of the membrane. The consequence of these events is the preferential accumulation of active Na/K-ATPase at the basolateral membranes (cell-cell contact sites) and the development of cell surface polarity of this important protein in polarized transporting epithelial cells.

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Role of the Actin Cytoskeleton in Ischemic Injury*

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Epithelial cell function is dependent upon the establishment and maintenance of surface membrane polarity. The actin cytoskeleton is being increasingly recognized as an important determinant of surface membrane polarity. In renal proximal tubule cells, we now interrelated the dysfunction of the cortical actin cytoskeleton and the loss of Na/K-ATPase surface membrane polarity during ischemia.

Using indirect immunofluorescence staining, ischemia was found to result in the rapid duration-dependent redistribution of actin from a microvillar-subapical (terminal web) pattern to a diffuse cytosolic distribution. This was associated with the rapid duration-dependent dissociation of Na/K-ATPase from its actin cytoskeletal attachments. The ratio of detergent (Triton-x-100) soluble (noncytoskeletal associated Na/K-ATPase) to detergent insoluble Na/K-ATPase (cytoskeletal associated) increased from 0.12 ± 0.03 to 0.21 ± 0.04 and 0.32 ± 0.05 during 15 and 30 min of ischemia, respec-

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Inflammatory Mediators and the Generation and Release of Reactive Oxygen Species by Airway Epithelium in Vitro*

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Epithelial cells lining the respiratory airways represent the first cells with which inhaled inorganic particulates and microbes come into contact upon inhalation, yet surprisingly, little is known about interactions between these substances and epithelial cells. Most studies of such interactions relate to airway epithelial response as a classical "target" type of cell, responding to exogenous stimuli or related local inflammatory reactions by altering one or more facets of epithelial function, such as mucin secretion, ion transport, and ciliary beating.1

However, recent studies have implicated airway epithelium as capable of acting as "effector" cells in response to exogenous stimuli, synthesizing and releasing a variety of mediators, such as eicosanoids,4 cytokines,5 and smooth muscle relaxing "factors"6 that can influence function of neighboring cells and tissues. Other potential mediators that may be produced and released by airway epithelial cells are ROS. We have reported previously7 that GPTE cells in air/liquid interface culture respond to oxidant stress by increasing mucin secretion via a mechanism apparently related to increased intracellular production or flux of additional ROS. In the present studies, we wished to determine directly whether or not GPTE cells were capable of generating and releasing ROS under normal, unstimulated conditions, and after exposure to PMA, known to stimulate oxygen radical production by phagocytic cells; or to the potent bronchoconstrictive inflammatory mediators, PAF and ET.

Materials and Methods

Cell Culture

Primary cultures of guinea pig tracheal epithelial cells were established using the liquid/air interface system developed in this laboratory and described in detail previously.8 Briefly, tracheal epithelial cells were dissociated with pronase and plated out on a collagen gel substrate in a Transwell COL culture chamber at a plating density of approximately 50,000 cells/cm². The cells were maintained in serumless medium containing antibiotics, with medium being changed every 2 days. Medium was placed beneath the cells only: they were exposed to a humidified 95% air/5% CO₂ interface on the apical side. When cultured in this manner, the cells maintain the morphologic and functional characteristics of GPTE cells in situ, as described previously. Near confluent (approximately 8-day-old) cultures were used in these studies. Prior to all biochemical assays, cells were washed with endotoxin-free Hanks balanced salt solution containing Ca²⁺ and Mg²⁺.

Measurement of Released Hydrogen Peroxide

Hydrogen peroxide released by the cells was measured spectrofluorometrically using a modification of the method of Ruch et al.7 The cells were incubated for 30 to 90 min with homovanillic acid (3-methoxy-4-hydroxy phenylacetic acid) and horseradish peroxidase, and H₂O₂ measured spectrofluorometrically at an emission wavelength of 425 nm and extinction wavelength of 321 nm. Proteins were assayed by a standard Lowry assay and lactate dehydrogenase measured spectrophotometrically.8

Release of Hydrogen Peroxide by GPTE Cells

Hydrogen peroxide was measured in both apical or basal sides of the cells at 30-min intervals for a period of 90 min. Cells were exposed to either solvent vehicle (control), PAF (1 to 100 nmol), PMA (10 to 200 ng/ml), or ET (50 to 200 nmol). In some experiments, the cells were preincubated for 15 min with the PKC inhibitor, staurosporine (50 nmol).

Results

Effects of Inflammatory Mediators on Hydrogen Peroxide Release

As illustrated in Table 1, GPTE cells released H₂O₂ into the medium, with about 95% of the H₂O₂ being released from the apical side of the cells. The rate of H₂O₂ release was approximately 0.04 nmol/min/mg protein, and was essentially steady state over the 90 min period of measurement. Removal of Ca²⁺ from the medium decreased H₂O₂ release by 50%. The PAF and PMA provoked a concentration-dependent increase in H₂O₂ release at 30 min, while

Table 1—Release of Hydrogen Peroxide by GPTE Cells*

<table>
<thead>
<tr>
<th>Addition</th>
<th>30 Min</th>
<th>60 Min</th>
<th>90 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>1.32 ± 0.15</td>
<td>2.44 ± 0.08</td>
<td>3.47 ± 0.51</td>
</tr>
<tr>
<td>PAF 1 nmol</td>
<td>1.36 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 nmol</td>
<td>2.11 ± 0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nmol</td>
<td>3.36 ± 0.32†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 nmol</td>
<td>4.73 ± 0.49†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMA 10 ng</td>
<td>2.93 ± 0.34†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 ng</td>
<td>3.55 ± 0.66†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 ng</td>
<td>2.77 ± 0.89†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET 50 nmol</td>
<td>1.42 ± 0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nmol</td>
<td>1.61 ± 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 nmol</td>
<td>1.66 ± 0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAF (100 nmol) + SST</td>
<td>0.97 ± 0.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Hydrogen peroxide expressed as nmol/mg protein. All values are means ± 1 SE from 3-7 replicate experiments. SST is staurosporine.
†Significantly different from control (p<0.05).