Oxygen, gastrin-releasing peptide, and pediatric lung disease: life in the balance

Mary E. Sunday*
Department of Pathology, Duke University Medical Center, Durham, NC, USA

INTRODUCTION

Oxygen (O₂) is essential for life. In aerobic animals, the lung evolved as a critical organ for gas exchange permitting species to move from water to land. Lungs are exposed to all the elements: air, earth, water, and fire/radiation. Homeostasis and health represent a natural equilibrium between opposing forces. Disease results when there is an imbalance between environmental exposures and host defense. Individual responses to diverse challenges can vary due to genetic factors.

Life hinges on a delicate balance. Too much or too little heat, humidity, or O₂ can be lethal. Although O₂ is essential for life, too much O₂ can lead to tissue injury, fibrosis, senescence, and death (1–4). For several decades my research has focused on O₂-sensing pulmonary neuroendocrine cells (PNECs) and their product gastrin-releasing peptide (GRP), a mammalian homolog of amphibian bombesin (5). GRP secretion can be induced by reactive oxygen species (ROS) from exposure to hyperoxia (6), ozone (7), or ionizing radiation (RT) (8). Furthermore, PNEC degranulation is known to be induced by hypoxia (9), which is also associated with increased ROS levels (10).

In the current review, I will introduce background information about PNECs as O₂-sensing cells. The discussion will then summarize the highlights of over 25 years of work from my laboratory regarding the role of GRP in lung development and postnatal lung diseases, especially bronchopulmonary dysplasia (BPD). Cumulatively, these studies provide the foundation for future exploration of how GRP could mediate lung injury including acute and chronic inflammation and pulmonary fibrosis (PF) (7, 8, 11).

OXYGEN-SENSING CELLS: PULMONARY NEUROENDOCRINE CELLS

O₂-sensing cells are important regulators of vascular tone and cardiac function. Historically, most research about O₂-sensing cell biology and physiology has been focused on cardiomyocytes (12), vascular smooth muscle cells (13), and carotid body cells (glomeruli) (14), although interest in PNEC biology is growing (15). Much has been written about all of these cells (Figure 1) and their collective tissues, with numbers of PubMed citations on July 5, 2014 as follows: 228,676 for cardiomyocyte(s), cardiac muscle cell(s), or cardiac muscle cells (vs. tissue = 61,228 vs. 213,615); 77,151 for vascular smooth muscle cell(s), vascular smooth myocyte(s), or vascular smooth muscle cells (vs. tissue = 53,567 vs. 72,628); 13,068 for carotid body cell(s), glomus cells(s) or carotid body (cells vs. tissue = 6,462 vs. 11,987). However, relatively little is known about PNECs or their clusters in pulmonary epithelium, called neuroepithelial bodies (NEBs): 3,547 total citations, representing 3048 for PNECs and/or 624 for NEB(s). If cancer is excluded from the search for the cardiac, vascular, or carotid cells or tissues, the numbers drop modestly (Figure 1, lower panel) with the percentage of non-cancer citations: cardiac muscle or myocytes and vascular smooth muscle or myocytes, and 79–80% for carotid body or glomus cells. In contrast, the numbers of non-cancer citations for PNECs is only 26%, providing objective evidence that PNEC research has been largely focused on lung cancer, especially small cell carcinoma of the lung, a highly malignant cancer apparently derived from PNECs (16). Although a PubMed search for NEBs yielded only 624 citations, 508 (81%)
cell proliferation both in culturing normal PNECs or NEBs, which have a low rate of cancer-related publications is also likely due in part to challenges has been relatively under-explored. The low number of injury/repair as well as lung carcinogenesis (17–21). The biology of non-neoplastic/homeostatic PNEC responses to environmental challenges has been relatively under-explored. The low number of cancer-related publications is also likely due in part to challenges in culturing normal PNECs or NEBs, which have a low rate of cell proliferation both in vitro (22, 23) and in vivo (24), although PNEC proliferation can occur in vivo following acute injury (25).

PNECs were first identified in the lung by Feyrter as part of a diffuse epithelial endocrine system (26, 27). Studying airway epithelium of human newborn lung, Lauweryns later identified clusters of similar amine-producing cells, which he called “NEBs,” containing dense-core neurosecretory vesicles (DCV) (28). He first studied hypoxia- or hypercarbia-induced exocytosis of DCV from NEBs (9). Second, by using cross-circulation studies in rabbits, he observed that airway hypoxia but not hypoxemia induced exocytosis of DCV from NEBs (30). He postulated that NEB react to the composition of inhaled air and by releasing serotonin or peptides could produce a local vasoconstriction and/or bronchoconstriction in hypoxically aerated lung areas, thus enabling intrapulmonary regulation of the V/Q ratio (30). Innervation of single PNECs and NEBs is extensive in newborn rabbits (32), consisting predominantly of vagal afferent sensory nerves (15, 33). Although the function of NEB innervation remains unclear, evidence suggests a role in the generation of dyspnea (34).

Investigating how PNECs sense hypoxia, Cutz et al. carried out patch-clamp analysis of intact NEBs stained with a vital dye. They found the key players in rabbit and human lung are a membrane-bound O$_2$-binding NADPH oxidase coupled to an H$_2$O$_2$-sensitive K$^+$ channel protein (35, 36), later confirmed in knockout mice as Nox2 (37). Although NEBs express multiple NADPH oxidases and diverse voltage-gated potassium channels (Kv) and tandem pore acid-sensing K$^+$ channels (TASK) (38), there is molecular complex formation between NOX2 (gp91 phox) and Kv but not TASK1. This observation implicates NOX2/Kv as the major O$_2$ sensor complex in PNECs (39, 40).

## GASTRIN-RELEASING PEPTIDE DURING PHYSIOLOGICAL HYPOXIA AND PHYSIOLOGICAL HYPEROXIA

Ernest Cutz is a pediatric pathologist who has carried out much of the seminal work on PNECs and GRP in pediatric lung diseases (17, 41). Writing a chapter together, we explored temporal and spatial expression of GRP expression during perinatal physiological processes versus postnatal disease states (42). This dichotomy can be viewed as functions of GRP in fetal lung development and perinatal transitioning (physiological hypoxia and physiological hyperoxia) versus GRP mediating pathological responses to sustained hyperoxic exposure, such as BPD.

In utero development can be considered a state of “physiological hypoxia.” Peak PNECs occur during the canalicular stage of development (at midgestation in primates and during late gestation in rodents), during which the foundation of the pulmonary capillary bed is established. At term, the umbilical artery pO$_2$ is ~16 mm Hg (~24% O$_2$ saturation), and umbilical vein pO$_2$ is ~27 mm Hg (~55% O$_2$ saturation), in contrast to postnatal arterial pO$_2$ of ~100 mm Hg with O$_2$ saturation >90% for term infants on room air (43).

Peak GRP mRNA levels are present in human fetal lung at midgestation (44), in the setting of physiological hypoxia (43). GRP (also known as bombesin, bombesin-like peptide or BLP) is initially synthesized as a 138–148 amino acid pro-hormone composed of three isoforms (45). These are all cleaved at methionine #27. This Met becomes the carboxy terminus of GRP that must be amidated to form the bioactive GRP peptide with GRP (14–27) amino acid sequence of – Met-Tyr-Pro-Arg-Gly-Asn-His-Leu-Met-NH$_2$ (17, 41). Writing a chapter together, we explored temporal and spatial expression of GRP expression during perinatal physiological processes versus postnatal disease states (42). This dichotomy can be viewed as functions of GRP in fetal lung development and perinatal transitioning (physiological hypoxia and physiological hyperoxia) versus GRP mediating pathological responses to sustained hyperoxic exposure, such as BPD.

In utero development can be considered a state of “physiological hypoxia.” Peak PNECs occur during the canalicular stage of development (at midgestation in primates and during late gestation in rodents), during which the foundation of the pulmonary capillary bed is established. At term, the umbilical artery pO$_2$ is ~16 mm Hg (~24% O$_2$ saturation), and umbilical vein pO$_2$ is ~27 mm Hg (~55% O$_2$ saturation), in contrast to postnatal arterial pO$_2$ of ~100 mm Hg with O$_2$ saturation >90% for term infants on room air (43).

Peak GRP mRNA levels are present in human fetal lung at midgestation (44), in the setting of physiological hypoxia (43). GRP (also known as bombesin, bombesin-like peptide or BLP) is initially synthesized as a 138–148 amino acid pro-hormone composed of three isoforms (45). These are all cleaved at methionine #27. This Met becomes the carboxy terminus of GRP that must be amidated to form the bioactive GRP peptide with GRP (14–27) amino acid sequence of – Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Lys-Asp-His-Leu-Met-NH$_2$ (14–27). This Met becomes the carboxy terminus of GRP that must be amidated to form the bioactive GRP peptide with GRP (14–27) amino acid sequence of – Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Lys-Asp-His-Leu-Met-NH$_2$ (14–27). This Met becomes the carboxy terminus of GRP that must be amidated to form the bioactive GRP peptide with GRP (14–27) amino acid sequence of – Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Lys-Asp-His-Leu-Met-NH$_2$ (14–27).
These observations were later confirmed by Fraslon and Bourbon in France (51) and Asokananthan and Cake in Australia (52), with the additional observation of GRP-induced surfactant secretion (52). We also determined that bombesin and a related frog peptide, Leu8-phyllolitorin, promote branching morphogenesis and cell proliferation in embryonic mouse lung buds (53).

In contrast to in utero development, postnatal adaptation is often referred to as “physiological hypoxia” in recognition of the sudden change in O2 levels in the infant from ~27 mm Hg in utero to 100 mm Hg on room air (43). Room air is essentially hypoxic to the newborn lungs. It has been recognized since the 1950s that postnatal lung development in premature infants is a unique medical situation, as first defined in pioneering work by Mel Avery that led to the discovery that respiratory distress syndrome (RDS) is due to a deficiency of surfactant. Consequently, preterm infants cannot readily expand their lungs with air to allow breathing (54). Before the arrival of surfactant therapy, premature infants often needed high levels of O2 therapy to survive. New challenges arose because prematurity is also associated with inadequate antioxidant defenses (55). Chronic lung disease of newborns, called BPD (56), was linked to O2 therapy, a mainstay of treatment for premature infants (57). The severity of BPD has decreased thanks to surfactant therapy and modern medical management such as low-barotrauma high-frequency ventilation and CPAP (58, 59). Despite improved medical care, the incidence of BPD has paradoxically increased or remains unchanged, which is puzzling regardless of how BPD is defined (57, 60, 61).

BPD: NEUROENDOCRINE CELLS AND GASTRIN-RELEASING PEPTIDE

Bronchopulmonary dysplasia remains a major cause of morbidity and mortality in very low birth weight infants with gestational age <28 weeks (60, 62). BPD is associated with persistent respiratory morbidity including increased hospital admissions for respiratory distress, bronchiolitis, status asthmaticus, and pneumonia (59). BPD is also associated with other complications including pulmonary hypertension, systemic hypertension, intraventricular hemorrhage, periventricular leukomalacia, neurocognitive delay, and cerebral palsy (62–65).

Early prediction of BPD has proven challenging. Relative numbers of GRP-positive PNECs normally decrease over the first postnatal months, and are markedly decreased in premature infants dying of RDS at postnatal day (PND) 1–7, thought to reflect PNEC degranulation (66). In contrast, PNECs are increased in bronchioles of infants dying with BPD at 2 weeks to 6 months of age (66). We hypothesized that elevated urine GRP levels precede BPD. One hundred thirty-two infants born at 28-weeks gestation or less, were studied. Urine GRP levels, determined by radioimmunoassay, were normalized for creatinine. BPD was defined as O2 dependence at 36 weeks post-menstrual age. Consistent with the increased number of PNECs, urine GRP was also elevated in a first urine sample at PND 1–5 in ≤28-week gestation infants who later developed BPD (67). GRP is excreted as a stable peptide in the urine; urine GRP levels are positively correlated with bronchoalveolar lavage (BAL) GRP levels (68). In the analysis by Anne Cullen (now Anne Cullen Twomey), a first urine specimen with GRP level greater than 20,000 pg/mg creatinine between PND 1–5 occurred among 54% of the infants who later developed BPD (p < 0.001), versus 10% among non-BPD infants (specificity 90%). Multivariable logistic regression analyses demonstrated that elevated urine GRP levels were associated with a 10-fold increased risk of BPD (p < 0.001) after adjusting for all confounding factors. Furthermore, urine GRP elevation occurs in parallel with markedly increased levels of GRP mRNA in newborn baboon lung (69). Utilizing urine GRP for screening might permit early therapeutic interventions to reduce disease progression and could provide a target for new preventive therapies.

We tested the hypothesis that GRP is linked to the pathogenesis of BPD through analysis of two baboon models of BPD: hyperoxia (140-day-old animals (~32 weeks human gestational equivalent) given 100% O2 for 10 days, vs. non-BPD 140-day-old animals given PRN O2) and barotrauma (125-day-old animals (~26 weeks human gestational equivalent) given PRN O2 for 14 days) in collaboration with Jackie Coalson and the NIH Program in BPD (70–72). In both BPD models, GRP was elevated at 24–72 h after birth. This GRP elevation was closely correlated with impaired respiratory function with increased oxygenation index, and also arrested alveolar number with alveolar wall thickening, decreased secondary alveolar septa, and blunted capillary tubulogenesis (69, 73). Remarkably, postnatal inhibition of GRP with a blocking anti-GRP antibody prevented the functional and histological changes of BPD in these animal models (69, 73). These observations suggest that GRP could be an important therapeutic target to decrease BPD prevalence and later pulmonary morbidity.

OXIDATIVE STRESS, NEUROENDOCRINE CELLS, AND GASTRIN-RELEASING PEPTIDE

PNEC hyperplasia occurs in weanling rat lungs in response to cigarette smoke (74) or hyperoxia (75). Elevated GRP has been associated with oxidative stress in humans including cystic fibrosis (CF) patients (76), asymptomatic smokers (68, 77), and patients with chronic obstructive pulmonary disease (78).

ROS, also known as oxygen free radicals, have been implicated in the pathogenesis of BPD. In the hyperoxic baboon model of BPD, inhibition of oxidative stress using a catalytically active metalloporphyrin (AEOL10113) decreased the number of PNEC cells, decreased GRP levels, and diminished BPD severity pathologically (6). The antioxidant not only decreased PNECs, but abrogated parenchymal mast cells and eosinophils (6). Subsequent work determined a direct link between GRP and mast cell accumulation (79). Despite the epidemiologic evidence that oxidative stress is linked to risk for BPD, this knowledge has not yet been translated into validated biomarkers for disease, or into mechanism-specific therapies to mitigate BPD morbidity.

Notably, several urine biomarkers of oxidative stress have been shown to be elevated in BPD in published clinical studies: F2-isoprostane (80, 81), 8-hydroxydeoxyguanosine (82, 83), and allantoin (84). F2-isoprostanes are increased in term infants ventilated with FiO2 of 1.0 for severe pulmonary disease due to meconium aspiration, neonatal pneumonia, or primary pulmonary...
hypertension (85) or in preterm infants with BPD. 8-hydroxy-
2′-deoxyguanosine is an established marker of in vitro and in vivo
oxidative stress and is increased in preterm infants (82), is greater
in sick vs. stable preterm infants (83), and is increased in patients
with chronic obstructive pulmonary disease (86), smokers (87),
and workers exposed to traffic exhaust (88).

The question arose whether administration of GRP alone during
perinatal transition could lead to histopathological and func-
tional perturbations similar to BPD, even in a clinical setting free of
abnormal oxidative stress. To test this hypothesis, we turned to
a mouse model, considering that basic molecular mechanisms of
lung development have often been explored in mice (89–94).

MODEL OF NEWBORN MICE TREATED WITH EXOGENOUS
GRP
Extending Koch’s postulates (95) to a non-infectious disease
process, we tested whether exogenous GRP would alter lung devel-
OPMENT in newborn mice. To recapitulate elevated GRP levels
shortly after birth, as observed in infants with BPD, we treated
newborn mice with bombesin or GRP twice daily from PND
1–3 (11). On Day 14, when alveolarization is normally about
half complete, we observed pathological effects similar to BPD
induced by bombesin or GRP: alveolar myofibroblast prolifer-
ation, increased alveolar wall thickness and diminished alveo-
larization. Compared with wild-type littersmates, bombesin or
GRP-treated GRP receptor (GRPR)-null mice (96) had reduced
defects in alveolarization, although bombesin-induced interstitial
fibrosis was the same as in wild-type littersmates. Neuromedin B
(NMB) receptor-null (97), and bombesin receptor subtype 3-null
(98) mice had the same responses as their wild-type littersmates
(11). Neither NMB nor a synthetic bombesin receptor type 3
ligand had any effect, consistent with effects of GRP being abro-
gated in GRPR-null mice. Bombesin/GRP can induce features of
BPD, including interstitial fibrosis and diminished alveolariza-
tion. GRPR appears to mediate all effects of GRP, but only part
of the bombesin effect on alveolarization, suggesting that novel
receptors may transduce some effects of amphibian bombesin in
newborn lung.

These observations in newborn mice indicate that excessive
GRP alone can alter normal lung development, potentially medi-
ating a cascade leading to abnormal pulmonary structure and
function weeks to months later. GRP levels are elevated in urine
and BAL of asymptomatic smokers (68), who also have elevated
oxidative stress markers in urine (99). Maternal smoking is associ-
ated with many pediatric lung diseases, including asthma (100). It
was hypothesized by Sam Aguayo that GRP could mediate tobacco-
related lung diseases (77). We began to explore whether GRP can
mediate lung injury due to oxidative stress in older patients, such
as that occurring secondary to radiation (RT) exposure.

GRP AND RADIATION-INDUCED PULMONARY FIBROSIS
RT-induced lung injury is a clinically relevant model for studying
PF in humans, including idiopathic pulmonary fibrosis (IPF). RT
produces ROS in target tissues, inducing acute and chronic radia-
tion pneumonitis, and ultimately leading to interstitial fibrosis. In
mice and other experimental animals, PF is similar to the human
disease caused by environmental exposures or autoimmune dis-
eases, and idiopathic PF. In humans, PF is progressive and irre-
versible, usually developing over 6–12 months post-RT. The mean
survival of patients following the diagnosis of idiopathic PF is 3–
5 years. There is no cure for PF except for lung transplantation,
which has limited accessibility and has its own set of morbidities.
We seek to reverse fibrotic responses in lung by identifying new
pathways and bridges preserving organ integrity and homeostasis.

Long-term survivors of childhood malignancies, especially
those treated with RT for thoracic tumors, are at a ninefold
increased risk of developing PF (101). Post-treatment pulmonary
disease is becoming less common with newer modalities of RT
therapy such as high-resolution RT and proton beam therapy. In
contrast, children undergoing total body irradiation (TBI) prior
to bone marrow transplantation frequently develop serious pul-
monary sequelae including interstitial fibrosis (102). Like IPF,
there is no effective treatment for this post-TBI PF. Similarly,
accidental nuclear exposure of children can lead to significant
interstitial (restrictive) lung disease that is greater in those individ-
uals exposed to the highest doses of radioactivity (103). Analysis
of GRP+ PNECs or urine GRP levels in patients post-RT could
clarify the disease pathogenesis and potentially set the stage for
GRP-blockade treatment to prevent the chronic lung disease in
similar clinical settings.

Considering that GRP-blockade abrogates pulmonary inflam-
mation and fibrosis in the hyperoxic baboon model of BPD, we
sought to determine whether GRP contributes to inflammatory
and fibrotic phases of RT induced lung injury. Using a well-
characterized mouse model of PF developing ~20 weeks after
high-dose thoracic RT (15 Gy) (104), we injected GRP blocking
small molecule 77427 h after RT then twice weekly for up to
20 weeks (8). Mice given RT plus PBS had increased interstitial
CD68+ macrophages 4 weeks later and increased GRP+/PGP9.5+PNECs 6 weeks later. Ten weeks post-RT, PBS controls had
increased pSmad2/3+ nuclei indicating active TGFβ signaling.
GRP-blockade with 77427 abrogated or significantly diminished
CD68+, GRP+, and pSmad2/3+ cells. Twenty weeks post-RT
interstitial fibrosis was demonstrated by α-smooth muscle actin
(SMA) immunostaining for myofibroblasts (105, 106), which exe-
cute organ fibrosis, and also by Mason’s trichrome histochemical
staining for interstitial collagen deposition (107, 108). Treatment
with 77427 abrogated both interstitial SMA and collagen. Sham
mice given 77427 did not differ significantly from PBS controls
(8). These observations indicate that GRP-blockade decreases
inflammatory and fibrotic responses to RT in mice. Similar to
our experiments with hyperoxia and ozone, we propose a gen-
eral working hypothesis, summarized in Figure 2. Environmental
exposures generating ROS trigger PNECs to secrete GRP, which
can act directly on target cells bearing cognate receptors, includ-
ing airway smooth muscle cells (109), macrophages (7), CD4+T
cells (7), neutrophils (7, 110), endothelial cells (69), and pul-
monary fibroblasts (69). Secondary effects could be due to GRP-
induced cell differentiation (46, 50) and/or secretion of cytokines
by macrophages and T cells (7, 111). Novel approaches to inter-
rupting GRP signaling could prevent or reverse lung injury and
fibrosis caused by RT, hyperoxia, or ozone.
Additional pediatric lung diseases have been associated with a distinct group of pediatric patients with clinical signs and symptoms. NEHI patients have improved (120). Thus, NEHI represents a disease that has been identified by Robin Deterding as a cause of chronic hypoxia in infants at risk (123, 124). Considering that PNECs function as airway O₂ sensors, Cutz suggested that GRP or another PNEC marker could herald airway chemoreceptor dysfunction as a risk factor for SIDS (125). However, GRP levels are low in SIDS victims, suggesting that another PNEC-derived product could play a role, such as calcitonin gene-related peptide (CGRP) (124). Moreover, parents of SIDS infants have a diminished ventilator response to acute hypercapnia (126), whereas hypercapnia has no effect on PNEC secretion (9).

Cystic fibrosis has also been associated with increased numbers of PNECs immunostaining for GRP, calcitonin, and serotonin (113). CF is a complex lung disease with altered mucus, chronic infection with lung inflammation, and destruction leading to bronchiectasis (127). Urine GRP levels are high postnatally in children with CF, in contrast to the decline in normal infants (76). PNECs express CFTR at the apical membrane, suggesting that NEBs could contribute to CF lung disease, including the early stages before establishment of chronic infection and progressive lung disease (128, 129). Although PNECs, airway innervation, and smooth muscle are altered in Cfr-null mice (130), it remains possible that PNEC abnormalities are secondary to infection and/or inflammation. For instance, NE cell differentiation can be induced by TNFα (131) or other cytokines. At this time, there is no clear-cut evidence for a pivotal role for GRP or PNEC in CF lung disease.

TIME FOR A PARADIGM SHIFT

Early and excessive GRP secretion is associated with chronic lung disease in infants. With regards to the variable interstitial fibrosis and arrested alveolarization that are characteristic of modern-day BPD, the body of evidence indicates a cause-and-effect relationship: elevated GRP can cause the clinical and pathological hallmarks of BPD in animal models. An NIH observational multicenter clinical investigation of premature infants is currently underway with Judy Vojnow and Mike Cotten as PIs, with outcomes including urine levels of GRP and oxidative stress markers. The focus of this collaborative work has now intensified: to determine how transient, early GRP elevation triggers chronic lung disease with fibrosis weeks to months later. Last, but not least, we are actively seeking an optimal approach for GRP-blockade to most effectively prevent BPD in infants and PF in older children and adults.

ACKNOWLEDGMENTS

I wish to acknowledge the major contributions of Drs. Jackie Coalson at UT San Antonio, Stella Kourembanas, Linda van Marter, and Mel Avery at Boston Children’s Hospital, and Shutang Zhou, Mike Cotten, Judy Vojnow, and Mark Dewhirst at Duke University Medical Center for all they have done to make this work possible (Judy Vojnow is now at Virginia Commonwealth University). This work has been supported by the following grants: NIH U01-HL52638 (Mary E. Sunday), RO1-HL50045.
REFERENCES

1. Yee M, Bucyznski BW, Lawrence BP, O’Reilly MA. Neonatal hyperoxia increases sensitivity of adult mice to bleomycin-induced lung fibrosis. *Am J Respir Cell Mol Biol* (2013) 48:258–66. doi:10.1165/rcmb.2012-0238OC

2. Saretski G, Feng J, von Zglinicki T, Villeponteau B. Similar gene expression pattern in senescent and hyperoxic-treated fibroblasts. *J Gerontol A Biol Sci Med Sci* (1998) 53:B838–42. doi:10.1093/gerona/53A.A.84B8

3. Cataldi A, Di Giulio C. “Oxygen supply” as modulator of aging processes: hypoxia and hyperoxia models for aging studies. *Curr Aging Sci* (2009) 2:95–102. doi:10.2174/1874469098001020095

4. Gore A, Murdahidur M, Espy MG, Degenhardt K, Mantell LL. Hyperoxia sensing: from molecular mechanisms to significance in disease. *J Immunotoxicol* (2010) 7:239–54. doi:10.1007/s10765-010-0422-4

5. Wharton J, Polak JM, Bloom SR, Ghatei MA, Solcia E, Brown MR, et al. Bombesin-like immunoreactivity in the lung. *Am J Physiol* (1998) 276:769–70. doi:10.1038/273769A0

6. Chang L, Subramaniam M, Yoder BA, Day BJ, Coalson JJ, Sunday M, et al. A parabronchial smooth muscle constitutes an airway epithelial stem cell niche. *Nature* (2009) 457:56–7. doi:10.1038/nature07538

7. Zhou S, Potts EN, Cuttitta F, Foster WM, Sunday ME. Gastrin-releasing peptide blockade as a broad-spectrum anti-inflammation therapy for asthma. *Proc Natl Acad Sci U S A* (2011) 108:21100–5. doi:10.1073/pnas.1014792108

8. Zhou S, Nissar E, Jackson IL, Leong W, Dancy L, Cuttitta F, et al. Radiation-induced lung injury is mitigated by blockade of gastrin-releasing peptide. *Am J Pathol* (2013) 182:1248–54. doi:10.1016/j.ajpath.2012.12.024

9. Lauweryns JM, Cokelaere M. Hypoxia-sensitive neuro-epithelial bodies. Intrapulmonary neuroendocrine cells. *Am J Respir Cell Mol Biol* (2005) 33:1248–54. doi:10.1165/rccmb.2005021536

10. Lauweryns JM, Cokelaere M, Detlefsenster M, Liebens M. Intrapulmonary neuroendocrine cells. *Invest* (1993) 70:379–89. doi:10.1007/BF03651350

11. Stevens TP, McBride JT, Peake JL, Pinkerton KE, Stripp BR. Cell proliferation of pulmonary neuroendocrine cells: diffuse idiopathic pulmonary neuroendocrine cell hyperplasia, tumorlet, and carcinoids. *Am J Respir Cell Mol Biol* (2000) 33:373–86. doi:10.1165/rccmb.20000309

12. Cadenas S, Aragones J, Landazuri MO. Mitochondrial reprogramming through hypoxia and hyperoxia, hypercapnia, nicotine, reserpine, L-DOPA and 5-HTP. *Cell Tiss Res* (1977) 182:425–50. doi:10.1007/BF00219827

13. Ashour K, Shan L, Lee JH, Wada SJK, Wada E, Sunday ME. Bombesin and lung disease—recent advances. *Pediatr Dev Pathol* (2014) 17:201–11. doi:10.1016/j.pdp.2013.02.009

14. Reynolds SD, Hong KU, Giangreco A, Mango GW, Guron C, Morimoto Y, et al. Fernando de Castro and the discovery of the arterial chemoreceptors. *Proc Natl Acad Sci U S A* (2010) 107:7936–41. doi:10.1073/pnas.1005753107

15. Feyrer F, Ecker Ueber Diffus Endokrine Epitheliale Organe. Leipzig: J.A. Barth (1938).

16. Feyrer F. Zur pathologie des argyrophilen helle-zellen-organes im bronchial-baum des menschen. *Virchows Archives* (1954) 325:723–32. doi:10.1007/BF00955103

17. Wharton J, Polak JM, Bloom SR, Ghatei MA, Solcia E, Brown MR, et al. Bombesin-like immunoreactivity in the lung. *Am J Physiol Lung Cell Mol Physiol* (1997) 273:L598–607. doi:10.1152/ajplung.00170.2012

18. Buttigieg J, Pan J, Yeger H, Cutz E. NOX2 (gp91phox) is a predominant O2 sensor in lung. *Cell Tiss Res* (1973) 145:521–40.

19. Yager H, Cutz E. Innervation of pulmonary neuroendocrine cells and neuroepithelial bodies in developing rabbit lung. *J Histochim Cytochem* (2004) 52:379–89. doi:10.1038/365153a0

20. Domnik NJ, Cutz E. Pulmonary neuroepithelial bodies as airway sensors: putative role in the generation of dyspnea. *Curr Opin Pharmacol* (2011) 11:211–7. doi:10.1016/j.coph.2011.04.003

21. Youngson C, Nurse C, Yeger H, Cutz E. Oxygen sensing in airway chemoreceptors and small pulmonary neuroepithelial bodies. Intrapulmonary secretory neuroepithelia, modulated by the CNS. *Cell Tiss Res* (1973) 167:377–85.

22. Linnoila RI, Nettesheim P, DiAugustine RP. Lung endocrine-like cells function as an oxygen sensor complex in airway chemoreceptors and small pulmonary neuroepithelial bodies in young rabbits. *Experientia* (1983) 39:1123–4. doi:10.1007/BF01943141

23. Lauweryns JM, Cokelaere M, Detlefsenster M, Liebens M. Intrapulmonary neuroepithelial bodies after vagotomy: demonstration of their sensory neuroreceptor-like innervation. *Experientia* (1983) 39:1123–4. doi:10.1007/BF01943141

24. Domnik NJ, Cutz E. Pulmonary neuroepithelial bodies as airway sensors: putative role in the generation of dyspnea. *Curr Opin Pharmacol* (2011) 11:211–7. doi:10.1016/j.coph.2011.04.003

25. Yager H, Cutz E. Hypoxia-induced changes in pulmonary and systemic vascular resistance: where is the O2 sensor? *Respir Physiol Neurobiol* (2013) 184:373–86. doi:10.1016/j.resp.2012.05.022

26. Brusa D, De Paoli T, Pinetel J, Timmermans JP, Ardaens D. Sensory receptors in the airways: neurochemical coding of smooth muscle-associated airway receptors and pulmonary neuroepithelial body innervation. *Auton Neurosci* (2006) 127:307–19. doi:10.1016/j.autneu.2006.02.006

27. Domnik NJ, Cutz E. Pulmonary neuroepithelial bodies as airway receptors: putative role in the generation of dyspnea. *Cure Pharm Oncol* (2011) 3:5170–4. doi:10.1073/pnas.0900668106

28. Wang D, Youngson C, Wong V, Yeger H, Dinauer MCV, Saenz Miera E, et al. NADPH-oxidase and a hydrogen peroxide-sensitive K+ channel may function as an oxygen sensor complex in airway chemoreceptors and small lung carcinoma cell lines. *Proc Natl Acad Sci U S A* (1996) 93:13182–7. doi:10.1073/pnas.93.23.13182

29. Fuk WA, Wang D, Nurse CA, Dinauer MCV, Cutz E. NADPH oxidase is an O2 sensor in airway chemoreceptors: evidence from K+ current modulation in wild-type and oxidase-deficient mice. *Proc Natl Acad Sci U S A* (2000) 97:4374–9. doi:10.1073/pnas.97.8.4374

30. Buttigieg J, Pan J, Yager H, Cutz E. NOX2 (gp91phox) is a predominant O2 sensor in a human airway chemoreceptor cell line: biochemical, molecular, and electrophysiological evidence. *Am J Physiol Lung Cell Mol Physiol* (2012) 303:L598–607. doi:10.1152/ajplung.00170.2012

31. Aparicio P, Nurse C, Cutz E. Characterization of slowly inactivating KV(alp) current in rabbit pulmonary neuroepithelial bodies: effects of hypoxia and nicotine. *Am J Physiol Lung Cell Mol Physiol* (2007) 293:L892–902. doi:10.1152/ajplung.00098.2007

32. Cutz E, Pan J, Yager H. The role of NOX2 and “novel oxidases” in airway chemoreceptor O2 sensing. *Adv Exp Med Biol* (2009) 648:427–38. doi:10.1007/978-90-481-2259-2_49
41. Cutz E. Neuroendocrine cells of the lung: an overview of morphologic characteristics and development. Exp Lung Res (1982) 3:185–208. doi:10.3109/01902148208969563

42. Sunday ME, Cutz E. Role of neuroendocrine cells in fetal and postnatal lung. In: Mendelsson CR editor. Endocriologia del Lung. Totowa, NJ: Humana Press (2000). p. 299–336.

43. Arikan GM, Scholz HS, Petru E, Hauesler MC, Haas J, Weiss PA. Cord blood oxygen saturation in vigorous infants at birth: what is normal? BJOG (2000) 107:987–94. doi:10.1111/j.1471-0528.2000.tb10401.x

44. Spindel ER, Sunday ME, Hoffler H, Wolfe HJ, Habener JF, Chin WW. Transient elevation of mRNAs encoding gastric-releasing peptide (GRP), a putative pulmonary growth factor, in human fetal lung. J Clin Invest (1987) 80:1172–9. doi:10.1172/JCI113176

45. Spindel ER, Chin WW, Price J, Rees LH, Besser GM, Habener JE. Cloning and characterization of cDNAs encoding human gastric-releasing peptide. Proc Natl Acad Sci U S A (1984) 81:5699–703. doi:10.1073/pnas.81.18.5699

46. Sunday ME, Hua J, Deputy HB, Nusrat A, Torday JS. Bombesin increases fetal lung growth and maturation in utero and in organ culture. Am J Respir Cell Mol Biol (1990) 3:199–205. doi:10.1165/ajrcmb.3.3.199

47. Sunday ME, Hua J, Torday JS, Reyes B, Shipp MA. CD10/neutral endopeptidase 24.11 in developing human fetal lung. Patterns of expression and modulation of peptide-mediated proliferation. J Clin Invest (1992) 90:2517–25. doi:10.1172/JCI116145

48. King KA, Hua J, Torday JS, Drazen JM, Graham SA, Shipp MA, et al. CD10/neutral endopeptidase 24.11 regulates fetal lung growth and maturation in utero by potentiating endogenous bombesin-like peptides. J Clin Invest (1993) 91:1369–73. doi:10.1172/JCI116417

49. Sunday ME, Hua J, Reyes B, Masui H, Torday JS. Anti-bombesin antibodies modulate fetal mouse lung growth and maturation in utero and in organ cultures. Anat Rec (1993) 236:25–32. doi:10.1002/ar.1092360107

50. Emanuel RL, Torday JS, Mu Q, Asokananthan N, Sikorski KA, Sunday ME. Bombesin-like peptides modulate alveolarization and angiogenesis in bronchopulmonary dysplasia. Am J Respir Crit Care Med (2002) 165:1093–7. doi:10.1164/ajrccm.165.8.2108044

51. Cullen A, Van Marter LJ, Moore M, Parad R, Sunday ME. Urine bombesin-like peptide elevation precedes clinical evidence of bronchopulmonary dysplasia. Am J Respir Crit Care Med (2002) 165:1093–7. doi:10.1164/ajrccm.165.8.2108044

52. Sunday ME, Torday JS, Kenya M, Sherrit KM, Silvers W, Nett LM, et al. Level of bombesin-like peptides in asymptomatic cigarette smokers: a potential risk marker for smoking-related diseases. Cancer Res (1992) 52:2727s–31s.

53. Coalson JJ, Winter VT, Silers-Khodr T, Yoder BA, Chang LX, et al. Bombesin-like peptides modulate alveolarization and angiogenesis in bronchopulmonary dysplasia. Am J Respir Crit Care Med (2007) 176:902–12. doi:10.1164/rccm.200611-1434OC

54. Coalson JJ, Kuehl TJ, Escobedo MB, Hilliard JL, Smith F, Meredith K, et al. A baboon model of bronchopulmonary dysplasia II. Pathologic features. Exp Mol Pathol (1982) 37:335–50. doi:10.1016/0014-4800(82)90046-6

55. Escobedo MB, Hilliard JL, Smith F, Meredith K, Walsh W, Johnson D, et al. A baboon model of bronchopulmonary dysplasia: I. Clinical features. Exp Mol Pathol (1982) 37:323–34. doi:10.1016/0014-4800(82)90045-4

56. Coalson JJ, Torday JS, Winter VT, Silers-Khodr T, Yoder BA. Neonatal chronic lung disease in extremely immature baboons. Am J Respir Crit Care Med (1999) 160:1333–46. doi:10.1164/ajrccm.160.4.9810071

57. Sunday ME, Yoder BA, Cuttitta F, Haley KJ, Emanuel RL. Bombesin-like peptide mediates lung injury in a baboon model of bronchopulmonary dysplasia. J Clin Invest (1998) 102:584–94. doi:10.1172/JCI23329

58. Joad JF, IC, Kott KS, Bric JM, Pinkerton KE. In utero and postnatal effects of sidestream cigarette smoke exposure on lung function, hyperresponsiveness, and neuroendocrine cells in rats. Toxicol Appl Pharmacol (1995) 132:63–71. doi:10.1006/taap.1995.1087

59. Shenberger JS, Shew RL, Johnson DE. Hyperoxia-induced airway remodeling and pulmonary neuroendocrine cell hyperplasia in the weaning rat. Pediatr Res (1997) 42:539–44. doi:10.1203/00006450-199710000-00020

60. Scher H, Miller YL, Aguayo SM, Johnson KJ, Miller JE, McCray PB Jr. Urinary bombesin-like peptide levels in infants and children with bronchopulmonary dysplasia and cystic fibrosis. Pediatr Pulmonol (1998) 26:326–31. doi:10.1002/(SICI)1099-0496(199811)26:5<326::AID-PPUL4>3.0.CO;2-J

61. Aguayo SM, Gomberg SM, Hoyt BA, Chang LX, et al. Determinants of susceptibility to cigarette smoke. Am J Respir Crit Care Med (1994) 149:1692–9. doi:10.1164/ajrccm.149.8.8917110

62. Meloni F, Ballabio P, Pistorio A, Todarello C, Montoli C, Berrayash L, et al. Urinary levels of bombesin-related peptides in a population sample from northern Italy: potential role in the pathogenesis of chronic obstructive pulmonary disease. Am J Med Sci (1998) 315:258–65. doi:10.1097/00000441-199804000-00008

63. Subramaniam M, Sugiyama K, Coy DH, Kong Y, Miller YE, Weller PF, et al. Bombesin-like peptides and mast cell responses: relevance to bronchopulmonary dysplasia? Am J Respir Crit Care Med (2003) 168:601–611. doi:10.1164/rccm.200212-1434OC

64. Ahola T, Fellman V, Kjellmer I, Raivio KO, Lapatto R. Plasma 8-isoprostane levels are increased in preterm infants who develop bronchopulmonary dysplasia. Pediatr Res (2006) 59:88–93. doi:10.1203/01.pdr.0000020134.07851.4a

65. Ahmed B. Regulatory peptides in the thyroid gland—a review on their localization and function. Acta Endocrinol (1991) 124:225–32.

66. Nasi N, Ponziani V, Becatti M, Galvan P, Donzelli G. Anti-oxidant enzymes and pulmonary neuroendocrine cell hyperplasia in the weanling rat. Pediatr Res (1985) 19:185–208. doi:10.1203/PDR.0000130478.05324.9D

67. Ahren B. Regulatory peptides in the thyroid gland—a review on their localization and function. Acta Endocrinol (1991) 124:225–32.
83. Tsukahara H, Toyoi-Oka M, Kanaya Y, Ogura K, Kawatani M, Hata A, et al. Quantitation of glutathione S transferase-pi in the urine of preterm neonates. Pediatr Int (2005) 47:528–31. doi:10.1111/j.1442-2003.2005.01213.x

84. Ogihara T, Kim HS, Hirano K, Imanishi M, Ogihara H, Tami H, et al. Oxidation products of uric acid and ascorbic acid in preterm infants with chronic lung disease. Biol Neonate (1998) 73:24–33. doi:10.1055/s-000013956

85. Goil S, Truog WE, Barnes C, Norberg M, Rezaiekhaligh M, Thibeault D. Effects of oxygen, GRP, and lung disease. Biol Neonate (1997) 71:75–81. doi:10.1111/j.1349-7207.1996.00988.x

86. Lai CH, Liu SH, Lin HC, Shih TS, Tsai PJ, Chen JS, et al. Exposure to traffic-related air pollutants and circulating biomarkers of oxidative stress in young children. Environ Health Perspect (2005) 113:148–56. doi:10.1289/ehp.534

87. Tamae K, Kawai K, Yamasaki S, Kawanami K, Hide M, Takahashi K, et al. Effects of age, smoking and other lifestyle factors on urinary 7-methylguanine and 8-hydroxydeoxyguanosine. Cancer Sci (2009) 100:715–21. doi:10.1111/j.1349-7006.2009.01088.x

88. Lach E, Haddad EB, Gies JP. Contractile effect of bombesin on guinea pig lung in vitro: involvement of gastrin-releasing peptide-prefering receptors. Am J Physiol (1993) 264:L823–38.

89. Buckingham S, Avery ME. Time of appearance of lung surfactant in the foetal lamb. Respir Physiol (1997) 98:49–56.

90. Schuger L, Varani J, Mitra R Jr, Gilbride K. Retinoic acid stimulates mouse lung development by a mechanism involving epithelial-mesenchymal interaction and regulation of epidermal growth factor receptors. Dev Biol (1999) 214:62–73. doi:10.1006/dbio.1998.2756

91. Kim C, Nielsen HC. Hox-5 in mouse developing lung: cell-specific expression and retinoic acid regulation. Am J Physiol Lung Cell Mol Physiol (2000) 279:L663–71.

92. Costa RH, Kalinichenko VV, Lim L. Transcription factors in mouse lung and development. Am J Physiol Lung Cell Mol Physiol (2001) 280: L823–38. doi:10.1152/ajplung.00052.2006

93. Auten RL, O’Reilly MA, Ourdy TD, Nozik-Grayck E, Whorton MH. Transgenic extracellular superoxide dismutase protects postnatal alveolar epithelial proliferation and development during hyperoxia. Am J Physiol Lung Cell Mol Physiol (2006) 290:L32–40. doi:10.1152/ajplung.00333.2005

94. Gonzalez N, Moody TW, Igarashi H, Ito T, Jensen RT. Bombesin-related peptides and their receptors: recent advances in their role in physiology and disease states. Curr Top Dev Biol (2007) 84:1–36.

95. Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmouliere A, Varga J, et al. Receptor-activated Smad localisation in bleomycin-induced pulmonary fibrosis. J Clin Pathol (2007) 60:283–9. doi:10.1136/jcp.2006.037606

96. Wada E, Watase K, Yamada K, Ogura H, Kikwaa K, Asano S, Kinoshita M. Receptor-activated Smad localisation in bleomycin-induced pulmonary fibrosis. J Clin Pathol (2007) 60:283–9. doi:10.1136/jcp.2006.037606

97. Ashcroft T, Simpson JM, Timbrell V. Simple method of estimating severity of pulmonary fibrosis on a numerical scale. J Clin Pathol (1998) 41:467–70. doi:10.1136/jcp.14.4.467

98. Higashiyama Y, Yoshimoto D, Okamoto Y, Kikwaa K, Asano S, Kinoshita M. Receptor-activated Smad localisation in bleomycin-induced pulmonary fibrosis. J Clin Pathol (2007) 60:283–9. doi:10.1136/jcp.2006.037606

99. Buckingham S, Avery ME. Time of appearance of lung surfactant in the foetal lamb. Respir Physiol (1997) 98:49–56.

100. Manoli SE, Smith LA, Vyhlidal CA, An CH, Porrata Y, Cardoso WV, et al. Maternal smoking and maternal stress during pregnancy, smoking and other lifestyle factors on urinary 7-methylguanine and 8-hydroxydeoxyguanosine. Cancer Sci (2009) 100:715–21. doi:10.1111/j.1349-7006.2009.01088.x
125. Cutz E, Perrin DG, Pan J, Haas EA, Krous HF. Pulmonary neuroendocrine cells and neuroepithelial bodies in sudden infant death syndrome: potential markers of airway chemoreceptor dysfunction. *Pediatr Dev Pathol* (2007) 10:106–16. doi:10.2350/06-06-0113.1

126. Schiffman PL, Westlake RE, Santiago TV, Edelman NH. Ventilatory control in parents of victims of sudden-infant-death syndrome. *N Engl J Med* (1980) 302:486–91. doi:10.1056/NEJM198002283020903

127. Salvatore D, Buzzetti R, Baldo E, Forneris MP, Lucidi V, Manunza D, et al. An overview of international literature from cystic fibrosis registries. Part 3. Disease incidence, genotype/phenotype correlation, microbiology, pregnancy, clinical complications, lung transplantation, and miscellaneous. *J Cyst Fibros* (2011) 10:71–85. doi:10.1016/j.jcf.2010.12.005

128. Yeger H, Pan J, Fu XW, Bear C, Cutz E. Expression of CFTR and Cl(–) conductances in cells of pulmonary neuroepithelial bodies. *Am J Physiol Lung Cell Mol Physiol* (2001) 281:L713–21.

129. Grasemann H, Ratjen F. Early lung disease in cystic fibrosis. *Lancet Respir Med* (2013) 1:148–57. doi:10.1016/S2213-2600(13)70026-2

130. Pan J, Luk C, Kent G, Cutz E, Yeger H. Pulmonary neuroendocrine cells, airway innervation, and smooth muscle are altered in Cftr null mice. *Am J Respir Cell Mol Biol* (2006) 35:320–6. doi:10.1165/rcmb.2005-0468OC

131. Haley KJ, Patidar K, Zhang F, Emanuel RL, Sunday ME. Tumor necrosis factor induces neuroendocrine differentiation in small cell lung cancer cell lines. *Am J Physiol* (1998) 275:L311–21.

**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 21 April 2014; accepted: 25 June 2014; published online: 18 July 2014.

Citation: Sunday ME (2014) Oxygen, gastrin-releasing peptide, and pediatric lung disease: life in the balance. *Front. Pediatr*. 2:72. doi: 10.3389/fped.2014.00072

This article was submitted to Neonatology, a section of the journal *Frontiers in Pediatrics*.

Copyright © 2014 Sunday. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.