ical pattern of reduced TM protein expression in CM. Persistence of staining for von Willebrand factor, which is stored in intracellular Weibel-Palade bodies, provided evidence that decreased TM staining was not due to endocardial loss. To assess the physiologic consequence of reduced TM expression, we measured in situ formation of APC using a chromogenic assay. Segments of control LV (n = 4) generated 2,100 ± 138 fmol APC/min/cm^2. In contrast, LV harvested after 8 and 15 weeks of pacing generated only 329 ± 35 and 307 ± 78 fmol APC/min/cm^2, respectively (p < .0001 vs control LV). **Conclusion:** Endocardial TM protein expression is reduced by 90% at 8 weeks and 92% at 15 weeks following rapid pacing. This decrease in TM results in an 85% reduced capacity of the EE to generate activated protein C compared to normal LV. Reduced TM expression in CM markedly impairs APC formation, thereby contributing to a significant loss of endocardial thromboresistance.

27

PROTECTIVE EFFECT OF PURINERGIC AGONIST ATPγS AGAINST ACUTE LUNG INJURY. L.A. Kolosova, T. Mirzapoiazova, L. Moreno, S. Sammani, J.G. Garcia, A.D. Verin, University of Chicago, Chicago, IL.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are major causes of acute respiratory failure that are associated with high morbidity and mortality. These disorders are characterized by a significant pulmonary inflammatory response resulting in injury to alveolar epithelial and endothelial barriers of the lung and protein-rich pulmonary edema. The pharmacological treatment of ALI/ARDS is nonspecific and relies on good supportive care and control of initiating cause. ALI/ARDS pathogenesis is still only partly understood; however, pulmonary endothelium plays a major role by changing its barrier permeability, thus promoting pulmonary edema formation. Pulmonary endothelial functional and structural alterations are key components of increased vascular leak. Consequently, endothelium-related therapies may have beneficial effects in ALI/ARDS. Recently, much attention has been given to the therapeutic potential of purinergic agonists and antagonists for the treatment of cardiovascular and pulmonary diseases. Extracellular purines (adenosine, ADP, and ATP) and pyrimidines (UDP and UTP) are important signaling molecules that mediate diverse biological effects via cell-surface P2Y receptors. We have shown that ATP promotes endothelial cell (EC) barrier enhancement via a complex cell signaling. We hypothesize that activation of endothelial purinoreceptors would exert anti-inflammatory barrier-protective effect. To test this hypothesis we used two model systems: cultured pulmonary EC and murine model of ALI induced by intratracheal administration of endotoxin/lipopolysaccharide (LPS). In cell culture model, ATP inhibited intracellular gap formation and junctional permeability induced by inflammatory mediator thrombin. In murine model of ALI, nonhydrolyzed ATP analogue ATPγS (50 μM final blood concentration) administered intravenously attenuated inflammatory response by decreasing accumulation of cells (48%, p < .01) and proteins (57%, p < .01) in bronchioalveolar lavage (BAL) and by reducing neutrophil infiltration into alveoli. ATPγS slightly reduced LPS-induced release of inflammatory cytokines (tumor necrosis factor alpha and interleukin-6) into BAL. These findings suggest that purinergic receptor stimulation exerts a protective role against ALI, probably via decreasing endothelial junctional permeability.

28

THE REGULATION OF NEURONAL OUTGROWTH AND RETRACTION BY SEROTONIN RECEPTORS 5-HT4a AND 5-HT7: THE ROLE OF LIM KINASE 1. A. Krbanjevic, G. Liu, J. Profirovic, T. Voyno-Yasenetskaya, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL.

Serotonin is a neurotransmitter with a prominent role in the central nervous system. The higher mammals express seven families of serotonin receptors. Functions of only some of the receptors have been determined. Most of the 5-HT functions are mediated through G protein–coupled receptors. We have shown that 5-HT$_{4a}$ serotonin receptor couples $G_{\alpha S}$ and $G_{\alpha 13}$ protein, causing RhoA-dependent neurite retraction and cell rounding (Ponimaskin et al, 2002). We also found that 5-HT$_7$ serotonin receptor couples to $G_{\alpha s}$ and $G_{\alpha 12}$ protein. 5-HT$_7$ receptor-$G_{\alpha 12}$ protein pathway induces activation of two small GTPases, RhoA and Cdc42, leading to pronounced cell rounding and filopodia formation, respectively (Kwachnina et al, 2005). LIM kinase 1 (LIMK1) is a serine-threonine kinase that negatively regulates function of actin depolymerizing protein cofilin. Therefore, activation of LIMK1 leads to actin polymerization. The small GTPases of Rho family are shown to stimulate LIMK1. In the present study, we are showing for the first time that 5-HT$_{4a}$ and 5-HT$_7$ receptor phosphorylate LIMK1. Therefore, this could be mechanism for regulation of cytoskeleton of cells transfected with 5-HT$_{4a}$ and 5-HT$_7$ receptors. When we down-regulated endogenous LIMK1 using siRNA technology we almost totally lost rounded cells. Also, when we cotransfected 5-HT$_{4a}$R and 5-HT$_7$ receptor with LIMK1-siRNA, we observed that the 5-HT$_7$ receptor led to an increase in the number of rounded cells and a decrease in the number of process-bearing cells. We are suggesting here that LIMK1 influences activity of small GTPases through the feedback loop between LIMK1 and small GTPases. This mechanism is important for precise control of LIMK1 activity in cells transfected with 5-HT$_{4a}$ and 5-HT$_7$ receptors. Our data give a new insight into serotonin, not only as a well-known neurotransmitter but also as a modulator of neuronal plasticity.

29

REGULATION OF APOPTOSIS SIGNAL-REGULATING KINASE 1 BY α SUBUNITS OF HETEROTRIMERIC G PROTEINS G12 AND G13. M.A. Kutuzov, A.V. Andreeva, T.A. Voyno-Yasenetskaya, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL.

Heterotrimeric G protein α subunits Gα12 and Gα13 are able to stimulate c-Jun N-terminal kinase (JNK) and induce apoptosis via more than one MAP kinase pathways. Gα12 and Gα13 have been reported to stimulate apoptosis signal-regulating kinase (ASK1), one of MAPKK kinases upstream of JNK; the mechanism of this effect has not been studied in detail. Here we report that Gα12 and Gα13 associate with ASK1, as demonstrated by immunoprecipitation assays. Gα12 and Gα13 binding to ASK1 occurs independently of their activation state, as evidenced by ASK1 binding to wild-type Gα subunits both in the presence and in the absence of AlF_4^-, as well as its similar binding to mutationally activated as compared to wild-type Gα12/Gα13. Using deletion mutants of ASK1, we show that both N- and C-terminal regulatory domains of ASK1 may be involved in the interaction with Gα12/Gα13, while its kinase domain is not required. Coexpression of ASK1 with wild-type Gα13 in COS-7 cells leads to strongly decreased levels of ASK1 protein. In contrast, coexpression of ASK1 with mutationally activated Gα13 increases ASK1 levels as compared to control. Analysis of ASK1 polyubiquitinylation shows that polyubiquitinylated high molecular mass forms of ASK1 are more pronounced in the presence of wild-type Gα13 but are alleviated in the presence of activated Gα13. These results indicate that, in addition to the stimulation of ASK1 kinase activity by Gα12/Gα13 reported previously, ASK1 expression levels can be regulated by these G proteins via control of polyubiquitinylation and following degradation of this kinase.

30

NOVEL REGULATION OF LIPID SYNTHESIS: THE SPOT 14 PROTEIN CONTROLS FATTY ACID SYNTHASE ACTIVITY IN VIVO AND IN VITRO. L.T. LaFave, L.B. Augustin, S. Chohnan, C.N. Mariash, University of Minnesota, Minneapolis, MN, and Ibaraki University, Ibaraki, Japan.

Spot 14 (S14) is a 17 kD protein located in tissues that synthesize fatty acids. In the lactating mammary glands of S14 null mice there is significantly reduced triglyceride content due to a 70% reduction in lipogenesis with a 90% reduction in medium-chain fatty acids. However, the site and role of the S14 protein in the regulation of lipid synthesis remains unknown. We observed no difference in gene expression or in vitro activity of all tested lipogenic enzymes in wild-type vs null mice. A sensitive recycling assay measuring mammary gland malonyl-CoA revealed that malonyl-CoA is significantly increased in S14 null mice vs wild type (7.19 ± 0.68 vs 5.44 ± 0.43 nmol/g, p < .05). Therefore, the block in lipogenesis occurs at the terminal step, fatty acid synthase (FAS). Based on this evidence, we hypothesized that S14 is integral to the function of FAS, which catalyzes the production of fatty acids from malonyl-CoA. We first tested whether S14 acts by binding fatty acids during their production or transport. Recombinant Spot 14 produced from baculovirus-infected insect cells was used to investigate whether S14 acts as a fatty acid binding protein (FABP). We utilized a well-established assay that binds a hydrophobic fluorescent probe, 1-anilinonaphthalene 8-sulfonic acid (ANS), to putative FABPs. We found that the S14 protein bound to ANS with positive cooperativity (Hill coefficient = 2.9), suggesting that S14 is a multimer, and the formation of the multimer creates the hydrophobic pocket for ANS binding. However, the fluorescent probe was not displaced by fatty acids or their CoA derivatives of varying lengths. Therefore, to investigate further which specific lipogenic proteins associate with S14 in vitro, we created an affinity agarose column to which we bound the purified S14. Elution at 3M NaCl followed by SDS-PAGE showed only a single protein of molecular weight greater than 200 kD. This high-affinity interaction is consistent with S14 binding to FAS. Our data suggest that S14 possesses a hydrophobic binding pocket and interacts with FAS in vivo and in vitro to regulate the rate of fatty acid synthesis in mammary tissue.

31

EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON BRONCHOALVEOLAR LAVAGE IMMUNOGLOBULIN G SUBCLASS LEVELS IN HIV-POSITIVE PATIENTS. S. Lodhi, R.B. Day, P.A. Smith, C. Huffer, H.L. Twigg III, Indiana University Medical Center, Indianapolis, IN.

Background and Objective: HIV infection in the lung is characterized by lumphocytic alveolitis and a state of chronic alveolar macrophage and lung lymphocyte activation resulting in enhanced cytokine secretion and hypergammaglobulinemia. It has been shown that HAART is associated with a generalized decline in cytokine secretion, especially interferon-gamma (IFN-γ), and reduced IgG and IgM production. We examined the effect of HAART on IgG subclasses, including the IFN-γ-dependent subclass IgG2. **Methods:** IgG subclass levels were measured in BAL of 20 HIV-infected patients before and at 24 weeks after initiation of HAART using a human IgG subclass ELISA kit. Cytokine concentrations were measured using a cytokine bead array assay. IgG and cytokine concentrations were corrected for BAL dilution by determining epithelial lining fluid (ELF) using the urea dilution technique. **Results:** Data expressed as mean ± SEM μg IgG/mL ELF (*p value ≤ .05).

	IgG1	*IgG2*	*IgG3*	*IgG4*	*IgG1:IgG2*
Entry	3.71 ± 0.44	7.55 ± 1.58	0.72 ± 0.17	0.86 ± 0.22	0.90 ± 0.12
Week 24	3.30 ± 0.34	5.32 ± 0.90*	0.29 ± 0.05*	0.76 ± 0.19	0.82 ± 0.17

HAART was associated with a significant decline in IgG2 and IgG3 levels. Unlike normal subjects, BAL IgG2 levels were higher than IgG1 levels, a difference persisting even after 24 weeks of HAART. A significant correlation was found between IFN gamma levels and IgG1 (R = .47, p = .03), IgG2 (R = .53, p = .02) and IgG3 (R = .53, p = .02) before initiation of HAART. This did not persist at week 24. IL-6 levels and IgG1 levels were found to be positively correlated at the time of entry (R = .46, p = .04). None of the other subclasses demonstrated any significant correlation with IL-6 levels. **Conclusion:** HIV infection is associated with increased IgG2 in the lungs of HIV infected patients, possibly due to elevated IFN-γ concentrations in the lung. While this is partially correcting after 6 months of HAART, IgG2 levels still remain elevated in the lung, probably due to the long half-life of IgG.

32

EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON BRONCHOALVEOLAR LAVAGE PNEUMOCOCCAL-SPECIFIC IMMUNOGLOBULIN A LEVELS IN HIV-POSITIVE PATIENTS. S. Lodhi, Y. Wang, R.B. Day, P.A. Smith, T. Lahm, C. Huffer, H.L. Twigg III, Indiana University Medical Center, Indianapolis, IN.

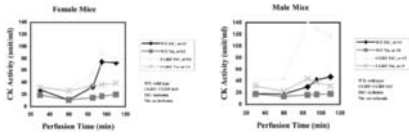
Background and Objective: The incidence of invasive pneumococcal disease among HIV-infected persons has declined in the post HAART era. Changes in the humoral immune responses have been implicated in this change. It has already been shown that HAART is associated with a decline in BAL total IgG and IgM concentrations and a decrease in BAL pneumococcal-specific IgG concentrations. We measured total and pneumococcal-specific IgA concentrations in BAL of 24 HIV-infected patients before and at 4 and 24 weeks after initiation of HAART, anticipating a similar response. **Methods:** BAL pneumococcal-specific IgA levels were measured using ELISA. The IgA concentrations were corrected for dilution by determining epithelial lining fluid (ELF) using the urea dilution technique. **Results:** Data expressed as mean ± SEM μg IgA/mL ELF (*p value ≤ .05).

	Baseline	*Week 4*	*Week 24*
Total IgA	495.13 ± 88.47	514.16 ± 145.07	389.59 ± 67.27
Pneumococcal-specific IgA	3.57 ± 0.82	2.96 ± 0.57	2.68 ± 0.66
Pneumococcal-specific IgA%	1.35% ± 0.004	1.16% ± 0.003	1.85% ± 0.009

Total and pneumococcal-specific IgA levels did not change after initiation of HAART. No change was detected in the levels of pneumococcal-specific IgA when expressed as a percentage of total IgA. No significant correlation was found between BAL IL-6 and IgA concentration or BAL percentage lymphocytes and IgA concentrations. **Conclusion:** HAART is not associated with significant changes in total or pneumococcal-specific IgA concentrations in the distal respiratory tract. We speculate that this reflects the more dominant role of IgG in protection of the alveolar space against bacterial pathogens.

33

BIOINFORMATIC CLASS DISCOVERY OF POTENTIAL BIOMARKERS IN VENTILATOR-ASSOCIATED LUNG INJURY. S.-F. Ma,[1] D.N. Grigoryev,[2] R.B. Easley,[3] S.Q. Ye,[1] B.A. Simon,[3] J.G N. Garcia,[1] [1]Critical Care, The University of Chicago, Chicago, IL; [2]Clinical Immunology and [3]Anesthesiology and Critical Care, Johns Hopkins University, Baltimore, MD.
Rationale: The unilateral (one lung injured and the contralateral lung intact) model of ventilator-associated lung injury (VALI) allows us to utilize uninjured lung as the systemic VALI effects indicator. We speculated that regulation of genes in uninjured lung will be attributed to blood or lymph carrying effectors produced by an injured lung and the analysis of upstream bioprocesses governed by affected genes will lead to the effectors' discovery. **Methods:** Dogs (n = 4) were intubated, left lung lavaged, and then both lungs were either independently ventilated (total Vt = 15 mL/kg) for 6 hours (injured and uninjured) or immediately harvested (control). Lung mRNA (n = 16 for all three groups) was hybridized to HG_U133A and analyzed by SAM using interspecies probe adjustment. Genes with the lowest false discovery rate (q = 0.124%) that imposed fold change (FC) range from −3.52 to −1.26 and 1.22 to 6.96 with corresponding FC averages −1.59 and 2.01 were considered affected by VALI. Gene ontology filtering for receptor activity term was conducted by MAPPFinder. **Results:** Our analyses revealed 22 receptor-related genes, of which 7 were growth factor receptors including EGFR (FC = −1.54), FGFR1 (FC = −1.59), FGFR2 (FC = −1.56), and PDGFR (FC = −1.30). The overall down-regulation of these receptors was concordant with decreased expression of their corresponding ligands in injured lung including EGF (FC = −1.39), FGF2 (FC = −1.39), PDGFA (FC = −1.41), and PDGFB (FC = −2.00). Fibroblast (Z = 6.17) and epidermal (Z = 4.49) growth factor receptor activity were the first and the third significantly (Z > 1.96) affected pathways. **Conclusions:** Our approaches effectively identify a class of potential biomarkers in VALI. Further investigation of this study may elucidate systemic VALI effects and facilitate the development of new diagnostic tools.



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34

MYOCARDIAL ISCHEMIA CAUSES HIGHER CREATINE KINASE RELEASE IN CALCITONIN GENE–RELATED PEPTIDE KNOCKOUT MALE MOUSE HEARTS. H. Ma, R Huang, A. Carve, I. Shah, S.C. Supowit,* D.J. DiPette,* G.S. Abela, Department of Medicine, Michigan State University, East Lansing, MI; *Texas A & M University, Scott and White Hospital, Temple, TX.
Background: Calcitonin gene–related peptide (CGRP) influences vasoregulatory activities. We determined the gender-specific effects of CGRP knockout (KO) on creatine kinase (CK) activity following ischemia. **Methods:** Ninety-six mice were studied in a Langendorff preparation using 50 mm Hg perfusion pressure. Myocardial ischemia was induced by stopping flow to the coronary arteries for 20 minutes. The perfusion buffer was collected from a small chamber that housed the heart. Perfusion buffer was collected over 2 hours and CK activity was measured. **Results:** CK activity was significantly greater in CGRP-KO mouse hearts compared to wild-type (see graphs). Male CGRP-KO hearts released 1.5 times more CK than female CGRP-KO hearts 15 minutes after ischemia (130.4 vs 90.2 unit/mL, $p < .003$). **Conclusions:** CGRP contributes significantly to CK release during ischemia. This effect is more prominent in the male compared to the female mouse heart.

35

OXIDIZED PHOSPHOLIPIDS MEDIATE INTERACTION BETWEEN ADHERENCE JUNCTION AND FOCAL ADHESION PROTEINS. I. Malyukova,[1] A. A. Birukova,[1] A. Rios,[2] K.G. Birukov,[1] [1] Department of Medicine, The University of Chicago, Chicago, IL; [2] Department of Medicine, Johns Hopkins University, Baltimore, MD.
Introduction: Oxidized phospholipids appear in the pulmonary circulation as a result of acute lung injury or inflammation. We have previously shown that oxidized phospholipids exhibit barrier-protective effects on pulmonary endothelial cell (EC) monolayers. However, effects of oxidized phospholipids on EC focal adhesion (FA) and adherence junction (AJ) remodeling have not been yet fully explored. **Goal:** To study molecular mechanisms of adherens junction and focal adhesion remodeling mediated by oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (OxPAPC). **Methods:** All experiments were performed on human pulmonary artery endothelial cells (HPAEC). Intracellular protein localization was analyzed by immunocytochemistry. Subcellular localization of the proteins of interest was determined using subcellular proteome extraction kit. Protein phosphorylation profile was assessed by Western blot analysis. Protein-protein interactions were analyzed by coimmunoprecipitation assays. **Results:** Enhancement of EC barrier in response to OxPAPC was accompanied by dynamic remodeling of focal adhesions and adherence junctions. Immunofluorescent analysis of OxPAPC-stimulated EC revealed dramatic translocation and peripheral and enhanced peripheral staining for AJ proteins beta-catenin and VE-cadherin. In addition, OxPAPC treatment increased tyrosine phosphorylation of FA proteins FAK at Tyr-576 and paxillin at Tyr-118, which was associated with peripheral redistribution of FA and AJ complexes. Furthermore, subcellular fractionation analysis showed increase of VE-cadherin, beta-catenin, and GIT2 in membrane fraction after OxPAPC challenge. Remarkably, coimmunoprecipitation studies indicated increased interaction of paxillin with FA components FAK, vinculin and GIT2, and AJ protein beta-catenin upon HPAEC stimulation with OxPAPC. Complementary experiments with immunoprecipitation beta-catenin followed by probing for paxillin confirmed these results. **Conclusions:** The results of these studies characterize OxPAPC-induced focal adhesion remodeling and determine for the first time the specific interactions between focal adhesion and adherens junction protein complexes in endothelial barrier-protective responses to OxPAPC

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36

***BACILLUS ANTHRACIS* SPORES STIMULATE CYTOKINE AND CHEMOKINE INNATE IMMUNE RESPONSES IN HUMAN ALVEOLAR MACROPHAGES THROUGH MULTIPLE MAPK PATHWAYS.** K. Chakrabarty, W. Wu, J.L. Booth, E.S. Duggan, K.M. Coggeshall, J.P Metcalf, Pulmonary and Critical Care Division, Department of Medicine, University of Oklahoma Health Sciences Center, and the Program in Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK.
Contact with the human alveolar macrophage plays a key role in the innate immune response to *Bacillus anthracis* spores. Because there is a significant delay between the initial contact of the spore with the host and clinical evidence of disease, there appears to be temporary containment of the pathogen by the innate immune system. Therefore, the early macrophage response to anthrax exposure is important in understanding the pathogenesis of this disease. We examined the initial events after exposure of human alveolar macrophages obtained by bronchoscopy to *Bacillus anthracis (Sterne)* spores. Spores were rapidly internalized as determined by confocal microscopy. Spore exposure also rapidly activated the MAPK signaling pathways ERK, JNK, and P38. This was followed by transcriptional activation of cytokine and primarily monocyte chemokine genes as determined by RNase protection assays. Transcriptional induction was reflected at the translational level as IL-1α and β, IL-6, and TNF-α cytokine protein levels were markedly elevated as determined by ELISA. Induction of IL-6 and TNF-α, and to a lesser extent IL-1α and -β, was partially inhibited by blockade of individual mitogen-activated protein kinases, while complete inhibition of cytokine induction was achieved when multiple signaling pathway inhibitors were used. Taken together, these data clearly show activation of the innate immune system in human alveolar macrophages by *Bacillus anthracis* spores. The data also show that multiple signaling pathways are involved in this cytokine response.

37

A REEVALUATION OF EVANS BLUE DYE AS A MARKER OF ALBUMIN CLEARANCE IN MOUSE MODELS OF ACUTE LUNG INJURY. S. Sammani, J. Moitra, J.G.N. Garcia, Department of Medicine, The University of Chicago, Chicago, IL.
Background: Quantifying the amount of albumin conjugated to Evans Blue dye (Alb-EB) fluxing across organ barriers is a popular technique to measure intactness of the physical barrier in rodent models of a variety of diseases. We have reevaluated this technique in terms of the correction factors required in a spectrophotometric assay. **Methods and Results:** Eight- to 10-week-old C57BL6/J mice received either pH-neutral water (controls) or LPS (treatment) by intratracheal instillation, and acute lung injury was allowed to develop for 24 hours. Both control and treatment mice were further injected with Alb-EB via the jugular vein, at doses of either 20 or 30 mg/kg body weight, at 30, 60, 120, or 180 minutes before termination of the LPS treatment (24 hours total). At the end of exposure, formamide extracts of lungs were prepared, and the centrifuged supernatants were measured at 620 and 740 nm in a spectrophotometer. Lungs from control mice that were not injected with Alb-EB were similarly extracted and measured. The linear regression equation between absorbances at 740 nm (X) and 620 nm (Y) in control lung extracts of animals that did not get Alb-EB was considered to be the tissue-specific correction factor. The observed absorbances of the control and treatment samples at 620 and 740 nm were then normalized using this factor. This tissue-specific correction resulted in control samples read as positive integers, as opposed to negative integers when using a serum correction factor commonly used in the literature. We also determined that adjusting the duration of the conjugated dye in circulation is critical for maximizing the signal-to-noise ratio. **Conclusion:** The Evans Blue dye extravasation method to quantify barrier dysfunction can be improved in terms of repeatability and sensitivity by using tissue-specific correction factors and maximized signal-to-noise ratios, respectively.
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38

NOX4, A HOMOLOGUE OF NOX2, REGULATES HYPEROXIA-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION AND ANGIOGENESIS IN HUMAN LUNG ENDOTHELIAL CELLS. S. Pendyala, I. Gorshkova, B. Gorshko, H. Donghong, R.K. Stern, P. Usatyuk, V. Natarajan, Department of Medicine, The University of Chicago, Chicago, IL.
Rationale: Nox4, a homologue of Nox2 ($gp91^{phox}$), is involved in ROS production and signal transduction in vascular cells. In human pulmonary artery endothelial cells (HPAECs), mRNA expression of Nox4 is several folds higher compared to Nox2 and exposure of cells to hyperoxia (95% O_2, 24 hours) resulted in up-regulation of Nox4 and $p22^{phox}$ but not Nox1 or Nox3. Nox4 siRNA partially reduced ROS formation and blocked cell motility and capillary tube formation in cells exposed to either normoxia or hyperoxia, suggesting a role for Nox4 in angiogenesis. **Methods/Results:** In HPAECs and human lung microvascular ECs, expression of Nox4 was several folds higher, as shown by real-time PCR, and exposure to hyperoxia (24 hours) up-regulated Nox4 mRNA as well as protein expression. The localization of Nox4 in HPAECs, as determined by immunofluorescence microscopy with Nox4 antibody, revealed that a majority of the native Nox4 protein was localized near the perinuclear region that stained positive for Golgi marker and a small fraction extended throughout the cytoplasm in internal membrane and vesicular structures. Exposure of cells to hyperoxia (3 hours) caused the Golgi to assume a rounded appearance from a saucer-shaped structure where in majority of Nox4 was colocalized. As hyperoxia-mediated cell motility was attenuated by Nox4 siRNA and was dependent on ROS production, we studied the role of Nox4 in capillary tube formation using matrigel assay. Exposure of HPAECs grown on matrigel to hyperoxia (24 hours) increased the number of capillary tubes compared to normoxia and Nox4 siRNA attenuated the capillary tube formation. **Conclusion:** Nox4 participates in ROS production and acts as a signaling protein that plays a pivotal role in regulating key EC functions such as migration and capillary tube formation.
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39

5-HYDROXYTRYPTAMINE 4 RECEPTOR IN THE ENDOTHELIAL CELLS. I. Profirovic, I. Vardya, T. Voyno-Yasenetskaya, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL.
Serotonin (5-hydroxytryptamine [5-HT]) is an important neurotransmitter that regulates multiple events in the central nervous system (CNS). We have recently demonstrated that 5-HT4 receptor couples to G13 protein to induce RhoA-dependent gene transcription, neurite retraction, and neuronal cell rounding (Ponimaskin et al, 2002). Although multiple