Prostaglandin F$_{2\alpha}$ - and 12-O-Tetradecanoylphorbol-13-Acetate–Induced Alterations in the Pathways of Renal Ammoniagenesis$^{1,2}$

Atul Sahai,$^3$ Itzhak Nissim, Rose S. Sandler, and Richard L. Tannen

A. Sahai, R.S. Sandler, R.L. Tannen, Department of Medicine, University of Southern California School of Medicine, Los Angeles, CA

I. Nissim, Division of Biochemical Development & Molecular Diseases, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA


ABSTRACT

The mechanisms whereby prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) and the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibit ammoniagenesis and the reason why they behave differently at pH 7.4, were examined with (15N)glutamine to assess (15N)ammonia formation and (2-15N)-labeled glutamine, reflecting reduced flux through the glutaminase pathway. They also qualitatively reversed the competitive inhibition of the Na$^+$/H$^+$ antiporter activity of rat renal cortical tubules incubated in vitro (1). Furthermore, the inhibitory effects of PGF$_{2\alpha}$ on ammoniagenesis were apparent at an acidic pH but not at pH 7.4. Subsequently, we found that LLC-PK$_1$ cells incubated in vitro provided a useful model for the study of ammoniagenesis (2). Although these cells use both the mitochondrial phosphate-dependent glutaminase (PDG) and glutamate dehydrogenase (GLDH) pathways to produce ammonia, the rate of flux through GLDH accounts for only approximately 15\% of the overall rate of ammonia production at pH 7.4 (3). In response to acute acidification to pH 7.0, intracellular pH (pH$_i$) is reduced and total ammonia production by LLC-PK$_1$ cells is stimulated, with an increase in flux through GLDH, providing an important component of the overall increment in ammoniagenesis (3,4). Recent studies have indicated that, at pH 6.8, acute acidosis stimulates flux through PDG as well as GLDH (5).

PGF$_{2\alpha}$ and also the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), a direct activator of protein kinase C, inhibit the acute low pH (pH 6.8)-induced increases in total ammonia formation by LLC-PK$_1$ cells (6). Subsequent studies have shown that both PGF$_{2\alpha}$ and TPA activation of protein kinase C is followed by the stimulation of Na$^+$/H$^+$ antiporter activity, suggesting that the inhibition of ammoniagenesis results from an increase in intracellular pH (7,8). For reasons that were unclear, TPA, but not PGF$_{2\alpha}$, also inhibited the basal rate of total ammonia formation under pH 7.4 conditions in LLC-PK$_1$ cells. These observations suggested that TPA might be a more potent stimulus of intracellular alkalization than PGF$_{2\alpha}$; however, the possibility that the mea-
measurements of total ammonia obscured small but significant PGF2α-induced changes in flux through the GLDH pathway at pH 7.4 could not be discounted.

To resolve the lack of a PGF2α response on the basal rate of total ammonia metabolism and to determine whether the metabolic sites for the inhibition of ammoniagenesis were consistent with an effect mediated by an increase in intracellular pH, we examined the effects of both PGF2α and TPA on the ammoniagenic pathways as well as Na+/H+ antiporter activity under basal (pH 7.4) and acute acidic conditions. The results of this examination of ammoniagenic pathways using [15N]glutamine are consistent with the view that both PGF2α and TPA inhibit the acute low pH-Induced increases in ammonia formation as a result of an increase in pH. Furthermore, at pH 7.4, TPA, but not PGF2α, inhibits flux through both PDG and GLDH and stimulates Na+/H+ antiporter activity, as well as increases pH, suggesting that the more intense activation of protein kinase C and thereby Na+/H+ antiporter activity accounts for the differing effects of TPA and PGF2α at pH 7.4.

METHODS

Materials

LLC-PK1 cells were purchased from American Type Culture Collection (CL 101; Rockville, MD). L-[5-15N]glutamine and L-[2-15N]glutamine were obtained from Cambridge Isotope Laboratory (Cambridge, MA). PGF2α and TPA were purchased from Sigma Chemical Co. (St. Louis, MO), and stock solutions were prepared in dimethyl sulfoxide at 10 mg/mL and 10−6 M concentrations, respectively. All other reagents were of high chemical grade.

Cell Culture

LLC-PK1 cells were routinely cultured under continuous rocked conditions as previously described (2). In brief, cultures were grown in a 50:50 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium supplemented with 10% fetal bovine serum and 2 mM glutamine. These cultures were maintained in a humidified atmosphere of 5% CO2−95% air at 37°C.

Studies With (15N)Glutamine

Chronically rocked LLC-PK1 cells were used for the assessment of ammonia and alanine formation as previously described (3). Briefly, stock cultures were trypsinized and subcultured in 60-mm dishes in minimal essential medium containing 10% fetal bovine serum and 2 mM glutamine. Subconfluent cultures were washed with phosphate-buffered saline and incubated in the absence or presence of PGF2α (0.1 ng/mL), TPA (1 μM), or vehicle in Krebs-Hensleit buffer of either pH 7.4 or pH 6.8 (achieved by appropriate modifications of bicarbonate concentration), supplemented with 5 mM glucose and 1 mM of either [5-15N]glutamine or [2-15N]glutamine. Cultures were allowed to incubate with gentle rocking for 1 h at 37°C in a 5% CO2−95% air-humidified incubator. At the end of each incubation, 1 mL of Krebs-Hensleit buffer from each dish was removed and mixed with 0.5 mL of 6% perchloric acid. The perchloric acid extracts were neutralized and subsequently used for the assessment of ammonia and amino acid production. Cells were digested with 0.5 N NaOH and used for protein determination by the method of Lowry et al. (9).

Analysis of (15N)Ammonia and (15N)Amino Acid Formation

Neutralized samples were analyzed for 5-15N and 2-15N enrichment in ammonia, alanine, glutamate, and aspartate by the method of gas chromatography-mass spectrometry as previously described (10).

Calculations of 15N Metabolites

The rate of appearance of 15N-labeled ammonia and 15N-labeled alanine, glutamate, and aspartate were determined by the product of their 15N enrichment (atom % excess/100) times concentration and are expressed as nanomoles per hour per milligram of protein. Flux through the PDG pathway was considered to be reflected by 15N-ammonia formation from [5-15N]glutamine and through the GLDH pathway by 15N-ammonia formation from [2-15N]glutamine.

Measurement of Na+/H+ Antipporter Activity

Measurements of Na+/H+ antipporter activity were carried out by the method of Grienstein et al. (11) and Horie et al. (12) as previously described (8). Briefly, confluent cultures of LLC-PK1 cells were lightly trypsinized and washed with balanced salt solution (BSS) containing in (in millimolar concentrations): NaCl, 140; KCl, 5.0; CaCl2, 1.0; MgCl2, 1.0; N-hydroxyethylamine-N’-2-ethanesulfonic acid (HEPES), 15.0; and glucose, 5.0 (pH 7.4). Cells were then resuspended in BSS and loaded with BCECF-AM (2 μM) for 30 min at 37°C. Cells were washed with BSS by centrifugation at 200 g and suspended in 1 mL of BSS. A 100-μL suspension of cells was added to a thermostatically controlled cuvette at 37°C in a fluorescence spectrophotometer (Hitachi F-2000, Hitachi Instruments, Inc.) and studied under continuous magnetic stirring. Fluorescence was studied at excitation wavelengths of 500 and 440 nm and an emission wavelength of 530 nm. Routinely calibration of BCECF fluorescence versus pH was carried out by the method of Thomas et al. (13) using the K/nigericin technique. The calibration curve was linear in the pH range of 6.45 to 7.6 (not shown).

Portions of the cells were acidified by incubation with 20 mM NH4Cl for 2 to 3 min. BCECF-labeled cells were centrifuged in a microfuge, washed, and resuspended in a small volume of sodium-free BSS. The Na+/H+ antipporter activity in acidified cells was assayed in sodium-containing BSS as described above. Parallel studies were also carried out in sodium-free buffer where sodium was replaced with an equimolar concentration of N-methyl-d-glucamine.

The effect of both TPA and PGF2α on Na+/H+ antipporter activity was assessed under acidic conditions with acidified cells and under basal conditions with unacidified cells. For the assessment of Na+/H+ antipporter activity under acidified conditions, BCECF-labeled, acid-loaded cells were transferred to a cuvette with sodium-containing BSS, followed immediately by the addition of TPA (0.1 μM), PGF2α (0.1 or 100 ng/mL), or vehicle (control), and fluorescence was monitored for up to 5 min. Control Na+/H+ antipporter activity was assessed both at the beginning and at the end of each experiment to ensure their reproducibility. Initial pH values (time zero pH) between controls and experimental conditions were comparable within each experiment and did not differ significantly between experiments. Na+/H+ antipporter activ-
ity was calculated during the linear phase 60 s after the addition of respective agents and expressed as ΔpH/minute.

To examine the Na⁺/H⁺ antiporter activity under pH 7.4 conditions, BCECF-loaded cells in sodium-free BSS were transferred to a cuvette with sodium-containing BSS, followed immediately by the addition of TPA, PGF₂α (0.1 ng/mL), or vehicle as described above. Na⁺/H⁺ antiporter activity was determined by the change in pH, over the initial 60 s, and steady-state alterations in pH were assessed by measurements 5 min after additions. Initial pH values were comparable within and between experiments. Intracellular pH calculations were carried out with the computer software program from Hitachi Instruments.

**Statistical Analyses**

All statistical analyses were carried out by the use of a t test or analysis of variance.

**RESULTS**

The effects of PGF₂α and of TPA on [¹⁵N]ammonia and [¹⁵N]amino acid enrichment and production in LLC-PK₁ cells were carried out in both simultaneous and separate experiments. The results were similar in both instances. However, because the combined controls for each condition differ, the results of each maneuver are presented separately.

**Effect of PGF₂α and TPA on [¹⁵N]enrichment**

As shown in Tables 1 and 2, ammonia enrichment was observed with both [⁵-¹⁵N] and [²-¹⁵N]glutamine, whereas amino acid enrichment was only detected with [²-¹⁵N]glutamine. Except for significantly reducing [¹⁵N]ammonia enrichment from [²-¹⁵N]glutamine at pH 6.8, PGF₂α had no effect on the [¹⁵N] enrichment of ammonia, alanine, glutamate, or aspartate. TPA significantly reduced [¹⁵N]ammonia enrichment from [²-¹⁵N]glutamine at both pH 6.8 and pH 7.4 and also enhanced the enrichment of glutamate at both pH levels.

**Effect of PGF₂α on [¹⁵N]Ammonia Production**

LLC-PK₁ cells incubated at pH 7.4 with [⁵-¹⁵N]glutamine produced substantial amounts of [²-¹⁵N]ammonia, which was increased significantly at pH 6.8 (Table 1 and Figure 1A). Cells exposed to PGF₂α markedly reduced [¹⁵N]ammonia formation at pH 6.8 to values comparable to pH 7.4. [¹⁵N]ammonia production at pH 7.4 from [⁵-¹⁵N]glutamine was unaltered by PGF₂α treatment (Figure 1A and Table 1).

The effect of PGF₂α on [¹⁵N]ammonia production from incubation with [²-¹⁵N]glutamine is shown in Table 1 and Figure 1B. Substantially lower amounts of [¹⁵N]ammonia were produced at pH 7.4 in comparison with production from [⁵-¹⁵N]glutamine. Incubation of cells at pH 6.8 significantly stimulated [¹⁵N]ammonia formation from [²-¹⁵N]glutamine. PGF₂α reduced [¹⁵N]ammonia formation at pH 6.8 to values comparable to pH 7.4 but had no significant effect on the basal (pH 7.4) rate of ammonia formation from [²-¹⁵N]glutamine (Figure 1B).

**Effect of Phorbol Ester TPA on [¹⁵N]Ammonia Production**

We further examined the effect of TPA, a direct activator of protein kinase C, on [¹⁵N]ammonia production and compared its effects with those of PGF₂α. The effect of TPA on [¹⁵N]ammonia production from incubations with [⁵-¹⁵N] and [²-¹⁵N]glutamine is shown in Table 2 and Figure 2. Control cultures exhibited qualitatively similar changes in [¹⁵N]ammonia production as in the PGF₂α experiments. Similar to the effect of PGF₂α, exposure of cells to TPA reduced [¹⁵N]ammonia formation at pH 6.8 to values comparable to pH 7.4. from incubations with both [⁵-¹⁵N]glutamine (Figure 2A) and [²-¹⁵N]glutamine (Figure 2B). In contrast to the effect of PGF₂α, TPA also significantly inhibited the basal rates of [¹⁵N]ammonia formation from [⁵-¹⁵N]glutamine (Figure 2A) as well as from [²-¹⁵N]glutamine (Figure 2B).

**Effect of PGF₂α and TPA on [¹⁵N]Amino Acid Production**

[¹⁵N]alanine formation from [²-¹⁵N]glutamine at pH 6.8 was significantly increased in comparison with
value of 7.10 ± 0.10 and an acidified pH value of 6.70. Bicarbonate-free media revealed a resting basal pH.

TPA, and PGF2α assessments of Na+/H+ antiporter activity did not differ significantly (control, 6.70 ± 0.03; TPA, 6.66 ± 0.03; PGF2α (0.1 ng/mL), 6.64 ± 0.05; and PGF2α (100 ng/mL), 6.67 ± 0.04). The addition of TPA (0.1 μM) to acidified cells resulted in a significant activation of Na+/H+ antiporter activity (Figure 3, left panel). The exposure of acidified cells to a low dose of PGF2α also increased Na+/H+ antiporter activity in a fashion similar to TPA (Figure 3, left panel). In contrast, a high dose of PGF2α exhibited significantly less activation of the Na+/H+ antiporter than was produced with a low dose of PGF2α (Figure 3, left panel).

The effects of TPA and a low dose of PGF2α on Na+/H+ antiporter activity and steady-state pH, were also examined under pH 7.4 conditions. Cells exposed to TPA (0.1 μM) exhibited significant increases in Na+/H+ antiporter activity (Figure 3, right panel). By contrast, a low dose of PGF2α (0.1 ng/mL) had no significant effect on the Na+/H+ antiporter activity (Figure 3, right panel). Furthermore, TPA also significantly increased steady-state pH, whereas PGF2α had no effect (steady state pH: control, 7.50 ± 0.06; TPA, 7.62 ± 0.09 [P < 0.025 compared with control]; and PGF2α, 7.51 ± 0.05).

**DISCUSSION**

Previous studies from our laboratory using the isolated perfused rat kidney, renal cortical tubules, and cultured LLC-PK1 cells indicated that PGF2α is the physiologic arachidonate metabolite that inhibits the ammoniagenic response to acute acidosis (1,6). Studies with LLC-PK1 cells demonstrated that PGF2α and also TPA dramatically inhibit the acute low pH (pH 6.8)-induced increase in ammonia formation and suggested that the mechanism involves the protein kinase C-mediated activation of the Na+/H+ antiporter with a resultant increase in pH. (6,7). Subsequent studies using a variant LLC-PK1 cell line, isolated by our laboratory, provided additional evidence that the protein kinase C-mediated activation of the Na+/H+ antiporter accounts for the inhibition of ammoniagenesis produced by both PGF2α and TPA (8). This is consistent with the findings in most mammalian cells, including renal proximal tubular cells, that the activation of protein kinase C leads to the stimulation of 

---

**TABLE 2. Effect of TPA (1 μM) on 15N enrichment and 15N production of ammonia and amino acids under basal (pH 7.4) and acute acidic (pH 6.8) conditions**

<table>
<thead>
<tr>
<th>Ammonia or Amino Acid</th>
<th>15N Enrichment (atom % excess)</th>
<th>15N Production (nmol/h per mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 6.8</td>
<td>pH 7.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>TPA</td>
</tr>
<tr>
<td>NH₃</td>
<td>68 ± 3</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>From (5-15N)Glutamine</td>
<td>27 ± 1</td>
<td>13 ± 1b</td>
</tr>
<tr>
<td>NH₃</td>
<td>47 ± 2</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>Ala.</td>
<td>65 ± 1</td>
<td>80 ± 2³</td>
</tr>
<tr>
<td>Glu.</td>
<td>36 ± 3</td>
<td>37 ± 3</td>
</tr>
</tbody>
</table>

---

²NH₃, ammonia; Ala, alanine; Glu, glutamate; Asp, aspartate.

³P < 0.01 compared with respective pH control values.

⁴P < 0.05 compared with respective pH control values.

---

The amiloride-sensitive Na+/H+ antiporter plays a major role in the regulation of pH in LLC-PK₁ cells (14). BCECF labeling of these cells incubated in a bicarbonate-free media revealed a resting basal pH value of 7.10 ± 0.10 and an acidified pH value of 6.70 ± 0.03. pH recovery from an acid load was almost completely inhibited by amiloride (8). Furthermore, no recovery from the acid load was observed in the absence of sodium (8).

We reported previously that TPA and a low dose of PGF2α (0.1 ng/mL) inhibits low pH-induced increases in ammonia production, whereas a high dose of PGF2α (100 ng/mL) is ineffective (6). Therefore, we first examined the effect of both a low and a high dose of PGF2α on Na+/H+ antiporter activity under acidified conditions. The initial pH of the cells used for control, TPA, and PGF2α assessments of Na+/H+ antiporter activity did not differ significantly (control, 6.70 ± 0.03; TPA, 6.66 ± 0.03; PGF2α (0.1 ng/mL), 6.64 ± 0.05; and PGF2α (100 ng/mL), 6.67 ± 0.04). The addition of TPA (0.1 μM) to acidified cells resulted in a significant activation of Na+/H+ antiporter activity (Figure 3, left panel). The exposure of acidified cells to a low dose of PGF2α also increased Na+/H+ antiporter activity in a fashion similar to TPA (Figure 3, left panel). In contrast, a high dose of PGF2α exhibited significantly less activation of the Na+/H+ antiporter than was produced with a low dose of PGF2α (Figure 3, left panel).

The effects of TPA and a low dose of PGF2α on Na+/H+ antiporter activity and steady-state pH, were also examined under pH 7.4 conditions. Cells exposed to TPA (0.1 μM) exhibited significant increases in Na+/H+ antiporter activity (Figure 3, right panel). By contrast, a low dose of PGF2α (0.1 ng/mL) had no significant effect on the Na+/H+ antiporter activity (Figure 3, right panel). Furthermore, TPA also significantly increased steady-state pH, whereas PGF2α had no effect (steady state pH: control, 7.50 ± 0.06; TPA, 7.62 ± 0.09 [P < 0.025 compared with control]; and PGF2α, 7.51 ± 0.05).

**DISCUSSION**

Previous studies from our laboratory using the isolated perfused rat kidney, renal cortical tubules, and cultured LLC-PK₁ cells indicated that PGF2α is the physiologic arachidonate metabolite that inhibits the ammoniagenic response to acute acidosis (1,6). Studies with LLC-PK₁ cells demonstrated that PGF2α and also TPA dramatically inhibit the acute low pH (pH 6.8)-induced increase in ammonia formation and suggested that the mechanism involves the protein kinase C-mediated activation of the Na+/H+ antiporter with a resultant increase in pH. (6,7). Subsequent studies using a variant LLC-PK₁ cell line, isolated by our laboratory, provided additional evidence that the protein kinase C-mediated activation of the Na+/H+ antiporter accounts for the inhibition of ammoniagenesis produced by both PGF2α and TPA (8). This is consistent with the findings in most mammalian cells, including renal proximal tubular cells, that the activation of protein kinase C leads to the stimulation of
Figure 1. Effect of PGF$_{2\alpha}$ on $^{15}$N ammonia production at pH 7.4 and pH 6.8 from incubations with either (5-$^{15}$N)glutamine (A) or (2-$^{15}$N)glutamine (B). LLC-PK$_1$ cells were incubated for 1 h in Krebs-Henseleit buffer of pH 7.4 or pH 6.8 in the absence or presence of PGF$_{2\alpha}$ (0.1 ng/mL) as described in Methods. Bars are means and error lines are ±SE of five separate determinations. * P < 0.01 and ** P < 0.001 compared with controls at pH 6.8 from (5-$^{15}$N) and (2-$^{15}$N)glutamine, respectively.

Figure 2. Effect of TPA on $^{15}$N ammonia production at pH 7.4 and pH 6.8 from incubations with either (5-$^{15}$N)glutamine (A) or (2-$^{15}$N)glutamine (B). LLC-PK$_1$ cells were exposed to TPA (1 μM) or vehicle in a fashion similar to that described in the legend to Figure 1. Bars are means and error lines are ±SE of six separate determinations. * P < 0.01 and ** P < 0.001 compared with controls at pH 6.8 from (5-$^{15}$N) and (2-$^{15}$N)glutamine, respectively. * P < 0.05 compared with respective controls at pH 7.4.

potent stimulus of intracellular alkalinization and thereby can alter ammonia metabolism at pH 7.4.

In order to determine whether the specific sites of glutamine metabolism inhibited by PGF$_{2\alpha}$ and TPA at pH 6.8 were indeed similar and consistent with an pH$_1$-mediated mechanism and to delineate why these compounds behave differently at pH 7.4, we used 5-$^{15}$N- and 2-$^{15}$N-labeled glutamine and gas chromatography–mass spectrometry methodology to delineate the precise alterations in ammoniagenic pathways and also examined changes in the Na$^+$/H$^+$ antiporter using BCECF to assess pH$_1$.
The results demonstrate that PGF$_2\alpha$ and TPA inhibit the low pH (pH 6.8)-induced increase in ammonia and modified amino acid (alanine and glutamate) formation in a similar fashion. Both PGF$_2\alpha$ and TPA inhibited the low pH-induced increases in ammonia formation by decreasing $^{15}$N ammonia formation from $[2,^{15}$N]glutamine, reflecting flux through GLDH, and also from $[5,^{15}$N]glutamate, which on the basis of our earlier studies reflects flux through PDG (Figure 4). Furthermore, both PGF$_2\alpha$ and TPA reduced the rate of flux through PDG and GLDH to values similar to those observed at pH 7.4 in the absence of these compounds. PGF$_2\alpha$ and TPA also decreased $^{15}$N alanine production at pH 6.8, reflecting a reduced rate of flux through the glutamine aminotransferase reaction (glutaminase II pathway) ((3); Figure 4) and increased media $^{15}$N glutamate accumulation. These observations indicate that PGF$_2\alpha$ and TPA alter glutamine metabolism in response to acute acidosis via similar mechanisms and that they both specifically alter all of those components of glutamine metabolism that are modified by acute acidosis.

Earlier studies from our laboratory demonstrated that the alteration in ammonia production produced by PGF$_2\alpha$ is accompanied by an increase in $\alpha$-keto glutarate concentration—an indirect reflection of a rise in pH—and that it can be inhibited by amiloride, suggesting a role for the Na$^+$/H$^+$ antiporter (7). Recent studies using variant LLC-PK$_1$ cells confirmed a direct linkage between the stimulation of the Na$^+$/H$^+$ antiporter and the inhibition of ammoniagenesis by both PGF$_2\alpha$ and TPA (8). In this study, both PGF$_2\alpha$ and TPA also activated the Na$^+$/H$^+$ antiporter in acidified cells in a similar fashion. These findings all strongly point toward an increase in pH, as the mechanism whereby these compounds inhibit ammonia production in acidified cells. In combination with the finding from the $^{15}$N glutamine studies that both PGF$_2\alpha$ and TPA inhibit the mitochondrial PDG and GLDH pathways and the cytosolic glutaminase II pathway in a fashion consistent with a reversal of acidosis, it appears highly likely that an increase in cytosolic and mitochondrial pH accounts for the alterations in ammoniagenesis produced by these agents.

In previous studies, we found that a high dose of PGF$_2\alpha$ (100 ng/mL) does not inhibit ammonia production in the same fashion as a lower concentration (0.1 ng/mL) (6). This study demonstrates that a high concentration of PGF$_2\alpha$ stimulates the Na$^+$/H$^+$ antiporter significantly less than a low dose, further establishing a direct relationship between Na$^+$/H$^+$ antiporter activity and the inhibition of ammoniagenesis. These findings are also consistent with the view that sufficient intracellular alkalization secondary to the activation of the Na$^+$/H$^+$ antiporter is required in order to inhibit ammoniagenesis.

In contrast to the findings at pH 6.8, PGF$_2\alpha$ and TPA altered glutamine metabolism differently at pH 7.4. TPA inhibited flux through the PDG, GLDH, and glutaminase II pathways. In contrast, PGF$_2\alpha$ had no...
significant effect on [15]N-glutamine metabolism at pH 7.4. These data clearly indicate that the different effect on overall ammonia production between TPA and PGF2alpha, which we described previously, was not due to insensitivity of the methodology but rather to an actual mechanistic difference between these two compounds at pH 7.4. We have shown previously that, under basal pH conditions, TPA activates protein kinase C to a greater extent than does PGF2alpha (7). The data from this study demonstrate that, at pH 7.4, TPA activates the Na+/H+ antiporter and increases steady-state pH, to a greater degree than PGF2alpha. Thus, the differential response in ammoniagenesis elicited at pH 7.4 is not secondary to an undetectable inhibition of flux through GLDH with PGF2alpha, but rather is the result of more vigorous protein kinase C and Na+/H+ antiporter activation by TPA than by PGF2alpha and thereby a greater degree of intracellular alkalinization.

The conclusion that TPA inhibits ammoniagenesis at pH 7.4 by increasing pH, might seem inconsistent with our earlier observation concerning the alterations in ammonia metabolism produced by incubating cells in an alkaline pH (pH 7.6) (3). Incubation at pH 7.6 decreased ammoniagenesis solely by inhibiting flux through GLDH, whereas the addition of TPA to cells incubated at pH 7.4 decreased flux through both PDG and GLDH. One possible explanation for this difference would be that TPA increases pH, to a greater degree than incubations at pH 7.6 and thereby inhibits flux through PDG as well as GLDH, analogous to the observation that a more severe degree of intracellular acidosis increases flux through PDG as well as GLDH (5). This possibility should not be discounted, even though the increase in pH, (0.12 U) produced by TPA in these studies at pH 7.4 measured with BCECF was higher than the increase (0.08 U) found with pH 7.6 previously with 5,5-(2-14C) dimethyloxazolidine-2,4-dione (DMO) method (4). The TPA measurements of pH, were performed in a bicarbonate- and CO2-free incubation medium in which cells exhibit a higher baseline pH, whereas the pH 7.6 measurements of pH, were made in the presence of bicarbonate and CO2, as were the metabolic measurements with both TPA and pH 7.6. In view of the different baseline pH, levels and the possibility that protein kinase C activation might influence pH, via effects on the bicarbonate exchanger as well as the Na+/H+ antiporter, it is not possible to quantitatively compare the pH, changes between these studies with TPA and our prior studies at pH 7.6 (4,18). Thus, the mechanism whereby TPA inhibits ammonia production at pH 7.4 is consistent with a mechanism mediated solely by an increase in pH, however, it is not possible from our data to exclude with certainty a potential role for other mechanisms as well.

In conclusion, these studies with LLC-PK1 cells indicate that both PGF2alpha and TPA inhibit the ammoniagenic response to acute acidosis by inhibiting flux through the mitochondrial PDG and GLDH pathways, which is virtually certainly the result of an increase in pH, secondary to the activation of the Na+/H+ antiporter. TPA, but not PGF2alpha, also inhibits the PDG and GLDH pathways under basal (pH 7.4) conditions, because TPA is a more potent activator of protein kinase C and the Na+/H+ antiporter than is PGF2alpha. At pH 7.4, it appears that PGF2alpha does not produce a sufficient increase in pH, to modify ammonia metabolism.

ACKNOWLEDGMENTS

This work was supported by Research Grants DK-25248 and DK-39348 from the National Institute of Diabetes and Digestive and Kidney Diseases.

REFERENCES