stem cell-based therapy for heart disease are unresolved. Here, we study stem cells from a cloned adult rat liver stem cell line (WB F344) that acquire a cardiac phenotype in a cardiac microenvironment. Neonatal cardiomyocytes were co-cultured with WB F344 or WBAB1 cells, a mutagenized subclone with poorly functioning gap junctions. WB F344 and WBAB1 cells, a priori, express GATA4, SRF, MEF2C, and connexin 43. By 24 hours after co-culture, de novo intracellular oscillating calcium ($[Ca^{2+}]_i$) spikes, synchronous with $[Ca^{2+}]_i$ transients in adjacent cardiomyocytes, were demonstrated in WB F344 cells but not in WBAB1 cells. Functioning gap junctions and intracellular communication between WB F344 cells and myocytes were demonstrated using fluorescence recovery after photobleaching. Carbenoxolone, a gap junction uncoupler, eliminated $[Ca^{2+}]_i$ oscillations in WB F344 cells but not in myocytes. Within 48 hours, de novo expression at the RNA level of NKx2.5, Tbx5, Tbx20, and myocardin was present in WB F344 cells co-cultured with cardiac cells while nifedipine decreased their expression. Cardiac troponin I (cTnI) and cTnT in a sarcomeric pattern appeared by five days. Our findings suggest that a signal from the myocyte, possibly Ca^{2+} , diffuses through shared connexin 43-derived gap junction channels and induces cardiac-like oscillating $[Ca^{2+}]_i$ spikes in WB F344 cells. We hypothesize that these $[Ca^{2+}]_i$ oscillations transduce the expression of a cardiac specifying gene program and induce the acquisition of a cardiac phenotype in WB F344 cells.

STUDIES ON FETAL PHYSIOLOGY DURING MATERNAL MANIPULATION

James Reynolds

Long-term Goals: To elucidate mechanisms of fetal brain injury produced by pathophysiologic insult or maternal drug exposure; to determine the roles these mechanisms play in postnatal central nervous system disorders; and, ultimately, to design techniques or treatments to improve fetal (and maternal) well-being in the presence of such insults.

Background: All studies are designed as basic science investigations of clinically-relevant situations. Previous work has investigated the actions of drugs that are abused during pregnancy (e.g. ethanol, cocaine) and the fetal effects of pathophysiologic insults (e.g. umbilical cord occlusion) that can occur during gestation. Current studies are focussed on understanding and improving fetal physiology in the presence of common stressors, specifically maternal surgery and exposure to anesthetics agents.

Methodologies: We have developed techniques for continuous monitoring of fetal cerebral oxygenation. Using technology in pregnant sheep in combination with more standard fetal systemic monitoring has allowed us to describe divergent effects of maternal manipulations on fetal systemic and central oxygenation as well describing the gestational development of control of oxygenation.

Current Studies: We have recently described the effects of maternal general anesthesia on the preterm and nearterm fetus and we are now currently investigating the effects of maternal surgery at these two developmental time points. The final step will be to test out a method for improving fetal physiologic status during surgery and possibly during periods of insult (e.g. umbilical cord occlusion).

A ROLE FOR MACROPHAGE MIGRATION INHIBITORY FACTOR IN NEONATAL RESPIRATORY DISTRESS SYNDROME

Katherine Kevill

Using a mouse model of neonatal respiratory distress syndrome (RDS), we demonstrate a central role for macrophage migration inhibitory factor (MIF) in lung maturation at the developmental stage when human neonates are most susceptible to RDS. We prematurely delivered mouse pups at embryonic day 18, which is developmentally equivalent to the 25-28 week gestation human lung. Only 8% of the prematurely delivered pups genetically deficient in MIF (MIF-KO) survived 8 hours versus 75% of WT controls ($p < 0.001$). Local production of MIF in the lung increased at embryonic day 18, continued until full-term at embryonic day 19.5, and decreased in adulthood, thus coinciding with this developmental window. The lungs of the MIF-KOs were less mature upon histological evaluation, and demonstrated lower levels of VEGF and corticosterone – two factors that promote fetal lung maturation. *In vitro* studies support a role for MIF in surfactant production by pulmonary epithelial cells. In a cohort of human neonates with RDS, higher intrapulmonary MIF levels were associated with a lower likelihood of developing bronchopulmonary dysplasia, a sequelae of RDS ($P < 0.03$). This study demonstrates for the first time a role for MIF in lung maturation, and supports a protective role for MIF in newborn lung disease.

MECHANISMS OF IRON ACCUMULATION IN AIRWAY EPITHELIAL CELLS IN CYSTIC FIBROSIS

Jennifer Turi

Rationale: Cystic fibrosis (CF) is a highly morbid and ultimately fatal disease characterized by persistent lung infections and chronic inflammation. We have found high iron levels in CF airways, which can increase inflammation and generate oxidative stress. Airway epithelial cells normally protect against the damaging extracellular effects of iron by taking up the metal via divalent metal transporter 1 (DMT1) for sequestration in ferritin. Prior to uptake by DMT1, Fe^{3+} must be reduced to Fe^{2+} by a ferri-reductase, such as Dcytb. In addition, the transport of iron into the cell appears to require concurrent Na^+ transport. The expression of DMT1 and Dcytb in airway epithelium can be regulated by iron concentration. Therefore, we hypothesize that DMT1 and Dcytb expression in cystic fibrosis airway epithelial cells will be elevated and will contribute to increased intracellular iron in the absence of inflammation. Further, that the increased activity of the epithelial sodium channel (ENaC) in CF will contribute to increased iron transport.

Methods: Airway epithelial cells (HBE) obtained at autopsy from patients with CF were grown at air-liquid interface until differentiated into a mucociliary epithelium.* DMT1 and Dcytb gene and protein expression were measured by real time PCR and Western blot analysis, respectively. Intracellular iron concentration was measured by inductively coupled plasma atomic emission spectroscopy (ICPAES) in CF HBE cells and in normal HBE cells in the presence and absence of the ENaC channel inhibitor, amiloride. Lung sections from transgenic mice over-expressing ENaC were evaluated by immunohistochemistry (IHC) for intracellular ferritin accumulation.†

Results: DMT1 and Dcytb gene expression, normalized to GAPDH, were significantly increased in unstimulated CF HBE cells compared to control cells. This was followed by increased protein levels for both DMT1 and Dcytb when compared to control. Intracellular iron content, measured by ICPAES, was greater in unstimulated CF airway epithelial cells than control cells. However, when ENaC channel was inhibited in normal HBE cells, intracellular iron uptake was significantly decreased. Associated with these *in vitro* findings, ferritin staining was markedly increased in the airway epithelium, interstitium, and mucus in lung sections from mice over-expressing ENaC.

Conclusions: These data suggest that the expression and/or function of iron transport proteins may be intrinsically altered in CF, resulting in elevated iron concentration in airway epithelial cells. In addition, elevated ENaC activity in CF may contribute further to altered iron homeostasis. Additional studies will be required to evaluate the effect of exogenous iron and inflammation on the regulation of iron transport proteins in CF airway epithelial cells and to develop a mechanistic understanding of the role of increased ENaC activity in altered iron transport in CF.

NITROSOETHANOL INHALATION PREVENTS HYPEROXIA IMPAIRED POSTNATAL LUNG DEVELOPMENT

Richard Auten

Rationale: Nitric oxide (NO) regulates several molecular pathways important to lung development. Inhaled NO has been shown to partly protect premature lung development, albeit through unknown mechanisms. Severe oxidative stress can deplete free NO when superoxide reacts with NO to form peroxynitrite. In contrast, nitrosoethanol is less vulnerable to oxidative reaction and could theoretically improve the biological availability of NO. **Hypothesis:** We tested whether or not inhaled nitrosoethanol (ENO) could protect postnatal lung development from severe oxidative stress. **Method:** Newborn rats were exposed to air or 95% O₂ ± ENO 0.2-20 ppm at birth for 8 days. Some animals were recovered in room air until age 14 days. We measured leukocytes in airway lavage and tissue myeloperoxidase activity, neutrophil cytokine CINC-1, TNF α , and VEGF-1 by ELISA, CINC-1 mRNA by real-time RT-PCR, 3-nitrotyrosine (detects peroxynitrite), and NF- κ B activation at day 8. In animals treated with air v. 95% O₂ + ENO 10 ppm and allowed to recover in room air until day 14, we measured static lung compliance and estimated alveolar number and surface area by morphometry, and secondary crests were labeled with Hart's elastin stain. **Result:** ENO dose-dependently prevented 95% O₂-induced leukocyte influx, with no advantage between 10 and 20 ppm. 95% O₂-induced CINC-1, TNF α , CINC-1 mRNA, and peroxynitrite were prevented in pups treated with ENO 10 ppm. Pups recovered in air after exposure to air, 95% O₂ ENO 10 ppm showed significantly improved static lung compliance, alveolar number, and alveolar surface density. Secondary crest abundance: air > 95% O₂+ENO > 95% O₂. **Conclusion:** Inhaled ENO prevents O₂-impaired postnatal effects on lung development, most likely *via* its effects on inflammation, without increasing nitrosative stress.

CXCR2 ANTAGONISM REDUCES TRACHEAL MAST CELL ACCUMULATION IN HYPEROXIA-EXPOSED NEWBORN RATS

Eric Schultz

Background: Premature infants are at increased risk of developing airway hyper-reactivity (AHR) as a result of oxidative stress inflammation. Mast cells contribute to AHR by mediator release. CXCR2 (IL-8 receptor) is expressed on mast cells regulates mast cell precursor homing in mouse gut. The function of CXCR2 in pulmonary mast cells during development or lung injury is unknown.

Objective: Determine if mast cell abundance and/or mast cell degranulation is increased by 60% O₂-exposure in newborn rats, and whether treatment with CXCR2 antagonist SB-265610 affects mast cell abundance /or degranulation in 60% O₂-exposed newborn rat trachea and airways.

Design/Methods: Rats were exposed at birth to air v. 60% O₂ 14 days SB-265610 5 g/kg v. vehicle i.p., N=6/group. Random sections (5/pup) from trachea and lung hilum were stained with toluidine blue or anti-rat mast cell protease II (RMCP-II, recognizes mucosal-type mast cells). Mast cells were counted in random fields that

contained airways with smooth muscle. Mast cell # in trachea and smaller airways were normalized to airway perimeter length by image analysis. Degranulated mast cells=granules visible outside the cell membrane. Normalized mast cell # and the proportions of degranulated/total mast cell # in each treatment group were compared

by ANOVA. Data are expressed as mean SEM, **p*<.05 v. air . O₂ + vehicle.

Results:

	Trachea	Hilar	Trachea, degranulated	Hilum, degranulated
Air	5.6±0.6	0.5±0.1	0.61±0.04	0.54±0.07
O ₂ + vehicle	5.4±0.5	0.9±0.2	0.66±0.05	0.59±0.05
O ₂ + CXCR2 antagonist	3.2±0.2*	0.8±0.2	0.71±0.07	0.51±0.06

O₂ tended to increase mast cells in peripheral airways, with no effect of SB265610. The proportion of degranulated mast cells was unaffected by O₂-exposure and SB265610. Fewer than 10% of the mast cells in each group were RMCP II, showing that the majority were of the connective tissue-type, as expected, with no apparent group differences. There was a trend toward higher mast cell accumulation in hilar airways.

Conclusions: CXCR2 antagonism during hyperoxia exposure decreases tracheal mast cell accumulation. We speculate that other ligand-receptor pathways are predominantly responsible for mast cell homing in smaller airways.

MECHANICAL ADAPTATION OF MATURING AIRWAY SMOOTH MUSCLE

Lu Wang

Deep inspiration (DI) counteracts bronchospasm in normal subjects but triggers further bronchoconstriction in hyperresponsive airways. It is known that healthy immature airways of both human and animal exhibit hyperresponsiveness and that DI has limited bronchodilatory and bronchoprotecting benefit in infants. We suggested that the phenomenon is related to changes in force generating ability of airway smooth muscle after mechanical stretches. We postulated that the profile of active force generation after mechanical stretches changes with maturation and that this change contributes to the expression of airway hyperresponsiveness in the young. Most recently we reported for the first time a potentiated contractile property of immature and hyperresponsive airway smooth muscle after DI-mimicking stretches. This can account for further bronchoconstriction triggered by DI in hyperresponsive airways. We also showed that inhibition of cyclooxygenase, one of the critical enzymes that catalyze prostanoid biosynthesis, abolishes this phenomenon in a dose-dependent manner. Currently we are studying the types of prostanoid receptors responsible for initiating the force potentiation and the subsequent signaling pathways which involve small GTP-Rho and cytoskeleton dynamics. The outcome will improve our understanding of the mechanisms underlying the effect of deep inspiration on hyperresponsive airways and its potential role in juvenile airway hyperresponsiveness and asthma.

BOMBESIN INHIBITS ALVEOLARIZATION AND PROMOTES PULMONARY FIBROSIS IN NEWBORN MICE: DIVERSE ROLES FOR GRP RECEPTORS

Khalid Ashour, MD, William Schlicher, DDS, Keiji Wada, MD, PhD, Etsuko Wada, MD, PhD, and Mary E. Sunday, M.D., PhD.

Bombesin-like peptide (BLP) promotes fetal lung development. Normally BLP levels normally drop postnatally, but these are elevated in newborns that develop bronchopulmonary dysplasia (BPD), characterized by arrested alveolar septation. In premature baboon models of BPD, anti-bombesin antibodies reduce lung injury and promote alveolarization. The present study tests whether exogenous BLP given prenatally or postnatally alters alveolar development in newborn mice. We observe multiple effects of BLP on postnatal lung analyzed at P14, when alveolarization is ~half-complete. First, alveolar myofibroblast proliferation is induced by BLP compared to saline-treated controls. Second, BLP increases alveolar wall thickness and prevalence of alveolar myofibroblasts. Third, BLP abrogates alveolarization in C57BL/6 (but not Swiss-Webster) mice. We utilized BLP receptor-null mice to explore which receptors might mediate these effects. Compared to wild-type littermates, GRPR-null mice have reduced BLP-induced defects in alveolarization, indicating that this response is mediated by GRPR. However, GRPR-null mice the same increase in numbers of BLP-induced myofibroblasts. In contrast, NMBR- and BRS-3-null mice have all BLP responses similar to wild-type littermates and there is no effect of either NMB or a synthetic BRS3 ligand in this system. Thus, BLP can induce several features of BPD, including interstitial thickening and delayed alveolar development. GRPR appears to mediate the BLP effect on alveolarization. However, none of the 3 receptors mediates BLP-induced interstitial fibrosis, suggesting that a novel receptor may be implicated in these responses in newborn lung. These observations indicate that elevated BLP alone could trigger the phenotypic changes in lung of BPD infants.