PKC activation is required by EGF-stimulated Na\(^+\)-H\(^+\) exchanger in human pleural mesothelial cells

Liaw, Yuy-Suang, Pan-Chyr Yang, Chong-Jen Yu, Sow-Hsong Kuo, Kwen-Tay Luh, Yuh-Jeng Lin, and Mei-Lin Wu. PKC activation is required by EGF-stimulated Na\(^+\)-H\(^+\) exchanger in human pleural mesothelial cells. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L665–L672, 1998.—Epidermal growth factor (EGF) stimulates the Na\(^+\)-H\(^+\) exchanger, leading to enhanced cell proliferation. In human pleural mesothelial cells (PMCs), the intracellular signaling mechanism mediating the EGF-induced stimulation of the Na\(^+\)-H\(^+\) exchanger has not yet been identified. Using a pH-sensitive fluorescent probe, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein, to measure changes in intracellular pH (pH\(_i\)), we found that 1) EGF and 12-O-tetradecanoylphorbol 13-acetate (TPA; a phorbol ester) both stimulate the epithilose-propyl amiloride-sensitive Na\(^+\)-H\(^+\) exchanger; 2) TPA-induced alkalosis can be blocked by protein kinase C (PKC) inhibitors (chelerythrine and staurosporine) or by PKC downregulation, indicating that PKC activation is involved in the stimulation of the Na\(^+\)-H\(^+\) exchanger. However, TPA-induced alkalosis is not blocked by tyrosine kinase inhibitors; and 3) the stimulatory effect of EGF on the Na\(^+\)-H\(^+\) exchanger acts via stimulation of tyrosine kinase-receptor activity because it is inhibited by tyrosine kinase inhibitors (genistein, lavendustin A, and herbimycin A). It also involves PKC activation because EGF-induced alkalosis was blocked by PKC inhibitors. These results suggest that PKC activation is one of the downstream signals for EGF-induced activation of the Na\(^+\)-H\(^+\) exchanger in primary cultures of human pleural mesothelial cells.

tyrosine kinase; epidermal growth factor; protein kinase C; sodium-hydrogen exchanger

**MESOTHELIAL CELLS**, which cover the surface of the parietal and visceral pleura, respond to both intrinsic stimuli, such as growth factors, and extrinsic stimuli, such as asbestos fibers (2). Some pleural diseases, including parapneumonic effusion, lupus, pleurisy, and metastatic malignancy, result in the loss of mesothelial integrity and the subsequent accumulation of pleural fluid, with an increase in permeability (15). Cell proliferation in response to growth factors contained in the pleural fluid may therefore be important in restoring the normal mesothelium after pleural injury.

The housekeeping Na\(^+\)-H\(^+\) exchanger isoform 1 (NHE1) is amiloride and ethylisopropyl amiloride (EIPA) sensitive and is expressed in most eukaryotic cells. It is usually activated when the intracellular pH (pH\(_i\)) falls and exchanges external Na\(^+\) for internal H\(^+\). It appears to be involved in multiple cellular functions including pH regulation, transepithelial transport, and cell volume control (13). Moreover, this exchanger can be rapidly activated by various mitogenic factors such as growth factors [e.g., epidermal growth factor (EGF)] and phorbol esters [e.g., 12-O-tetradecanoylphorbol 13-acetate (TPA)], resulting in DNA synthesis and cell proliferation (8, 13, 27, 35).

At least two major mechanisms have been proposed to be involved in the activation of the Na\(^+\)-H\(^+\) exchanger by various stimulants. The first is a protein kinase C (PKC)-dependent pathway involving either G protein-activated phospholipase C (PLC)-β or tyrosine kinase receptor-activated PLC-γ (11, 19); activation of either system leads to the production of inositol 1,4,5-trisphosphate and diacylglycerol (5), and the latter then activates PKC, resulting in PKC translocation from the cytosol to the membrane. This membrane association-activation event can be mimicked by phorbol esters such as TPA, resulting in the irreversible insertion of PKC into the lipid bilayer (24). In fibro-blasts, it has been shown that addition of phorbol esters or growth factors increases the phosphorylation of the Na\(^+\)-H\(^+\) exchanger (31), thereby leading to an alkaline shift in the resting pH, that can easily be detected under nominally bicarbonate-free conditions. Recent studies (34, 35) have also shown that deletion of all the major potential phosphorylation sites only resulted in a 50% decrease in exchanger activation in response to phorbol esters or growth factors, suggesting that PKC and growth factors do not activate the exchanger exclusively by direct phosphorylation (34, 35).

The second mechanism is a PKC-independent pathway in which the Na\(^+\)-H\(^+\) exchanger is activated either by growth factors that stimulate the tyrosine kinase receptor (3, 10, 12, 35) or by Ca\(^{2+}\)- and/or calmodulin-dependent disinhibition of the exchanger (6, 21, 33, 35). There is evidence that, rather than directly phosphorylating the Na\(^+\)-H\(^+\) exchanger, tyrosine kinase phosphorylates an ancillary protein, and the latter then activates the exchanger, resulting in the modulation of a critical cytoplasmic region and the alteration of the "set point" of the exchanger, leading to intracellular alkalosis (34, 35).

In a previous study (16), we showed the housekeeping Na\(^+\)-H\(^+\) exchanger to be one of the three major pH regulators in cultured human pleural mesothelial cells (PMCs). Human PMCs possess EGF receptors (28), but little is known about the cellular signaling pathway involved in EGF-induced Na\(^+\)-H\(^+\) exchanger activation. Using 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) to continuously monitor pH\(_i\) in human PMCs, we have found that EGF-stimulated PKC activation, presumably as a result of receptor-linked tyrosine kinase activity, is required for activation of the exchanger.
MATERIALS AND METHODS

Chemicals and solutions. Unless otherwise stated, all chemicals were purchased from Sigma (St. Louis, MO) and all experiments were performed in HEPES-buffered solution consisting of (in mM) 130 NaCl, 5.0 KCl, 1.0 MgCl₂, 2.0 CaCl₂, 10 glucose, and 10 HEPES, pH adjusted to 7.4 at 37°C with NaOH. In Na⁺-free Tyrode solution, NaCl was totally replaced by 130 mM N-methyl-D-glucamine (NMDG). EGF was purchased from Collaborative Biomedical Products (Bedford, MA); and herbimycin A, lavendustin A, and chelerythrine were purchased from Research Biochemicals International (Natick, MA).

Isolation and cell culture of human PMCs. Human PMCs were obtained from patients with transudative pleural effusion, e.g., patients with liver cirrhosis or heart failure, as described in our previous report (16). Briefly, after removal of red blood cells from the pleural effusion by gradient centrifugation, the pellet was washed once in RPMI 1640, resuspended in the same medium to a volume of 8–10 ml, and cooled to 37°C. The 530-nm emission ratio (ratio of 490- to 440-nm excitation) from the intracellular BCECF was calculated and converted to a linear pH scale (see below) by in situ calibration at pH 4.5 and 9.5 at the end of the experiment using the nigericin technique (29). The following equation was used to convert the fluorescence ratio into pH i: 

\[ \text{pHi} = \log\left( \frac{R_{440\text{nm}}}{R_{490\text{nm}}} - \log(R_{440\text{nm}}) \right) \]

where pK is the negative log of dissociation constant for the dye measured at pH 7.16; R is the ratio of the 530-nm fluorescence at 490-nm excitation to that at 440-nm excitation; Rmax and Rmin are the maximum and minimum ratio values, respectively, from the data curve; and F440min and F440max are the minimum and maximum fluorescence values, respectively, at 440-nm excitation.

Measurement of pH i. Measurement of pH i has been described in detail in previous work by Wu and colleagues (36). Briefly, human PMCs, grown on a cover glass, were loaded with 5 µM BCECF-AM (Molecular Probes, Kent, OR) for 20 min at room temperature in HEPES-buffered solution, then washed with HEPES-buffered solution, and excited alternately by 490- and 440-nm wavelength light. The excitation light was transmitted to the cell with a 510-nm dichroic mirror under the microscope nosepiece, and the resulting signals was collected by a ×40 oil-immersion lens. The overall sampling rate was 0.5 Hz. The 530-nm emission ratio (ratio of 490- to 440-nm excitation) from the intracellular BCECF was calculated and converted to a linear pH scale (see below) by in situ calibration at pH 4.5 and 9.5 at the end of the experiment using the nigericin technique (29). The following equation was used to convert the fluorescence ratio into pH i:

\[ \text{pHi} = \log\left( \frac{R_{440\text{nm}}}{R_{490\text{nm}}} - \log(R_{440\text{nm}}) \right) \]

RESULTS

EGF stimulates the Na⁺-H⁺ exchanger in human PMCs. Under nominally bicarbonate-free conditions (i.e., HEPES-buffered medium), the addition of 100 ng/ml of EGF resulted in a slight initial acidosis (0.02 ± 0.01 pH units; n = 6) of the resting pH i, followed by a more marked pH i increase (0.12 ± 0.05 pH units; n = 6 preparations; Fig. 1A, Table 1) similar in magnitude to the EGF-induced alkalosis seen in rat hepatocytes (~0.1 pH unit) (23).
In HEPES-buffered medium, the Na\(^{+}\)-H\(^{+}\) exchanger is the main pH \(i\) regulator in human PMCs (16). We therefore tested whether the EGF-induced alkalosis was due to activation of this exchanger. Figure 1B shows that a pH \(i\) decrease occurs on transfer to Na\(^{+}\)-free medium, probably due to inhibition of the exchanger, resulting in accumulation of metabolic acid. Under these conditions, however, the initial EGF-induced acidosis (Fig. 1B, arrowhead) was more marked (Table 1) and the expected EGF-induced alkalosis (Fig. 1A) was completely abolished (Fig. 1B, Table 1). Similar results were seen when the Na\(^{+}\)-H\(^{+}\) exchanger was inhibited by EIPA (10 µM; Fig. 1C, Table 1), indicating that the EGF-induced alkalization seen in Fig. 1A was due to activation of the Na\(^{+}\)-H\(^{+}\) exchanger.

The statistical data for the above results are summarized in Table 1.

Tyrosine kinase-receptor activity is involved in the EGF-mediated stimulation of the Na\(^{+}\)-H\(^{+}\) exchanger. The EGF signaling pathway is known to act via activation of the tyrosine kinase receptor (5), and the EGF receptor has recently been found in human PMCs (28). We therefore tested whether tyrosine kinase activation is involved in stimulation of the Na\(^{+}\)-H\(^{+}\) exchanger. Genistein and lavendustin A are both potent specific blockers of EGF-stimulated tyrosine kinase activity (1, 25). Figure 2, A and B, shows that either 30 µM genistein or 50 µM lavendustin A itself caused a decrease in pH \(i\) (Table 1) by an unknown mechanism. However, in the presence of either of these inhibitors, a further decrease in pH \(i\) occurred on addition of 100 ng/ml of EGF (Fig. 2, A and B, arrowheads; Table 1). Herbimycin A, which binds to the thiol groups in tyrosine kinase, is another potent blocker. After over-

---

**Table 1.** EGF-induced intracellular alkalosis and acidosis after various treatments

<table>
<thead>
<tr>
<th></th>
<th>EGF (100 ng/ml, 15-20 min)</th>
<th>Na Free (130 mM NMDG, 30-40 min)</th>
<th>EIPA (10 µM, 20-25 min)</th>
<th>Genistein (30 µM, 25-30 min)</th>
<th>Lavendustin A (50 µM, 25-30 min)</th>
<th>Herbimycin A (0.5 µM, overnight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta pHi) Blocker</td>
<td>-0.20 ± 0.05 (n = 4)</td>
<td>-0.14 ± 0.03 (n = 5)</td>
<td>-0.09 ± 0.02 (n = 4)</td>
<td>-0.07 ± 0.01 (n = 4)</td>
<td>-0.12 ± 0.01 (n = 4)</td>
<td>-0.08 ± 0.03 (n = 4)</td>
</tr>
<tr>
<td>Blocker + EGF (Total acidosis)</td>
<td>-0.36 ± 0.05† (n = 4)</td>
<td>-0.24 ± 0.02† (n = 5)</td>
<td>-0.11 ± 0.02 (n = 4)</td>
<td>-0.12 ± 0.01† (n = 4)</td>
<td>-0.05 ± 0.01* (n = 5)</td>
<td>-0.08 ± 0.03 (n = 4)</td>
</tr>
<tr>
<td>EGF-induced acidosis</td>
<td>-0.16 ± 0.02 (n = 4)</td>
<td>-0.10 ± 0.01 (n = 5)</td>
<td>0.02 ± 0.01 (n = 4)</td>
<td>0.05 ± 0.01 (n = 4)</td>
<td>-0.08 ± 0.03 (n = 4)</td>
<td></td>
</tr>
<tr>
<td>(\Delta pHi)</td>
<td>0.12 ± 0.05 (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE in pH units; n, no. of preparations. EGF, epidermal growth factor; Na free, 130 mM NaCl totally replaced by N-methyl-D-glucamine (NMDG); EIPA, ethylisopropyl amiloride; \(\Delta pHi\), change in intracellular pH. Data are from experiments shown in Figs. 1 and 2. Significantly different (P < 0.05) from: *EGF-induced alkalosis (+\(\Delta pHi\)) by Mann-Whitney U-test; †blocker by paired t-test.
night pretreatment with herbimycin A (0.5 µM), the EGF-mediated stimulatory effect was lost (Fig. 2C, arrowhead; Table 1). These results all indicate that tyrosine kinase activation is involved in the activation of the Na\(^{+}\)-H\(^{+}\) exchanger.

The statistical data for the above results are summarized in Table 1.

TPA, a potent phorbol ester, also stimulates the Na\(^{+}\)-H\(^{+}\) exchanger. The membrane association-activation event during PKC activation can be mimicked by TPA (24). Figure 3A shows that, on addition of 1 µM TPA, a slight initial acidosis was seen (0.02 ± 0.01 pH units; n = 8 preparations), followed by a significant increase in pHi (0.11 ± 0.03 pH units; n = 8 preparations; Table 2). On removal of all extracellular Na\(^{+}\) (Fig. 3B) or on addition of EIPA (10 µM; Fig. 3C), a pHi decrease was seen due to inhibition of the Na\(^{+}\)-H\(^{+}\) exchanger; however, the subsequent addition of TPA (1 µM) induced a greater acidosis (Fig. 3, B and C, arrowheads; Table 2) compared with the control value (Fig. 3A), indicating that the TPA-induced alkalization (Fig. 3A) was due to stimulation of the Na\(^{+}\)-H\(^{+}\) exchanger.

Chelerythrine, staurosporine, and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7), potent PKC blockers (14, 17, 30), were used to determine whether the TPA-induced stimulation of the Na\(^{+}\)-H\(^{+}\) exchanger is mediated via activation of PKC. Figure 4, A and B, shows that chelerythrine (7 µM) or staurosporine (0.1 µM) completely abolished the TPA-induced alkaline effect (Table 2). TPA caused irreversible insertion of PKC into the lipid bilayer, resulting in cumulative, long-term PKC stimulation (24), and overnight TPA treatment caused complete depletion of endogenous PKC (i.e., downregulation) (38). After overnight treatment with 1 µM TPA, the alkalization induced by the subsequent addition of 1 µM TPA (Fig. 4C, arrowhead) was abolished (Table 2). These results indicate that PKC activation is indeed involved in the stimulation of the Na\(^{+}\)-H\(^{+}\) exchanger in PMCs. After treatment with PKC blockers, the initial slight TPA-induced acidosis (Fig. 3A) became even larger (Fig. 4, A–C, arrowheads), although the difference did not always reach statistical significance (Table 2). The mechanism responsible for the initial intracellular acidosis induced by these PKC blockers is unknown.

The statistical data for the above results are summarized in Table 2.

PKC activation is required for EGF-mediated stimulation of the Na\(^{+}\)-H\(^{+}\) exchanger. The above results indicate that activation of either tyrosine kinase or PKC can result in stimulation of the Na\(^{+}\)-H\(^{+}\) exchanger (Figs. 1–4). PLC-\(\gamma\) phosphorylation, resulting in PKC stimulation, is one of the downstream signaling pathways for tyrosine kinase-receptor activation (5). Thus the stimulatory effect of EGF on the Na\(^{+}\)-H\(^{+}\) exchanger is probably due to PKC activation via tyrosine kinase-receptor phosphorylation of PLC-\(\gamma\)1. We used PKC blockers to test this possibility (Fig. 5, A–C).

Chelerythrine (7 µM; Fig. 5A), staurosporine (0.1 µM; Fig. 5B), or TPA (1 µM, overnight; Fig. 5C) all completely inhibited EGF-induced Na\(^{+}\)-H\(^{+}\) exchanger activation (Fig. 5, A–C, arrowheads). In contrast, two
potent tyrosine kinase blockers, genistein (30 µM; Fig. 5D) and lavendustin A (50 µM; Fig. 5E), failed to inhibit the TPA-stimulated alkalization (Fig. 5, D and E, arrowheads). These results suggest that PKC activation is involved in the downstream signaling pathway of EGF-induced Na\(^{+}\)-H\(^{+}\) exchanger activation.

The statistical data for the above results are summarized in Table 3.

**DISCUSSION**

Activation of the Na\(^{+}\)-H\(^{+}\) exchanger by growth factors, including EGF, was originally thought to utilize signaling pathways acting directly through tyrosine kinase because the receptor has intrinsic tyrosine kinase activity (3, 9, 10, 12). An earlier study by Moolenaar et al. (22), however, showed that when the tyrosine-specific protein kinase was activated by monoclonal antibodies, the Na\(^{+}\)-H\(^{+}\) exchanger could not be activated, indicating that the pH\(_{i}\) signal could be dissociated from tyrosine kinase activity. At a later date, growth factor was suggested to induce tyrosine phosphorylation of PLC-\(\gamma\)1, thereby activating the PKC-dependent pathway (20). Direct support for this hypothesis has been provided by at least one recent related study by Ma et al. (19), who used cells expressing platelet-derived growth factor receptors that lacked the PKC- and phosphatidylinositol 3-kinase-mediated signaling pathways and showed that both pathways are required for platelet-derived growth factor-induced activation of the NHE1 in transfected epithelial cells.

On the basis of the following evidence in human PMCs, we suggest that PKC activation is also involved in EGF activation of the Na\(^{+}\)-H\(^{+}\) exchanger, possibly due to tyrosine kinase-receptor phosphorylation of PLC-\(\gamma\)1 (19, 20):

1. The pH\(_{i}\) increase induced by EGF or TPA can be blocked by either EIPA or the use of

**Table 2. TPA-induced intracellular alkalosis and acidosis after various treatments**

<table>
<thead>
<tr>
<th>Blocker</th>
<th>Na Free (130 mM NMDG, 30–40 min)</th>
<th>EIPA (10 µM, 20–25 min)</th>
<th>Chelerythrine (7 µM, 20–25 min)</th>
<th>Staurosporine (0.1 µM, 25–35 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-£pH(_{i}) Blocker + TPA (Total acidosis)</td>
<td>-0.24 ± 0.06† (n = 5)</td>
<td>-0.11 ± 0.04 (n = 5)</td>
<td>-0.12 ± 0.02† (n = 4)</td>
<td>-0.15 ± 0.04 (n = 4)</td>
</tr>
<tr>
<td>TPA-induced acidosis</td>
<td>-0.09 ± 0.02* (n = 5)</td>
<td>-0.07 ± 0.03* (n = 5)</td>
<td>-0.03 ± 0.01* (n = 4)</td>
<td>-0.05 ± 0.02* (n = 4)</td>
</tr>
<tr>
<td>+£pH(_{i})</td>
<td>0.11 ± 0.03 (n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE in pH units; n, no. of preparations. TPA, 12-O-tetradecanoylphorbol 13-acetate. Data are from experiments shown in Figs. 3 and 4. Significantly different (P < 0.05) from: *TPA-induced alkalosis (+£pH\(_{i}\)) by Mann-Whitney U-test; †blocker by paired t-test.
Na\(^+\)-free conditions, indicating that it is due to activation of the Na\(^+\)-H\(^+\) exchanger by these two mitogens (Figs. 1 and 3, Tables 1 and 2); 2) the TPA-induced alkalosis can be blocked by PKC inhibitors (chelerythrine and staurosporine) and PKC downregulation (Fig. 4, Table 2), indicating that PKC is indeed involved in the activation of the Na\(^+\)-H\(^+\) exchanger; and 3) the EGF-induced alkalosis was blocked not only by tyrosine kinase inhibitors (genistein, lavendustin A, and herbimycin A; Fig. 2, Table 1) but also by PKC inhibitors (chelerythrine and staurosporine) and PKC downregulation (Fig. 5, A–C; Table 3). Because the tyrosine kinase inhibitors did not block the TPA-mediated activation of the Na\(^+\)-H\(^+\) exchanger (Fig. 5, D and E; Table 3), we cannot completely exclude the possibility that they themselves did not induce an alkalosis after extended treatment (Fig. 5, D and E). However, we found that once the steady state of the pH\(_i\) was reached after addition of these blockers, the pH\(_i\) did not change to any great extent until the end of the experiment (data not shown), suggesting that the TPA-induced alkalosis seen in Fig. 5, D and E, is most probably due to TPA-mediated activation of the exchanger and that PKC activation is one of the downstream signals for EGF-mediated activation of the Na\(^+\)-H\(^+\) exchanger in human PMCs, as shown in other cells (19, 20). In the present study, however, we cannot completely rule out the possibilities that a mutual effect of tyrosine kinase and PKC activation is simply expressed at the EGF receptor or that multiple effects of various kinases and phosphatases may also be involved in the EGF-induced Na\(^+\)-H\(^+\) exchanger. The upward shift in the resting pH\(_i\) produced by the addition of EGF reflects a change in the set point, which determines the pH\(_i\) sensitivity of

Table 3. pH\(_i\) changes induced by EGF and TPA

<table>
<thead>
<tr>
<th>pH(_i) Changes</th>
<th>EGF</th>
<th>TPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF-induced pH(_i) changes</td>
<td><strong>0.12 ± 0.05</strong> (n = 6)</td>
<td><strong>0.11 ± 0.03</strong> (n = 8)</td>
</tr>
<tr>
<td>TPA-induced pH(_i) changes</td>
<td><strong>0.04 ± 0.01</strong> (n = 4)</td>
<td><strong>0.11 ± 0.01</strong> (n = 4)</td>
</tr>
</tbody>
</table>

Values are means ± SE in pH units; n, no. of preparations. Data are from experiments shown in Fig. 5. Intracellular alkalosis induced by addition of EGF can be blocked by protein kinase C inhibitors. TPA-induced alkalosis cannot be blocked by tyrosine kinase inhibitors. *Significantly different from EGF-induced alkalosis (EGF alone), P < 0.05 by Mann-Whitney U-test. No significant difference between addition of TPA in presence of tyrosine kinase inhibitors and TPA-induced alkalosis (TPA alone), P > 0.05 by Mann-Whitney U-test.
the exchanger. According to this model, the set point of the H⁺ sensor is adjusted upward by 0.15–0.3 pH unit (13), the magnitude of the alkalosis recorded in the present study.

The molecular mechanism involved in the PKC-mediated activation of the Na⁺-H⁺ exchanger is still unclear. In fibroblasts, it has been shown that phorbol esters increase NHE1 phosphorylation (31); therefore, it is possible that PKC directly phosphorylates a serine residue and activates the NHE1. However, with the use of deletion mutants expressed in fibroblasts, a recent study (34) has shown that replacement of this serine residue with alanine results in only a 50% reduction in phorbol ester-induced alkalinization, clearly indicating that PKC does not activate the NHE1 by direct phosphorylation (35); therefore multiple effects of various kinases and phosphatases may be involved.

It is interesting that, in the presence of Na⁺-H⁺ exchanger blockers or tyrosine kinase and/or PKC inhibitors (Figs. 1–5), although not always statistically significant, a greater pH decrease was normally seen on addition of EGF and TPA (Tables 1 and 2). Because a small, but consistent, initial pH decrease was seen on addition of these two mitogens (Figs. 1A and 3A), we suggest that the larger pH decrease seen after abolition of the alkalization (i.e., activation of the Na⁺-H⁺ exchanger) was probably due to unmasking of the initial acidification induced by these two mitogens. However, the mechanism for this initial small pH decrease seen on the addition of EGF or TPA (Figs. 1A and 3A) is unknown. One possibility is that EGF and TPA cause transient increases in intracellular Ca²⁺ levels, resulting in displacement of H⁺ from common buffering sites (32); another is overproduction of H⁺ (e.g., via stimulation of the metabolism) by the addition of EGF or TPA.

Recently, the EGF receptor has been shown to be present in human PMCs (28) and rat mesothelial cells (15, 26), and its activation stimulates cell proliferation (15, 26). However, the physiological role of EGF-mediated stimulation of the Na⁺-H⁺ exchanger, resulting in alkalosis in human PMCs, is not clear. Activation of the Na⁺-H⁺ exchanger, resulting in intracellular alkalosis, is a common phenomenon that stimulates cell proliferation in a variety of cell types (8, 13, 27), with small decreases in pH, causing reduced cell proliferation (8) and a rise in pH, being associated with increased cell numbers (4). However, the role of the Na⁺-H⁺ exchanger in stimulating cell growth as a result of intracellular alkalosis is still debatable (13) because some studies show that growth factors or phorbol esters fail to induce a detectable rise in pH, in physiological bicarbonate-containing media (7, 13). In such cases, pH changes induced by the Na⁺-H⁺ exchanger are clearly not of significance in the initiation of cell growth. The possibility of the Na⁺-H⁺ exchanger being involved in restoring normal human PMCs after pleural injury requires further investigation.

We thank Chiu-Mei Wu for excellent technical help. P.-C. Yang and M.-L. Wu made equal contributions to this paper.

This work was supported by National Taiwan University Hospital Grant NTUH 87-2008.

Address for reprint requests: M.-L. Wu, Dept. of Physiology, College of Medicine, National Taiwan Univ., No. 1, Sec. 1, Jen-Ai Rd., Taipei 100, Taiwan, ROC.

Received 27 August 1997; accepted in final form 16 January 1998.

REFERENCES


