ride (LPS) for 24 h. Proteins were separated by SDS-PAGE and Type II NOS protein was detected by western blot analysis using anti-Type II NOS antibodies. Type II NOS protein was not induced by hypoxia in aVSM or in mVSM cells. However, Type II NOS protein expression was increased in both mVSM and aVSM cells with hypoxia in the presence of LPS. LPS alone had no effect on the level of Type II NOS protein in either cell type.

The difference in the functional activity of the Type II NOS promoter in aVSM cells and mVSM cells in hypoxia is unclear. There appears to be an apparent contradiction between the results obtained with the endogenous gene in the western blot analysis and that of the transient transfection. There are several possible explanations for these results. First, elements contained in the 3′-flanking region of the Type II NOS gene may contribute to the hypoxic response. Second, a number of plasmid isolation procedures (silica slurry and CsCl gradient) are known to be contaminated with LPS. It is possible that the plasmids used for the transient transfections presented here are contaminated with LPS. However, the same plasmid preparations were used in both cell types. Thus, it is possible that the response depends on a certain threshold level of cytokine induced by the LPS and the mVSM cells have a lower threshold than the aVSM cells.

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REFERENCES
2 Wang GL, Semenza GL. General involvement of hypoxia inducible factor-1 in the transcriptional response to hypoxia. Proc Natl Acad Sci USA 1993; 90:4304-08
3 Gleadle JM, Ebert BL, Firth JD, et al. Regulation of angiogenic growth factor expression by hypoxia, transition metals, and chelating agents. Am J Physiol 1995; 268:C1362-68
5 Xue C, Johns R. Upregulation of nitric oxide synthase corre-
lates temporally with onset of pulmonary vascular remodeling in the hypoxic rat. Hypertension 1996; 28:743-53

Cultured Rat Main Pulmonary Artery Smooth Muscle Cells Exhibit at Least Two Polyamine Transport Phenotypes That Differ Morphologically and in Response to Hypoxia*

Pavel Babal, MD; Mark N. Gillespie, PhD

Emerging evidence suggests that pulmonary artery medial cells exhibit different smooth muscle, or smooth muscle-like, phenotypes, with a discrete population responsible for smooth muscle responses in hypoxic pulmonary vascular remodeling. Because the polyamines, putrescine, spermidine, and spermine, are required for cell proliferation and differentiation, we developed a new method to evaluate polyamine disposition by fluorescence detection and used it to localize polyamine uptake within rat main pulmonary artery (MPA) explants and to determine if cultured rat pulmonary artery smooth muscle cells (PASMCs) exhibit multiple polyamine transport phenotypes that are differentially responsive to hypoxia. Monodansylated polyamines were synthesized in a 1:1 stoichiometric reaction between dansyl chloride and the natural polyamines; chemical structure was confirmed by mass spectrometry. Fluorescence microscopy showed a diffuse labeling pattern for dansyl-polyamines in normoxic rat MPA explants. After 48 h in hypoxia, however, dansyl-polyamine localization was accentuated and focal, and intense labeling was evident in cells near the adventitial-medial border. These differences in the disposition of dansyl-polyamines in normoxic and hypoxic MPA explants were mimicked by 14C-polyamines localized autoradiographically. Using microfluorometric detection, studies in rat cultured PASMCs showed that uptake of the dansyl derivatives was similar to the natural polyamines in terms of time, concentration, and temperature dependence. Dansyl-derivatives also inhibited uptake of their 14C-counterparts. Fluorescence microscopy of PASMCs labeled with dansyl-spermidine or -spermine revealed a homogeneous cellular localiza-

*From the Departments of Pharmacology (Dr. Gillespie) and Pathology (Dr. Babal), University of South Alabama, Mobile.
Remodeling of the Na/H Antiport and the Cytoskeleton of Human Pulmonary Artery Endothelial Cells*

Response to an Oxygen Deficit

Michael Cutaia, MD, FCCP; N. Parks; Sharon I. S. Roudes, MD; K. F. Yip

(CHEST 1998; 114:355S)

The pulmonary vasculature undergoes remodeling after hypoxic exposure, but little is known about how cell membranes adapt to an oxygen deficit. We determined the effect of environmental hypoxia (H) and chemical hypoxia (CH) on the localization of a specific membrane ion transport site, the Na/H antiport (NHE1), in human pulmonary artery endothelial cells (HPAEC) by using immunofluorescent staining. Staining for F-actin was used as a control to determine the effects of H and CH on the cytoskeleton.

MATERIALS AND METHODS

Monolayers were incubated in Eagle’s minimal essential medium/10% fetal calf serum under hypoxic conditions (95% N₂/5% CO₂) for 72 h. CH was induced by incubating monolayers in Eagle’s minimal essential medium/pH-6.2/potassium thiocyanate/iodoacetate (2.5 mM)/iodoacetate (0.5 mM) for 3 h. The cells were stained for NHE1 and F-actin in a double-label immunofluorescent method. The primary NHE1 antibody (polyclonal antirabbit IgG) was recognized by a fluorescein isothiocyanate-conjugated antirabbit IgG secondary antibody.

*From the Veterans Administration Medical Center and Brown University, Providence, RI.

F-actin was labeled with rhodamine-conjugated phalloidin. Controls were incubated under normoxic (21% O₂/5% CO₂) conditions or without potassium thiocyanate/iodoacetate for the same time. Monolayers were viewed with an inverted microscope coupled to a confocal scanning unit.

RESULTS

NHE1 was localized to the basolateral surface of control cells. H did not alter NHE1 visualization or localization but led to a change in cytoskeletal morphology, that is, a loss of central F-actin fibers in comparison with controls. In contrast, CH attenuated the intensity of NHE1 staining and also decreased the number of central F-actin bands in comparison with controls. The change in F-actin fibers after CH was more pronounced than that observed after H. Cell viability was unchanged after H or CH under these experimental conditions in separate experiments.

CONCLUSIONS

The similarity of the response of the F-actin cytoskeleton after H and CH suggests that a shared feature (adenosine triphosphate depletion?) is responsible for these findings. Diminished NHE1 visualization after CH suggests a defect in NHE1 protein expression and/or protein insertion into the membrane. Diminished NHE1 visualization only after CH suggests an important difference in the mechanisms underlying the membrane response to these two distinct models of oxygen deficit.

The pathophysiological significance of remodeling of membrane ion transport sites in response to an oxygen deficit is unknown. Whereas NHE1 plays a key role in endothelial cell signal transduction and mediator release, we speculate that this remodeling may contribute to the development of pulmonary hypertension. Future experiments will focus on the mechanisms of this remodeling and the relation to altered vascular function.

Effects of Chronic Hypoxia and Altered Hemodynamics on Endothelial Nitric Oxide Synthase and Preproendothelin-1 Expression in the Adult Rat Lung*

Timothy D. Le Cras, PhD; Robert C. Tyler, PhD; Marilee P. Horan, BS; Ken C. Morris, MS; Ivan F. McMurty, PhD; Roger A. Johns, MD; and Steven H. Abman, MD

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*From the Departments of Pediatrics (Drs. Le Cras and Abman and Ms. Horan) and Medicine (Drs. Tyler and McMurty and Mr. Morris), University of Colorado Health Sciences Center, Denver, and the Department of Anesthesiology (Dr. Johns), University of Virginia Health Sciences Center, Charlottesville.