

CALCIUM MOBILIZATION IN HUMAN AIRWAY EPITHELIAL CELLS BY AN AIRWAY DISEASE-RELEVANT EXTRACT OF HOG-BARN DUST

P. R. Dodmane¹, N. A. Schulte¹, D. J. Romberger¹, M. L. Toews¹

¹University of Nebraska Medical Center, Omaha, NE, United States of America

Corresponding author's email: mtoews@unmc.edu

RATIONALE. Workers in swine confinement facilities are susceptible to development of chronic inflammatory lung disease, but the active components in the dust and the cellular and molecular mechanisms involved in the disease remain poorly defined. An aqueous hog-barn dust extract (HDE) stimulates release of cytokines IL6 and IL8 from cultured BEAS-2B human airway epithelial cells. Because this response involves activation of the Ca²⁺-dependent protein kinase C-alpha isozyme, we hypothesized that HDE would stimulate Ca²⁺ mobilization in these cells also. **METHODS.** HDE was subjected to gel filtration to separate its components by size, and HDE or fractions were treated with proteinase K to hydrolyze protein components. Intracellular Ca²⁺ mobilization in confluent BEAS-2B cells was monitored fluorometrically with a Flex Station Ca²⁺-monitoring system. **RESULTS.** HDE caused a concentration-dependent increase in Ca²⁺ mobilization that was comparable to that induced by 10 μM lysophosphatidic acid, with half-maximal effects at 0.7% HDE. Gel filtration of HDE on Sephadex G-100 showed two distinct peaks of Ca²⁺-mobilizing activity, one with relatively low activity in ~30-50 kDa MW fractions and a broad peak of strong activity in the very low MW fractions eluting near the end of the run. These Ca²⁺-mobilizing peaks overlapped with two of the three peaks of IL6- and IL8-releasing activity of HDE identified in separate studies. HDE-induced stimulation of IL6 and IL8 release is reduced but not eliminated by proteinase K treatment of HDE; the larger MW Ca²⁺-mobilizing activity was also sensitive to proteinase K, but the low MW activity was not. Complete HDE stimulates rapid phosphorylation of EGF receptors but the Ca²⁺-mobilizing fractions did not. The higher MW peak exhibited very slow Ca²⁺ elevation (1-2 min) whereas the low MW peak exhibited rapid Ca²⁺ elevation (2-10 sec), suggesting different mechanisms for these two components. **CONCLUSIONS.** These data add Ca²⁺ mobilization to the signaling mechanisms activated by HDE, consistent with the involvement of protein kinase C-alpha in HDE responses. Multiple Ca²⁺-mobilizing factors are present in HDE and mediate their Ca²⁺ responses by different mechanisms. These Ca²⁺-mobilizing factors likely contribute to the cytokine responses to HDE in isolated cells and to dust-induced lung disease in hog-barn workers.

This abstract is funded by: NIH 1-R01-OH008539

Am J Respir Crit Care Med 181;2010:A6379

Internet address: www.atsjournals.org

Online Abstracts Issue