Thyrotropin-releasing hormone activates Na^+/H^+ exchange in rat pituitary cells

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The effects of thyrotropin-releasing hormone (TRH) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) on cytosolic pH (pH_i) were studied on GH_4C_1 pituitary cells loaded with the fluorescent pH indicator bis(carboxyethyl)carboxyfluorescein (BCECF) and the fluorescent Ca^{2+} indicator quin2. TRH, which was minimally effective at around 10^{-9} M, and TPA, 100 nM, produced very small elevations in pH_i of about 0.05 pH units from the normal basal resting pH_i of GH_4C_1 cells of around 7.05. The effects were more marked after acid-loading the cells using $1 \mu\text{g}$ of nigericin/ml. Preincubation with amiloride or replacing the extracellular Na^+ with choline⁺ completely blocked the elevations stimulated by TRH or TPA, consistent with an activation of the Na^+/H^+ antiport mechanism. The effects were completely independent of the cytoplasmic free calcium concentration ($[\text{Ca}^{2+}]_i$). The calcium ionophore ionomycin produced an elevation in $[\text{Ca}^{2+}]_i$ with no concomitant effect on pH_i , and amiloride, although completely inhibiting the pH change stimulated by TRH, failed to affect the initial stimulated $[\text{Ca}^{2+}]_i$ transient. Although the data are consistent with an elevation in pH_i by TRH which is caused by stimulation of a protein kinase C and subsequent activation of the antiporter, the rapidity of the onset of the pH_i response to TRH could not be mimicked by a combination of TPA and ionomycin. These results, together with previous findings which show that secretion can be mimicked by TPA and ionomycin, suggest that TRH-stimulated Na^+/H^+ exchange plays no part in the acute stimulation of secretion, but that TRH increases the pH-sensitivity of the antiport system during increased synthesis of prolactin and growth hormone.

INTRODUCTION

TRH stimulates the secretion and the synthesis of prolactin and growth hormone by the rat pituitary cell strain GH_4C_1 (Tashjian, 1979). Several biochemical consequences of the interaction of TRH with its receptor have been observed; for example, increased Ca^{2+} fluxes across the plasma membrane and release of Ca^{2+} from intracellular stores (Tan & Tashjian, 1981; Gershengorn & Thaw, 1983; Albert & Tashjian, 1984; Snowdowne & Borle, 1984), Ca^{2+} -dependent action potentials (Kido-koro, 1975; Ozawa & Kimura, 1979), increased inositol lipid hydrolysis leading to the enhanced formation of diacylglycerol and inositol trisphosphate (Martin, 1983; MacPhee & Drummond, 1984; Gershengorn, 1985), as well as protein phosphorylation (Drust & Martin, 1982; Sobel & Tashjian, 1983). Previous studies have shown that the activity of protein kinase C, a Ca^{2+} - and phospholipid-dependent enzyme, is stimulated by TRH (Drust & Martin, 1985). Recent studies have reconstituted the complex biphasic stimulation of hormone secretion normally observed with TRH with a combination of the Ca^{2+} ionophore ionomycin and TPA, a phorbol ester known to activate protein kinase C (Albert & Tashjian, 1985).

In several cell types, including HL-60 cells (Besterman & Cuatrecasas, 1984), neutrophils (Grinstein *et al.*, 1985a)

and T-lymphocytes (Grinstein *et al.*, 1985b), cytoplasmic fluorescent pH indicators have been used to show that phorbol esters can cause changes in pH_i . These elevations in pH_i by TPA, which occur over 2–3 min, are thought to be caused by the activation of a Na^+/H^+ antiport mechanism in the plasma membrane (Busa & Nuccitelli, 1984; Aronson, 1985). Phorbol esters have been shown to increase Na^+ uptake and pH_i in cultured cells (Dicker & Rozengurt, 1981; Rosoff & Cantley, 1983) and in T-lymphocytes (Grinstein *et al.*, 1985b) and the effects were inhibited by amiloride and dimethyl-amiloride (Besterman *et al.*, 1985), known inhibitors of Na^+/H^+ exchange. The actions of individual peptide hormones on the Na^+/H^+ exchange system in differentiated target cells has differed in various cells. In Swiss 3T3 cells insulin alone caused an increase in pH_i (Vara & Rozengurt, 1985) while in chick muscle insulin had no effect (Vigne *et al.*, 1985). Glucagon did not alter pH_i in hepatocytes (Deutsch *et al.*, 1985).

It is clear that GH_4C_1 cells contain protein kinase C and that several of the physiological actions of TRH may be caused by the activation of this enzyme together with the elevation in the concentration of $[\text{Ca}^{2+}]_i$ (Ronning & Martin, 1985; Albert & Tashjian, 1985). In the present study we report the effects of TRH on pH_i and the dependency of those effects on $[\text{Ca}^{2+}]_i$. We show that TRH can stimulate a rapid increase in pH_i in partially

Abbreviations used: BCECF, bis(carboxyethyl)carboxyfluorescein; BCECF-AM, acetoxymethyl ester of BCECF; quin2-AM, acetoxymethyl ester of quin2; $[\text{Ca}^{2+}]_i$, cytosolic free calcium concentration; Ca_o^{2+} , extracellular Ca^{2+} ; Na_o^{+} , extracellular Na^+ ; pH_i , cytosolic pH; TPA, 12-*O*-tetradecanoylphorbol 13-acetate (phorbol ester); TRH, thyrotropin-releasing hormone; TRH-OH, TRH lacking the C-terminal amide (TRH free acid).

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acidified cells, back to the normal resting pH_i , that this appears to be caused by activation of a Na^+/H^+ exchanger and that it is independent of a rise in $[\text{Ca}^{2+}]_i$. The role of stimulated Na^+/H^+ exchange in pituitary cell activation is discussed.

MATERIALS AND METHODS

Materials

TPA was purchased from LC Services (Woburn, MA, U.S.A.). BCECF-AM was from Molecular Probes Inc. Quin2-AM and ionomycin were from Calbiochem. Nigericin was from Sigma. The sources of all other materials were as described previously (Albert & Tashjian, 1984; Hallam & Rink, 1985).

Cell culture

The methods of cell culture have been described in detail previously (Albert & Tashjian, 1984). In brief, GH_4C_1 cells were grown in monolayer culture for 6–10 days, fed freshly about 24 h prior to harvest, collected with 0.02% EDTA in medium containing 118 mM-NaCl, 4.6 mM-KCl, 10 mM-D-glucose and 20 mM Hepes, pH 7.2, washed in the same medium but containing 1 mM- CaCl_2 and used within 1 h.

Measurement of pH_i

pH_i was determined fluorimetrically using BCECF (Rink *et al.*, 1982; Paradiso *et al.*, 1984). Cells (2×10^7) in Ca^{2+} -containing medium (2 ml) were incubated with 5 μM -BCECF-AM for 45 min at 37 °C. At the end of this incubation the cells were washed twice in assay medium and then resuspended in the same medium for measurement. pH_i was manipulated by addition of nigericin, 1 $\mu\text{g}/\text{ml}$ final concn., from a 1 mg/ml stock in dimethyl sulphoxide. Fluorescence measurements were made in a Perkin-Elmer MPF 44A spectrofluorimeter modified to allow continuous stirring of the cells in a round cuvette which was held at 37 °C. The fluorescence signals from the cells, containing approx. 10 μM -BCECF, were monitored at an excitation wavelength of 500 nm with a 5 nm slit width and emission of 530 nm, slit width 8 nm. To calibrate the signals at the end of each experiment, the cells were lysed with 100 μM -digitonin to release the dye into the medium and then the signal was recorded at known pH values. This procedure is not entirely accurate because the dye behaves slightly differently in the cells than it does in the extracellular medium. The excitation spectrum was shifted by approximately 5 nm toward the red causing the signals to appear more acid. The calibration scales in all the Figures have been corrected by 0.12 of a pH unit to allow for this. This correction factor was obtained by imposing known pH values inside the cells by altering the extracellular pH in medium containing 130 mM-KCl and in the presence of the H^+/K^+ antiporter, nigericin, at 50 $\mu\text{g}/\text{ml}$. As $[\text{H}^+]_i/[\text{H}^+]_o = [\text{K}^+]_i/[\text{K}^+]_o$ a calibration curve could be constructed for intact cells. Digitonin was then added to lyse the cells and another calibration curve constructed. The difference in the two calibration curves gave the correction factor (Rink *et al.*, 1982).

Measurement of $[\text{Ca}^{2+}]_i$

Where $[\text{Ca}^{2+}]_i$ was to be determined, the cells were loaded with both 5 μM -BCECF-AM and 40 μM -quin2-

AM for 45 min at 37 °C and the cells prepared as described above. Quin2 fluorescence was measured at excitation 340 nm and emission 500 nm with 4 and 8 nm slit widths, respectively. Calibration was essentially as described previously using digitonin and Mn^{2+} to give F_{max} . and F_{Mn} . F_{min} . was then calculated (Hallam *et al.*, 1984) as:

$$F_{\text{min.}} = F_{\text{Mn}} + (F_{\text{max.}} - F_{\text{Mn}})/6$$

Leakage rates of dyes

Quin2 and BCECF were found to be lost from the cells to the extracellular medium at a rate of approx. 10%/h and this rate was unaffected by stimulation of the cells. The calibration scales were adjusted accordingly.

Presentation of results

All traces are from single experiments but are typical of at least four experiments.

RESULTS AND DISCUSSION

Effect of TRH and TPA on pH_i at resting pH

The resting value of pH_i in the cytoplasm of GH_4C_1 cells loaded with BCECF in the presence of normal- Na^+ medium with 1 mM- Ca^{2+} and pH of 7.2 extracellularly was calculated to be 7.04 ± 0.01 (mean $\pm$ S.E.M., $n = 6$). Fig. 1 shows the results of adding a maximal concentration of either TRH, 1.5 μM , or TPA, 100 nM, to cells under these conditions. The data show small increases in pH_i of approx. 0.05 units.

Effect of TRH and TPA on pH_i in acidified cells

In many cells the compensating mechanism for restoring pH_i from acid, caused by production of lactic acid and HCO_3^- , back to neutral is to increase the activity of the Na^+/H^+ antiporter. To determine whether this mechanism may be activated on stimulation of pituitary cells it was necessary to first increase the size of the stimulated shift in pH_i by introducing an acid load into the cytoplasm. This can be achieved in various ways, the most simple of which are either by adding extracellular bicarbonate, or by adding the ionophore nigericin. In our hands bicarbonate produced a transient decrease in pH_i , returning to the resting level within 2–3 min, and was thus not simple to use as a method of introducing a maintained acidic pH_i from which to study

Fig. 1. Effect of TRH (1.5 μM) and TPA (100 nM) on pH_i in GH_4C_1 cells

Cells were prepared and loaded with BCECF as described in the Materials and methods section. Cells suspended in physiological saline containing 1 mM- Ca^{2+} were stirred continuously in the fluorimeter at 37 °C. The time-scale is shown by the 2 min time bar. The pH_i scale has been corrected for spectral shift as described in the Materials and methods section.

the effects of TRH and TPA. Nigericin, however, proved a far better controlled way of decreasing pH_i to around 6.6. Fig. 2 shows the effect on pH_i of adding nigericin ($1 \mu\text{g}/\text{ml}$) in the presence of physiological concentrations of extracellular Na^+ (145 mM). The pH_i decreased to about pH 6.75 after 30–60 s and remained at about this level for several minutes (Fig. 2e). This decreased pH presumably represents the steady-state elevated concentration of H^+ ions as a consequence of the K^+/H^+ exchange brought about by nigericin and the cell's compensatory basal Na^+/H^+ exchange. Larger doses of nigericin produced a more substantial drop in pH_i to below pH 6.6, whereupon pH_i rapidly returned to the resting level, presumably because the Na^+/H^+ antiporter had been fully activated by the large decrease in pH_i . Fig. 2(a) shows the effect of adding TRH, $1.5 \mu\text{M}$, after a steady-state acidification was attained with nigericin ($1 \mu\text{g}/\text{ml}$). Here a rapid alkalinization ensued immediately after adding TRH with no measurable delay. The signal reached a plateau at a new steady-state level and the acute response is over after 45–60 s. Lower concentrations of TRH, 15 and 1.5 nM , caused a response which was equally as immediate in onset and occurred over the same time-frame (about 1 min) but the magnitude of these responses was smaller (Figs. 2b and 2c). This dose-dependency for TRH-induced alkalinization correlates closely with the previously reported dose-dependency for $[\text{Ca}^{2+}]_i$ transients in the same cells. The data in Fig. 2(d) show no effect by the biologically inactive analogue of TRH, TRH-OH, which lacks the C-terminal amide group.

It is known that TRH can stimulate the translocation to the plasma membrane of protein kinase C while

TRH-OH cannot, and that phorbol ester, TPA, in many other cell types can stimulate the activation of Na^+/H^+ exchange, presumably via activation of C-kinase, suggesting that TRH may be changing pH_i by stimulating protein kinase C. Fig. 3(a) shows the effect of an optimal concentration of TPA, 100 nM , on the pH_i . The active phorbol ester, TPA, but not the inactive unesterified 4β -phorbol nucleus, which is ineffective at stimulating protein kinase C, produced after a delay of some 20–30 s a marked cytoplasmic alkalinization. The result confirms that direct stimulators of C-kinase can cause an increase in pH_i in GH_4C_1 cells and are consistent with the proposal that TRH may also be acting through stimulated production of diacylglycerol and activation of C-kinase to cause its effect. However, without a specific inhibitor of C-kinase this is not proven.

Effect of amiloride or Na^+ replacement on stimulated elevation of pH_i

To determine whether the observed increases in pH_i following stimulation with TRH or TPA could be attributed to an increased Na^+/H^+ antiport activity, two approaches were taken. The first was to use an inhibitor of the Na^+/H^+ exchanger. Amiloride, $100 \mu\text{M}$, was added to the cell suspension 2 min before nigericin. As expected, amiloride had little effect on the abrupt decrease in pH_i on addition of nigericin, which bypasses the antiport system (Fig. 4). However, the subsequent stimulated elevation in pH_i which normally occurred on addition of TRH or TPA was completely inhibited by pretreatment with amiloride. A problem concerning the

Fig. 2. Effect of TRH and TRH-OH on pH_i in GH_4C_1 cells acid-loaded with nigericin

TRH and TRH-OH, at the concentrations indicated, and nigericin (Nig.; $1 \mu\text{g}/\text{ml}$) were added where indicated to cells loaded with BCECF and suspended in normal physiological saline.

Fig. 3. Effect of phorbol ester, 100 nM -TPA, and the phorbol nucleus, 100 nM - 4β -phorbol, on pH_i in GH_4C_1 cells loaded with BCECF and acid-loaded by pretreatment with nigericin (Nig.; $1 \mu\text{g}/\text{ml}$)

Fig. 4. Effect of amiloride (100 μM) on the increase in pH_i stimulated by (a) 1.5 μM -TRH or (b) 100 nM-TPA in GH_4C_1 cells acid-loaded with nigericin (Nig.; 1 $\mu g/ml$) in normal physiological saline containing 1 mM- Ca^{2+} .

use of amiloride recently highlighted by Besterman *et al.* (1985) is that it has some inhibitory action on protein kinase C activity. However, at the dose used here, 100 μM , only at most 20% inhibition of protein kinase C is expected yet the increase in pH_i was blocked completely. The second approach was to remove extracellular Na^+ and replace it with another monovalent cation such as choline $^+$ and show that TRH and TPA are not capable of stimulating a subsequent elevation in pH_i . After replacing extracellular Na^+ with choline $^+$ the pH_i decreased to below pH 6.6. This suggests that, under resting physiological conditions, i.e. $[Na^+]_o = 145$ mM, there is a low constitutive Na^+/H^+ antiport activity and that when Na^+ is removed from the medium the extrusion of H^+ ions can no longer occur and the cells

become acidic. Under these conditions, TRH (Fig. 5a) or TPA (results not shown) were unable to stimulate an increase in pH_i . This result is unlikely to be caused by decreased hormone binding; binding of TRH to membranes is not affected by Na^+ concentration (Hinkle & Tashjian, 1973). Addition of 40 mM- Na^+ back to the medium caused an immediate increase in the pH_i which returned to the normal resting level of about pH 7.0 within 2–3 min and was similar in extent to that obtained on adding 40 mM- Na^+ without prior addition to TRH (Fig. 5b). Preincubation with nigericin in these conditions caused a yet further decrease in pH_i to approx. 6.3 but had no qualitative effect on the requirement of TRH for extracellular Na^+ to return the pH_i to resting. This result suggests that a large decrease in pH_i activates Na^+/H^+

Fig. 5. Effect of Na^+ -free medium on the pH_i response of GH_4C_1 cells to TRH

Cells were loaded with BCECF in Na^+ -containing medium as described in the Materials and methods section. After loading, the cells were washed twice and finally resuspended in medium in which choline $^+$ was substituted for Na^+ and the experiment was performed in the presence of 1 mM-extracellular Ca^{2+} . TRH (1.5 μM) and NaCl (40 mM final concn. in the cuvette) were added where indicated.

exchange to restore pH_i towards 7.0 and is supported by the observation that large doses of nigericin in normal- Na^+ medium, which reduce pH_i to below 6.6, do not produce a maintained low pH_i but appear to stimulate a compensatory mechanism which elevates pH_i (see above).

Are stimulated elevations of $[\text{Ca}^{2+}]_i$ and pH_i independent?

It has been suggested in other systems that stimulation of Na^+/H^+ exchange by growth factors is caused by prior elevation of the concentration of $[\text{Ca}^{2+}]_i$ (Owen & Villereal, 1982). It is known that TRH elicits a very brisk increase in $[\text{Ca}^{2+}]_i$ in GH_4C_1 cells. The possible dependence on $[\text{Ca}^{2+}]_i$ of the stimulated increase in pH_i was studied using batches of GH_4C_1 cells loaded with both BCECF and the fluorescent Ca^{2+} indicator quin2. Fig. 6(a) shows the effect of TRH on both pH_i and $[\text{Ca}^{2+}]_i$ following prior addition of nigericin. Acid-loading caused no detectable change in the quin2 fluorescence, suggesting that the resting level of $[\text{Ca}^{2+}]_i$ is little affected by pH_i . However, on addition of TRH ($1.5 \mu\text{M}$) a transient increase in $[\text{Ca}^{2+}]_i$ and a simultaneous increase

in pH_i was observed. To determine whether the rise in $[\text{Ca}^{2+}]_i$ was caused by activation of Na^+/H^+ exchange, for example by possibly increasing $\text{Na}^+/\text{Ca}^{2+}$ exchange, the effect of amiloride was studied. Fig. 6(b) shows that $100 \mu\text{M}$ -amiloride completely blocked any change in pH_i stimulated by TRH but was without effect on the initial rapid rise in $[\text{Ca}^{2+}]_i$. This finding indicates that stimulated Na^+/H^+ exchange does not cause the initial elevation in $[\text{Ca}^{2+}]_i$. {The initial rapid rise in $[\text{Ca}^{2+}]_i$ has previously been attributed to the discharge of Ca^{2+} from an intracellular store (Gershengorn & Thaw, 1983; Albert & Tashjian, 1984; Snowdowne & Borle, 1984) and therefore blocking $\text{Na}^+/\text{Ca}^{2+}$ exchange would not be expected to have any effect. However amiloride did appear to have an effect on $[\text{Ca}^{2+}]_i$ after the initial stimulated transient elevation in $[\text{Ca}^{2+}]_i$. It is not clear whether this effect is attributable to a direct effect of amiloride on $\text{Na}^+/\text{Ca}^{2+}$ exchange or whether it is as a consequence of inhibition of TRH-stimulated Na^+/H^+ exchange. The role of $\text{Na}^+/\text{Ca}^{2+}$ exchange in stimulated $[\text{Ca}^{2+}]_i$ responses to TRH is the subject of further study.}

To examine whether the converse might be true, i.e. that elevated $[\text{Ca}^{2+}]_i$ causes Na^+/H^+ exchange, the calcium ionophore, ionomycin, was added after acid-loading with nigericin (Fig. 6c). Although $[\text{Ca}^{2+}]_i$ was elevated rapidly to the levels normally evoked by TRH there was no detectable concomitant increase in pH_i . Furthermore, TRH caused an increase in pH_i after ionomycin treatment (Fig. 6c). The results show that the changes in pH_i and $[\text{Ca}^{2+}]_i$ are independent of each other and are consistent with the concept that TRH promotes Na^+/H^+ exchange via stimulation of inositol lipid metabolism leading to the production of diacylglycerol and subsequent activation of protein kinase C.

Do elevated $[\text{Ca}^{2+}]_i$ and C-kinase interact synergistically to stimulate Na^+/H^+ exchange?

The increase in pH_i stimulated by TPA is consistently and appreciably much slower in onset than that stimulated by TRH under identical conditions (cf. Figs. 2, 3 and 4). In many systems it is clear that $[\text{Ca}^{2+}]_i$ and activators of C-kinase act synergistically to produce a very rapid response (Rink *et al.*, 1983; Di Virgilio *et al.*, 1984; Nishizuka, 1984). In the platelet TPA-evoked secretion occurs slowly and only after a 30–45 s delay (Rink *et al.*, 1983). Pre-elevation of $[\text{Ca}^{2+}]_i$ from the basal level of about 100 nM to 600 nM with ionomycin, which by itself does not induce secretion, markedly quickens the onset of a subsequent TPA-evoked secretion (Rink *et al.*, 1983). To test whether the rapid pH_i response to TRH may be caused by some synergistic interaction of elevated $[\text{Ca}^{2+}]_i$ acting together with increased levels of diacylglycerol in the GH_4C_1 cells, ionomycin, which fails to cause any change in pH_i , was added together with TPA to attempt to promote a more rapid elevation of pH_i . Fig. 6(d) shows the result of such an experiment. Nigericin ($1 \mu\text{g}/\text{ml}$) was added to a suspension of GH_4C_1 cells in normal- Na^+ medium in the presence of $1 \text{ mM}-\text{Ca}_0^{2+}$ and an acid-load was produced. After 2–3 min, when the pH_i had reached a plateau at approx. 6.7, ionomycin and TPA were added simultaneously. Although the expected sustained rise in $[\text{Ca}^{2+}]_i$ was observed, a parallel experiment showed that the kinetics of the increase in pH_i were not detectably different from the effect of TPA alone. These results show that the rapid action of TRH on pH_i was not mimicked by simultaneous elevation of

Fig. 6. Elevations in $[\text{Ca}^{2+}]_i$ and pH_i by TRH in acid-loaded GH_4C_1 cells containing both BCECF and quin2 are independent

Cells contained approx. $10 \mu\text{M}$ -BCECF and 1 mM -quin2. The cells were stirred throughout at 37°C in normal physiological saline containing $1 \text{ mM}-\text{CaCl}_2$. Nigericin (Nig.; $1 \mu\text{g}/\text{ml}$), TRH ($1.5 \mu\text{M}$), amiloride (Amil.; $100 \mu\text{M}$), ionomycin (Iono.; 200 nM) and TPA (100 nM) were added as indicated. Each experiment shows two parallel experiments from the same batch of cells monitoring BCECF fluorescence in the upper trace and quin2 fluorescence in the lower trace. In (d) ionomycin and TPA were added simultaneously.

$[Ca^{2+}]_i$ and activation of C-kinase by TPA. Either activation of protein kinase C by TPA is slower than activation by TRH or some other, as yet unidentified, component of the stimulus-transduction mechanism accounts for the rapidity of the onset of Na^+/H^+ exchange induced by TRH.

Conclusion

We have shown that TRH and TPA can cause alkalization of the cytosol in acid-loaded GH_4C_1 cells and that the response is faster and more rapid in onset to TRH; the response to TPA occurs after a lag of 30–45 s. The finding that (a) amiloride or (b) replacement of extracellular Na^+ with choline $^+$ completely blocked the elevation in pH_i strongly suggests that the activity of a Na^+/H^+ antiporter is stimulated by TRH and TPA. The rapidly enhanced exchange of Na^+ for H^+ across the plasma membrane appears to be independent of the equally rapid increase in $[Ca^{2+}]_i$ on stimulation with TRH because: (a) inhibition of Na^+/H^+ exchanger with amiloride failed to affect the stimulated elevation of $[Ca^{2+}]_i$ and (b) selective elevation of $[Ca^{2+}]_i$ with ionomycin to levels similar to those evoked by TRH failed to elicit any detectable increase in pH_i .

The very small elevation in pH_i with TRH observed on stimulation with a resting basal pH_i of around pH 7.0 makes it unlikely that activation of Na^+/H^+ exchange is required for the acute TRH-induced secretion of prolactin and growth hormone in non-acidified cells. This conclusion is supported by the findings that the combination of TPA plus ionomycin can mimic the burst phase of secretion typical of stimulation with TRH (Albert & Tashjian, 1985) but they do not induce an acute change in pH_i (Fig. 6d). Elevated intracellular Na^+ or elevated pH_i have been suggested to provide the signal for proliferation (Dicker & Rozengurt, 1981) and differentiation (Rosoff & Cantley, 1983) in other cell systems. It is more likely that the activation of Na^+/H^+ exchange in GH_4C_1 cells acts to counter the acidification which will occur as a result of the increased metabolic activity of the cell as long-term stimulated synthesis of prolactin and growth hormone is induced by TRH.

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