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CFTR-mediated anion secretion in parathyroid hormone-treated Caco-2 cells is associated with PKA and PI3K phosphorylation but not intracellular pH changes or Na^+/K^+-ATPase abundance

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ABSTRACT

Parathyroid hormone (PTH) has previously been shown to enhance the transepithelial secretion of Cl^- and HCO_3^- across the intestinal epithelia including Caco-2 monolayer, but the underlying cellular mechanisms are not completely understood. Herein, we identified the major signaling pathways that possibly mediated the PTH action to its known target anion channel, i.e., cystic fibrosis transmembrane conductance regulator anion channel (CFTR). Specifically, PTH was able to induce phosphorylation of protein kinase A and phosphoinositide 3-kinase. Since the apical HCO_3^- efflux through CFTR often required the intracellular H^+/HCO_3^- production and/or the Na^+-dependent basolateral HCO_3^- uptake, the intracellular pH (pH_i) balance might be disturbed, especially as a consequence of increased endogenous H^+ and HCO_3^- production. However, measurement of pH_i by a pH-sensitive dye suggested that the PTH-exposed Caco-2 cells were able to maintain normal pH despite robust HCO_3^- transport. In addition, although the plasma membrane Na^+/K^+-ATPase (NKA) is normally essential for basolateral HCO_3^- uptake and other transporters (e.g., NHE1), PTH did not induce insertion of new NKA molecules into the basolateral membrane as determined by membrane protein biotinylation technique. Thus, together with our previous data, we concluded that the PTH action on Caco-2 cells is dependent on PKA and PI3K with no detectable change in pH_i or NKA abundance on cell membrane.

1. Introduction

Parathyroid hormone (PTH)—a peptide secreted from the parathyroid gland—not only acts as the major regulator of calcium and phosphorus homeostasis [1], but is also crucial for the anion secretion across the intestinal epithelium and epithelial-like Caco-2 monolayer [2–4]. This PTH action is probably essential for fine-tuning of luminal calcium and phosphate solubility, thereby altering their absorption rates (for review, please see Ref. [5]). In kidney, PTH also participates in Cl^- transport, intracellular pH (pH_i) regulation and acid-base balance [6].

The cystic fibrosis transmembrane conductance regulator anion channel (CFTR) has been shown to mediate Cl^- and HCO_3^- efflux after the enterocytes were exposed to PTH [4]. The transporting process is partially energized by sodium gradient across the basolateral membrane, which is created by Na^+/K^+-ATPase (NKA), as in Fig. 1A. This sodium gradient is postulated to induce the basolateral HCO_3^- uptake via Na^+/HCO_3^- co-transporter (NBCe1) and H^+ efflux through Na^+/H^+ exchanger (NHE)-1 during carbonic anhydrase-mediated HCO_3^- production [7]. Inadequate H^+ handling either from intracellular or extracellular sources may disrupt epithelial integrity and transporting function [8]. However, the underlying cellular mechanisms including signaling pathways, relevant ion and pH handling are not completely understood.

Although the CFTR-mediated anion secretion is often dependent on protein kinase A (PKA), phosphoinositide 3-kinase (PI3K) or an increase

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in intracellular calcium ($[Ca]_i$) [9–11], it is unclear whether PTH directly alters PKA/PI3K phosphorylation or $[Ca]_i$ all of which are crucial for CFTR activity. Meanwhile, NHE1—known to be abundantly expressed in the basolateral membrane of enterocytes—is postulated to help maintaining normal pH_i during anion secretion [12–14]. Nevertheless, whether prolonged PTH-induced HCO_3^- efflux eventually disturbs pH_i balance and hence ends the entire transport process remains unknown. Finally, in addition to the enhanced function of NKA that energizes a number of crucial basolateral ion transporters (e.g., NBCe1 and NHE1 in Fig. 1A) [15], the enterocytes may also increase insertion of new NKA molecules into the plasma membrane.

Therefore, in the present study, we performed a series of experiments to demonstrate whether phosphorylation of PKA, PI3K and/or $[Ca]_i$ contributed to the PTH action, and whether prolonged PTH exposure disturbed pH_i balance or induced NKA insertion into the basolateral membrane of Caco-2 cells.

2. Materials and methods

2.1. Cell culture

The Caco-2 cells [HTB-37; American Type Culture Collection (ATCC), VA, USA] and T84 cells (CCL-248; ATCC) were maintained in 25 cm^2 T-flask (Corning, NY, USA) containing Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO, USA) supplemented with 15% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY, USA), 1%

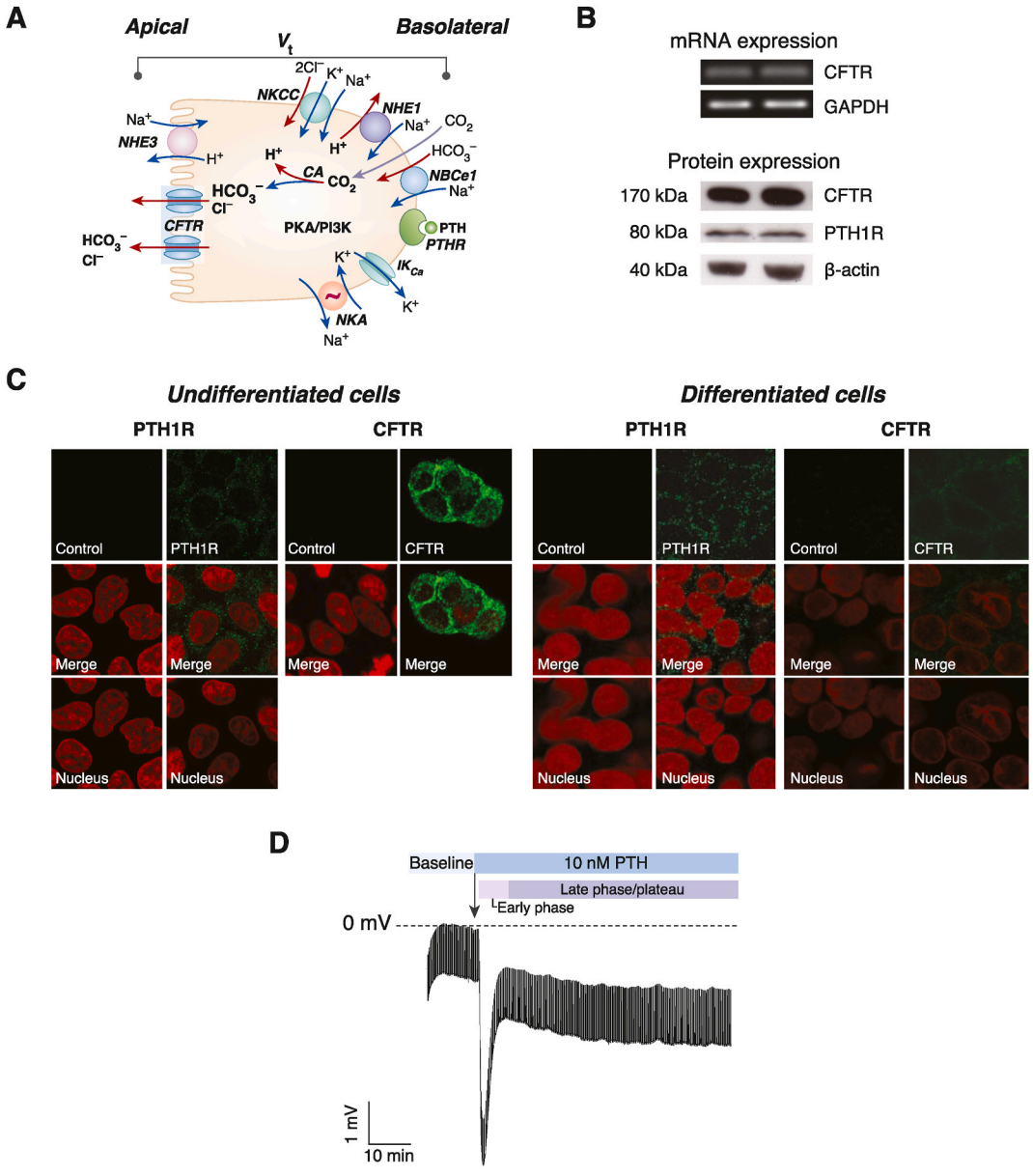

Fig. 1. (A) A model of the PTH-induced transport of anions (Cl^- and HCO_3^-) across intestinal epithelial-like Caco-2 cells. HCO_3^- is transported from extracellular fluid via NBCe1 or produced intracellularly by CA, which also liberates H^+. Without proper H^+ handling by NHE, the intracellular pH might change. Several transporters, e.g., NBCe1, NHE and NKCC, are dependent on the activity of NKA. CA, carbonic anhydrase; CFTR, cystic fibrosis transmembrane conductance regulator anion channel; IK_{Ca}, intermediate-conductance Ca^{2+}-activated K^+ channel; NBCe, Na^+/HCO_3^- cotransporter; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; NHE, Na^+/H^+-exchanger; NKA, Na^+/K^+-ATPase; NKCC, $Na^+/K^+/2Cl^-$ cotransporter; PTH1R, parathyroid hormone 1 receptor; V_t, transepithelial voltage. (B) Representatives of CFTR mRNA expression and protein expression of CFTR and PTH1R in Caco-2 cells. (C) Expression and localization of CFTR and PTH1R in undifferentiated cells (2-day culture) and differentiated cells (14-day culture) as detected by CLSM (using objective lens of 20× and digital zoom to make final magnification of 252×). (D) Tracing of electrogenic anion transport across Caco-2 monolayer after exposure to 10 nM PTH, as indicated by V_t changes.

nonessential amino acid (Sigma), 1% L-glutamine (GIBCO), and 100 U/mL penicillin-streptomycin (GIBCO) [16]. Cells were maintained at 37 °C under humidified air with 5% CO_2.

2.2. Immunocytochemistry

Caco-2 or T84 cells were grown on coverslips in 6-well plates for 7 days. Culture medium was removed from the wells, and cells on coverslips were washed with phosphate-buffered saline (PBS) and then fixed with iced-cold 100% methanol for 5 min. Cells were permeabilized with 0.1% Triton X-100 in PBS followed by 5-min washing with PBS. Non-specific bindings were blocked by 4% bovine serum albumin (BSA; Sigma) and 0.1% Tween-20 in PBS for 2 h. Cells were incubated with primary antibodies, i.e., 1:20 rabbit polyclonal *anti*-CFTR (catalog no. Sc-10747; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or 1:20 rabbit polyclonal *anti*-PTH1R (catalog no. Sc-20749; Santa Cruz Biotechnology) overnight at 4 °C. Thereafter, cells were washed with PBS and incubated with secondary antibody, i.e., goat anti-rabbit IgG conjugated with biotin (catalog no. Sc-2040; Santa Cruz Biotechnology), for 1 h at room temperature, followed by 1 h incubation with streptavidin-horseradish peroxidase (catalog no. SA10001; Invitrogen, CA, USA). Finally, cells were incubated with 3,3′-diaminobenzidine chromogen (Pierce, Rockford, IL, USA) and were visualized by a light microscope.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

Caco-2 cells were grown in 6-well plates for 14 days. Total RNA was extracted by using TRIzol reagent (Invitrogen) and converted to cDNA by iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) by a thermal cycler (model MyCycler; Bio-Rad). Forward and reverse primers used in the present study were: CFTR, 5′-ACCTGCACAGCCTCATCTTCA-3′ and 5′-CACACAGCCACGAAGACAGC-3′; PTH1R, 5′-CACTCCTCATGGGGCTAATCT-3′ and 5′-CTGATCTTCCCAGCTCTCTGA-3′; and GAPDH, 5′-CTGGTAAAGTGGATATTGTTG-3′ and 5′-GAGGCTGTTGTCATACTTCTC-3′ [4]. GoTaq Master Mix (Promega, Madison, WI, USA) was used to perform RT-PCR. PCR products were visualized on 1.5% agarose gel stained with 1 mg/mL ethidium bromide under a UV transilluminator (Alpha Innotech, San Leandro, USA).

2.4. Western blot analysis

Caco-2 cells were seeded at 5×10^5 cells on polyester Transwells (Corning) with 0.4-μM pore size. Confluent cells were treated with 10 or 100 nM recombinant human PTH 1–34 (catalog no. P3796; Sigma) in the basolateral compartment for 15, 30, 45 or 60 min. Total protein was extracted using radioimmunoprecipitation (RIPA) lysis buffer (catalog no. Sc-24948; Santa Cruz Biotechnology) and 100-μg protein was separated by 8% SDS-PAGE and transferred onto nitrocellulose membrane. The membrane was blocked by 5% BSA in Tris-buffered saline-Tween (TBST) at 25 °C for 1 h and probed overnight at 4 °C with primary antibody (Cell Signaling Technology, MA, USA), i.e., monoclonal *anti*-human CFTR C-terminus (catalog no. 2269), polyclonal *anti*-phospho-PI3K (catalog no. 4228), *anti*-phospho-PKA (catalog no. 4781), *anti*-PKA C-α (catalog no. 4782) or *anti*-PI3K p85 (catalog no. 4292). After being washed, the membrane was incubated with anti-rabbit IgG (HRP-linked; catalog no. 7074; Cell Signaling Technology) for 1 h at 25 °C. Bands were detected using Luminata Crescendo Western HRP substrate (catalog no. WBLUR0500; Merck, Darmstadt, Germany).

2.5. Immunofluorescent imaging by confocal laser-scanning microscopy (CLSM)

Caco-2 cells were seeded on coverslips in 6-wells plates for 24 h, and fixed with an iced-cold ethanol for 30 min. Permeabilization was performed using 0.3% Triton X-100 in TBST for 30 min followed by washing twice. Coverslips were blocked with 3% BSA in TBST. Incubation with primary antibody, i.e., rabbit polyclonal *anti*-CFTR (catalog no. Sc-10747; Santa Cruz Biotechnology) or rabbit polyclonal *anti*-PTH1R (catalog no. ab75150; Abcam, Cambridge, UK), was carried out overnight. After being washed, cells were incubated with anti-rabbit IgG tagged with Alexa Fluor 488 for 1 h at room temperature followed by washing with TBST 3 times. The coverslips were mounted with 85% glycerol. Expression and localization of CFTR and PTH1R were visualized by CLSM (Olympus FV10i).

2.6. Measurement of transepithelial voltage

The electrical parameter, i.e., transepithelial voltage (V_t), was determined by Ussing chamber technique as described previously [3]. It was used as a proxy indicator to represent electrogenic anion flux across the monolayer [4]. A Snapwell (Corning) containing 14-day-old Caco-2 monolayer was placed in Ussing chamber between two pairs of Ag/AgCl electrodes (World Precision Instruments, Sarasota, FL, USA) connected to salt bridges made of 2 M KCl and 2% agar. V_t-sensing electrodes were placed near Snapwell, whereas current-passing electrodes were placed at the end of each hemichamber. All electrodes were connected to a preamplifier/current generating unit (model ECV-4000, World Precision Instruments) and PowerLab/4SP (operated with Chart version 5.4.1; ADInstrument, CO, USA). Brief current pulses (3-μA amplitude, 800-ms pulse duration, 0.1-Hz frequency) were injected by a pulse generator across the monolayer and created a brief voltage deflection (ΔV_t).

2.7. Intracellular calcium measurement

Caco-2 cells were seeded on 8-well chambered coverglass (Nunc Lab-Tek, Thermo Fisher, MA, USA). Cells were washed with Hanks' balanced salt solution containing 10 mM HEPES, 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$, and then loaded with a mixture of 4 μM Fluo-4 acetoxymethyl (Fluo-4 AM; Thermo Fisher) and 20% pluronic acid (Sigma) for 45 min at 37 °C. The solution was discarded, and buffer was added into the chamber for 30 min to ensure complete de-esterification of AM ester. Loaded cells were mounted under a CLSM for live-cell imaging and were sequentially incubated for 3 min each with buffer (baseline), 100 nM PTH, and 5 μM ionomycin (positive control). Images were acquired every 5 s and quantified for fluorescence intensities.

2.8. Membrane protein biotinylation

Caco-2 cells were grown on 6-well plates. Confluent cells were treated with PBS (control) or PTH for 60 min. Then they were brought on ice and washed with PBS containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ (pH 8.2; PBS/$CaCl_2$/$MgCl_2$). According to the manufacturer's instruction, EZ-Link™ Sulfo–NHS–SS biotin (0.5 mg/mL; catalog no. 21331; Thermo Fisher) was added to each well and incubated for 60 min at 4 °C with gentle shaking. Biotinylated cells were incubated in quenching solution (50 mM glycine in PBS/$CaCl_2$/$MgCl_2$) for 5 min. Cells were collected, washed twice with PBS/$CaCl_2$/$MgCl_2$, and then lysed in RIPA buffer. Protein samples were collected by centrifugation at 12,000 rpm for 20 min at 4 °C. Protein (10 mg) was incubated with 400 μL Pierce NeutrAvidin beads (catalog no. 29201; Thermo Fisher) and incubated overnight at 4 °C on rotating shaker. The bead-biotin-membrane protein complexes were then washed with RIPA buffer. The eluted fractions, total and unbound proteins were subjected to Western blot analysis using NKA antibody (catalog no. ab76020; Abcam).

2.9. Intracellular pH measurement

Caco-2 cells were seeded in 96-well plate at 3000 cells/well and cultured at 37 °C with 5% CO_2 overnight, then washed twice with PBS. The pH-sensitive fluorescent dye BCFL-AM (catalog no. ab228552; Abcam) was added into each well, and cells were incubated in the dark

at 37 °C for 30 min. Thereafter, the solutions of various pH were added to verify that BCFL-AM dye was able to detect pH_i changes (Supplementary Fig. S1). In this verification study, fluorescent signals were determined at 490/535 nm (excitation/emission; cut-off wavelength of 515 nm) and 430/535 nm to obtain the fluorescence ratio by using a fluorescent microplate reader (model Spark™ 10 M multimode microplate reader; Tecan, Switzerland).

In the subsequent experiment, Caco-2 cells were grown on 8-well chambered coverglass for 14 days. BCFL-AM was loaded into the cells for 30 min at 37 °C. Then the chamber was mounted on Zeiss LSM 800 confocal microscope (Zeiss, Oberkochen, Germany), which was set at 37 °C under 5% CO_2. Signals were first recorded every 7.43 s for 30 cycles as a baseline, followed by treatment with either HHBS buffer (Hanks' buffer with 20 mM HEPES) or 100 nM PTH, and the signals were recorded for another 30 cycles. The series of treatment, baseline, buffer and then PTH in the same well were carried out to exclude the volume changing effect on fluorescent signals. The signals obtained at 490/535 nm were normalized with those at 430/535 nm.

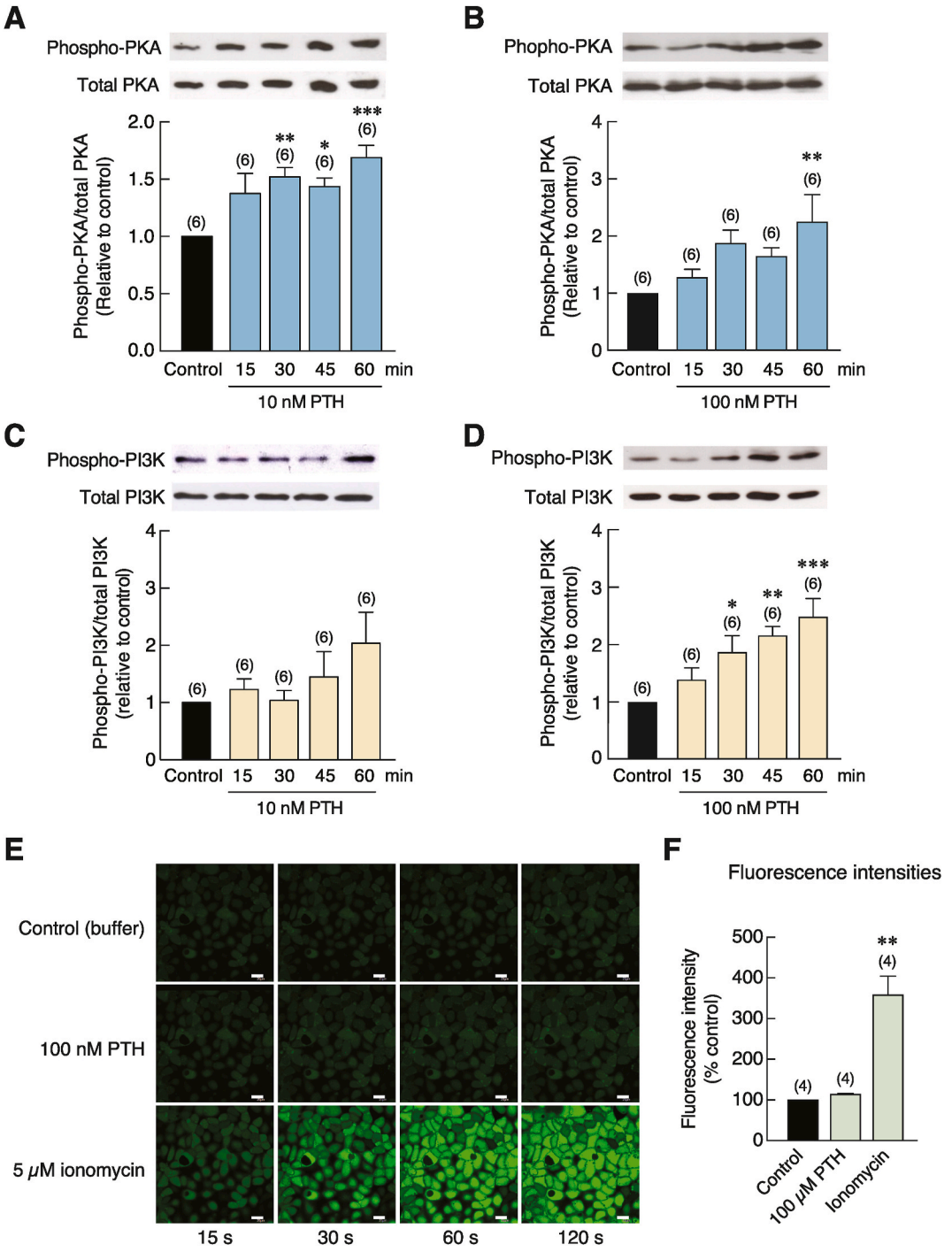

Fig. 2. Expressions of phospho-PKA relative to total PKA in Caco-2 cells treated with 10 and 100 nM PTH (A–B). Expressions of phospho-PI3K relative to total PI3K in control and after being treated with 10 and 100 nM PTH (C–D). (E) Representative images of intracellular calcium changes (fluorescent signals) in Caco-2 cells. Cells were incubated with buffer containing Fluo-4 probe alone (Control), 100 nM PTH or 5 μM ionomycin for 3 min each, and images were acquired at 15, 30, 60 and 120 s. Scale bars; 20 μm. (F) Fluo-4 fluorescence intensities at 120 s after being incubated with buffer (control), 100 nM PTH or 5 μM ionomycin. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Control. Numbers in parentheses represent the number of independent samples.

2.10. Statistical analysis

The results are expressed as means ± SE. Data were compared by student *t*-test (two groups of data) or one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test (multiple groups of data). Statistical significance was considered with $P < 0.05$. All data were analyzed by GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

Prior to the investigation of PTH actions on Caco-2 cells, the expression of its principal receptor PTH1R and the key transporter CFTR was confirmed as shown in Fig. 1B and C. Specifically, we found that PTH1R and CFTR were expressed in differentiated Caco-2 cells (14-day culture) as detected by Western blot analysis and PCR (Fig. 1B). The CFTR antibody was also validated in T84 colonic cells (Supplementary Fig. S2), which are known to abundantly express CFTR [17]. CLSM also confirmed protein expression and localization of both PTH1R and CFTR in proliferative (2-day culture) and differentiated Caco-2 cells (14-day culture) (Fig. 1C). After direct exposure to 10 nM recombinant human PTH 1–34, anion secretion was observed in Caco-2 monolayer (Fig. 1D), similar to that reported previously [2]. The acute response (peak) occurred within 10 min, followed by a steady anion flux (plateau) during prolonged PTH exposure (Fig. 1D).

Further investigation on PTH signaling revealed that 10 nM PTH (low-dose PTH) was able to induce PKA phosphorylation at 30, 45 and 60 min, but not at 15 min of PTH exposure (Fig. 2A). PKA phosphorylation was increased only at 60 min after exposure to 100 nM PTH (high-dose PTH; Fig. 2B). High-dose (100 nM) but not low-dose PTH (10 nM) was required for a significant induction of PI3K phosphorylation (Fig. 2C and D). On the other hand, the live-cell CLSM imaging using Fluo-4 indicator did not reveal any increase in $[Ca]_i$ in 100 nM PTH-treated Caco-2 cells although 5 μM ionomycin—a Ca^{2+}-selective pore-forming molecule used as a positive control—was capable of increasing $[Ca]_i$ (Fig. 2E). The fluorescence intensities at 120 s confirmed that the signals of 100 nM PTH-treated cells were not different from that of the control group ($P = 0.917$; Fig. 2F). Thus, PTH did not alter $[Ca]_i$ to trigger cell response.

To investigate whether PTH enhanced insertion of new NKA molecules into the basolateral membrane to produce a larger sodium gradient for more ion transport, membrane proteins were first labeled with biotin prior to PTH exposure. Our results showed that 100 nM PTH did not alter the expression level of biotinylated NKA (Fig. 3). Furthermore, since prolonged production of HCO_3^- as seen in PTH-treated Caco-2 cells could inadvertently increase pH_i, we used a ratiometric pH probe, i.e., BCFL-AM, to confirm the efficiency of H^+-handling in Caco-2 cells. It was found that 100 nM PTH did not significantly shift the 490/430 nm fluorescence ratio from the control (buffer-treated cells) as determined by a fluorescent microplate reader (Fig. 4A). In another experiment (Fig. 4B; live-cell CLSM), Caco-2 cells were first treated with HHBS (control buffer) followed by 100 nM PTH; however, PTH did not alter the 490/430 nm fluorescence ratio. These results indicated that there was no change in pH_i during anion secretion.

4. Discussion

Besides being a calcium/phosphate-regulating hormone, PTH has been reported to control transepithelial transport of several other ions, e.g., Na^+, Cl^- and HCO_3^-, in the intestine and kidney [3,4,18]. Although PTH is believed to have indirect action on the intestine through renal conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol [19], the expression of PTH1R in the enterocytes strongly suggests that the intestine is a potential direct target of this hormone. Indeed, PTH was previously found to directly stimulate a rapid uptake of calcium across the avian and rodent intestinal epithelia—a phenomenon known as transcaltachia [1,20].

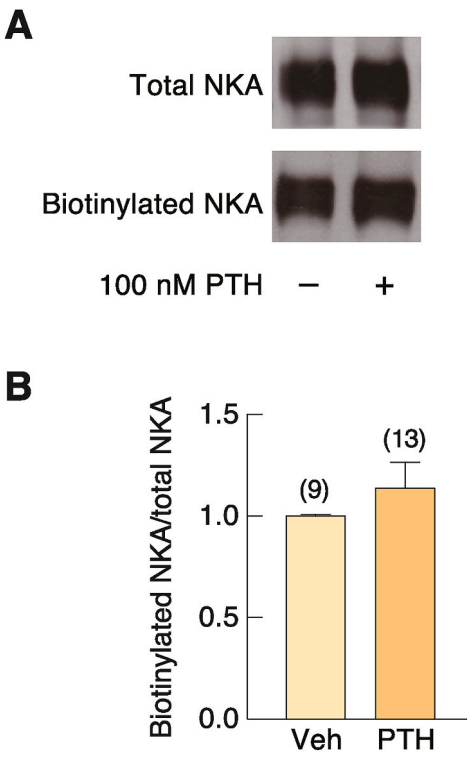

Fig. 3. (A–B) Representatives and quantitative expression of biotinylated (membrane-bound) NKA and total NKA expression in Caco-2 cells treated with 100 nM PTH for 60 min. Numbers in parentheses represent the number of independent samples. Veh, vehicle (buffer).

Regarding the anion transport, we have previously demonstrated that a direct exposure to PTH was capable of enhancing an acute high-rate electrogenic secretion of Cl^- and HCO_3^-, followed by a long-lasting anion efflux but with a slower rate across the Caco-2 monolayer [3]. In our previous work, we have already performed the HCO_3^--free and 5-(*N*-ethyl-*N*-isopropyl)-amiloride (EIPA) studies to confirm contribution of HCO_3^- efflux and NHE1, respectively, in PTH-treated Caco-2 cells [4]. Thus, in the present study, we moved further to demonstrate that Caco-2 cells were able to maintain intracellular pH during prolonged PTH-induced HCO_3^- transport, and the results that show no change in intracellular pH support our hypothesis. Nevertheless, besides NHE1, PTH probably used other transporters or expanded intracellular buffer capacity to regulate intracellular pH. In several ion-transporting epithelia including the intestine and kidney, anion exchangers (e.g., SLC26A3 and SLC26A4) contribute to intracellular pH regulation [21,22] therefore, PTH may modulate some of them to maintain intracellular pH in Caco-2 cells during HCO_3^- efflux.

In rats, intravenous PTH infusion also increased fluid secretion in the ileum [23]. The anion efflux across the enterocytic apical membrane was found to occur via CFTR, and was dependent on the carbonic anhydrase-mediated intracellular HCO_3^- production [4] and NBCe1-induced HCO_3^- uptake from the basolateral fluid compartment [23]. This CFTR-mediated anion transport was partially inhibited by PKA and PI3K inhibitors [4], but the contribution of both PKA and PI3K is still inconclusive due to inhibitors being non-specific.

In the present study, we used PTH1R- and CFTR-positive Caco-2 cells to demonstrate that PTH could directly induce phosphorylation of PKA and PI3K, indicating that both signaling proteins are involved in the intestinal response to PTH. PKA and PI3K have been reported to activate CFTR as well as other epithelial transporters, e.g., NHE1, NHE3, NBCe1 and NKA, to enhance ion transport [4,23–27]. In the renal epithelial cells, PTH1R was found to exert downstream signals via G_s/cAMP/PKA-

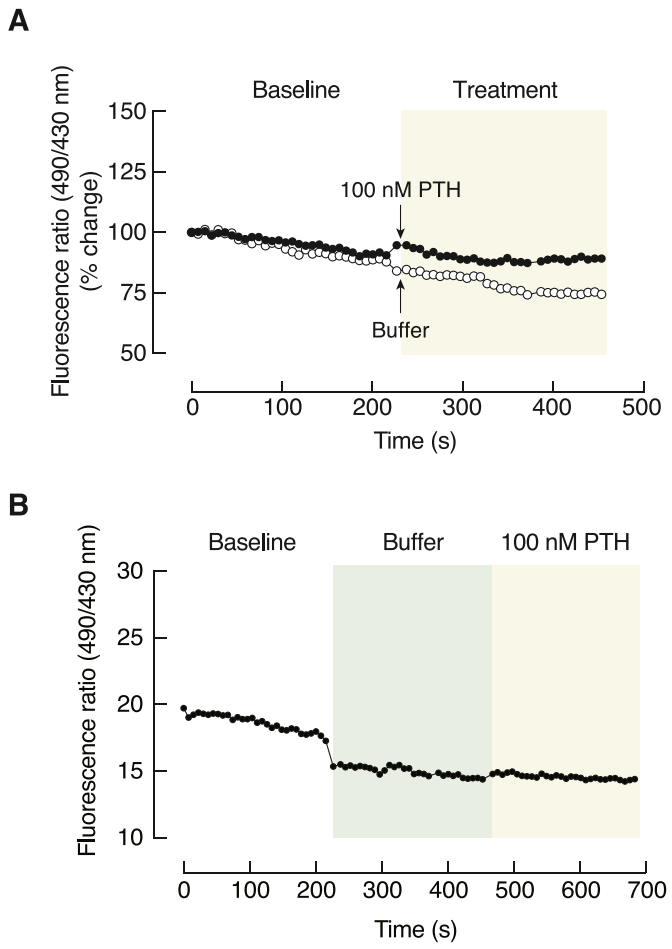

Fig. 4. (A) Normalized 490/430-nm fluorescent signals of Caco-2 cells treated with 100 nM PTH or buffer (control). (B) The series of 490/430-nm fluorescent signals consisting of baseline fluorescent signal, buffer (control) and PTH treatment in the same well. This serial experiment was carried out to exclude the volume-changing effect on the fluorescent signals.

and PI3K-dependent pathways [27,28]. Although PTH1R can be coupled with $G_{q/11}$ [29], PTH did not alter $[Ca]_i$ level in Caco-2 cells as determined by Fluo-4 probe, suggesting that the $G_{q/11}/[Ca]_i$ pathway was not induced in the stimulation of enterocytes by PTH. Similarly, osteoblasts, the well-known target of PTH, can also respond to PTH in $G_{q/11}/[Ca]_i$-independent manner [29].

A robust and prolonged HCO_3^- efflux often requires an efficient pH_i regulation and NKA function. Specifically, during endogenous HCO_3^- production by cytoplasmic carbonic anhydrase, H^+ must be eliminated by NHE1 across the basolateral membrane. At the same time, NKA is required to generate sodium gradient, which in turn is crucial for the optimal activity of NHE1 as well as that of other essential transporters, including NBCe1 [15,30]. As mentioned earlier, we used a pH-sensitive probe and live-cell CLSM technique to demonstrate that there was no significant cytoplasmic pH imbalance during PTH stimulation, thus implying that NHE1 was efficient in maintaining H^+ level. Furthermore, by using membrane protein biotinylation, we were able to show that there was no alteration in the NKA level in the plasma membrane. Therefore, PTH relied on the intact NKA function rather than new NKA insertion into the plasma membrane. However, one may argue that the PTH action is transient and the changes may disappear at the end of 60-min experimental period. Thus, a time-dependent study is required to reveal whether or not the PTH effect on NKA is transient and/or occurs rapidly. It is noteworthy that PTH as well as forskolin—a potent activator of cAMP/PKA pathway—were reported to increase both CFTR activity and the insertion of CFTR-laden vesicles onto the apical membrane in order to augment the apical anion efflux in the intestine [2]. In other words, the PTH-induced anion secretion required the insertion of CFTR, but not NKA into the plasma membrane of Caco-2 cells.

In conclusions, we have provided evidence that recombinant human PTH 1–34 was capable of increasing phosphorylation of PKA and PI3K in PTH1R- and CFTR-expressing intestinal epithelial-like Caco-2 cells. Despite a huge amount of HCO_3^- production and secretion [2–4], PTH did not induce disturbance in cytoplasmic pH or require insertion of new NKA molecules into the basolateral membrane. Although our results can shed some light on the underlying cellular mechanism of PTH actions in the intestinal epithelial-like cells, further investigation is required to confirm such actions in enterocytes from each intestinal segment in vivo.

Author contributions

Conception and design of the experiments: NC and NK; performed the research: RC, WJ and SK; analysis and interpretation of data: RC, JT, WJ, KL, SK, NP, NK and NC; drafting the article or revising it critically for important intellectual content: RC, JT, WJ, KL, SK, NP, NK and NC. All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.101054.

References

[1] K. Wongdee, N. Charoenphandhu, Vitamin D-enhanced duodenal calcium transport, Vitam. Horm. 98 (2015) 407–440.
[2] W. Jantarajit, K. Lertsuwan, J. Teerapornpuntakit, N. Krishnamra, N. Charoenphandhu, CFTR-mediated anion secretion across intestinal epithelium-like Caco-2 monolayer under PTH stimulation is dependent on intermediate conductance K^+ channels, Am. J. Physiol. Cell Physiol. 313 (2017) C118–C129.
[3] W. Jantarajit, K. Wongdee, K. Lertsuwan, J. Teerapornpuntakit, R. Aeimlapa, J. Thongbunchoo, B.S.J. Harvey, D.N. Sheppard, N. Charoenphandhu, Parathyroid hormone increases CFTR expression and function in Caco-2 intestinal epithelial cells, Biochem. Biophys. Res. Commun. 523 (2020) 816–821.
[4] S. Laohapitakworn, J. Thongbunchoo, L.I. Nakkrasae, N. Krishnamra, N. Charoenphandhu, Parathyroid hormone (PTH) rapidly enhances CFTR-mediated HCO_3^- secretion in intestinal epithelium-like Caco-2 monolayer: a novel ion regulatory action of PTH, Am. J. Physiol. Cell Physiol. 301 (2011) C137–C149.
[5] K. Chanpaisaeng, J. Teerapornpuntakit, K. Wongdee, N. Charoenphandhu, Emerging roles of calcium-sensing receptor in the local regulation of intestinal transport of ions and calcium, Am. J. Physiol. Cell Physiol. 320 (2021) C270–C278.
[6] F.A. Gesek, P.A. Friedman, On the mechanism of parathyroid hormone stimulation of calcium uptake by mouse distal convoluted tubule cells, J. Clin. Invest. 90 (1992) 749–758.
[7] C. Aalkjaer, E. Boedtkjer, I. Choi, S. Lee, Cation-coupled bicarbonate transporters, Comp. Physiol. 4 (2014) 1605–1637.

[8] N. Charoenphandhu, K. Wongdee, K. Tudpor, J. Pandaranandaka, N. Krishnamra, Chronic metabolic acidosis upregulated claudin mRNA expression in the duodenal enterocytes of female rats, Life Sci. 80 (2007) 1729–1737.
[9] M.K. Ahsan, L. Figueroa-Hall, V. Baratta, R. Garcia-Milian, T.T. Lam, K. Hoque, P. J. Salas, N.A. Ameen, Glucocorticoids and serum- and glucocorticoid-inducible kinase 1 are potent regulators of CFTR in the native intestine: implications for stress-induced diarrhea, Am. J. Physiol. Gastrointest. Liver Physiol. 319 (2020) G121–G132.
[10] R.J. Lee, J.K. Foskett, Ca^{2+} signaling and fluid secretion by secretory cells of the airway epithelium, Cell Calcium 55 (2014) 325–336.
[11] C. Mihályi, I. Iordanov, B. Töröcsik, L. Csanády, Simple binding of protein kinase A prior to phosphorylation allows CFTR anion channels to be opened by nucleotides, Proc. Natl. Acad. Sci. U.S.A. 117 (2020) 21740–21746.
[12] C. Bookstein, A.M. DePaoli, Y. Xie, P. Niu, M.W. Musch, M.C. Rao, E.B. Chang, Na^+/H^+ exchangers, NHE-1 and NHE-3, of rat intestine. Expression and localization, J. Clin. Invest. 93 (1994) 106–113.
[13] R.S. Haworth, C. McCann, A.K. Snabaitis, N.A. Roberts, M. Avkiran, Stimulation of the plasma membrane Na^+/H^+ exchanger NHE1 by sustained intracellular acidosis. Evidence for a novel mechanism mediated by the ERK pathway, J. Biol. Chem. 278 (2003) 31676–31684.
[14] Y. Yu, A. Seidler, K. Zhou, Z. Yuan, S. Yeruva, M. Amiri, C.C. Yun, K. Nikolovska, U. Seidler, Expression, localization and functional activity of the major Na^+/H^+ exchange isoforms expressed in the intestinal cell line Caco-2BBe, Cell. Physiol. Biochem. 52 (2019) 1017–1038.
[15] M.A. Gurney, D. Laubitz, F.K. Ghishan, P.R. Kiela, Pathophysiology of intestinal Na^+/H^+ exchange, Cell Mol. Gastroenterol. Hepatol. 3 (2017) 27–40.
[16] N. Thongon, L.I. Nakkrasae, J. Thongbunchoo, N. Krishnamra, N. Charoenphandhu, Prolactin stimulates transepithelial calcium transport and modulates paracellular permselectivity in Caco-2 monolayer: mediation by PKC and ROCK pathways, Am. J. Physiol. Cell Physiol. 294 (2008) C1158–C1168.
[17] J.A. Cohn, A.C. Nairn, C.R. Marino, O. Melhus, J. Kole, Characterization of the cystic fibrosis transmembrane conductance regulator in a colonocyte cell line, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 2340–2344.
[18] B. Lacour, T. Drueke, E. Pierandrei, B. Nabarra, J.L. Funck-Brentano, Rat enterocyte Na^+ transport in vitro. Action of parathyroid hormone and calcitonin, Biochim. Biophys. Acta 648 (1981) 151–161.
[19] H.L. Brenza, C. Kimmel-Jehan, F. Jehan, T. Shinki, S. Wakino, H. Anazawa, T. Suda, H.F. DeLuca, Parathyroid hormone activation of the 25-hydroxyvitamin D_3-1α-hydroxylase gene promoter, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 1387–1391.
[20] I. Nemere, A.W. Norman, Parathyroid hormone stimulates calcium transport in perfused duodena from normal chicks: comparison with the rapid (transcaltachic) effect of 1,25-dihydroxyvitamin D_3, Endocrinology 119 (1986) 1406–1408.
[21] H. Amlal, S. Petrovic, J. Xu, Z. Wang, X. Sun, S. Barone, M. Soleimani, Deletion of the anion exchanger Slc26a4 (pendrin) decreases apical Cl^-/HCO_3^- exchanger activity and impairs bicarbonate secretion in kidney collecting duct, Am. J. Physiol. Cell Physiol. 299 (2010) C33–C41.
[22] A.K. Singh, B. Riederer, M. Chen, F. Xiao, A. Krabbenhoft, R. Engelhardt, O. Nylander, M. Soleimani, U. Seidler, The switch of intestinal Slc26 exchangers from anion absorptive to HCO_3^- secretory mode is dependent on CFTR anion channel function, Am. J. Physiol. Cell Physiol. 298 (2010) C1057–C1065.
[23] N. Charoenphandhu, S. Laohapitakworn, K. Kraidith, L.I. Nakkrasae, P. Jongwattanapisan, P. Tharabenjasin, N. Krishnamra, Electrogenic Na^+/HCO_3^- co-transporter-1 is essential for the parathyroid hormone-stimulated intestinal HCO_3^- secretion, Biochem. Biophys. Res. Commun. 409 (2011) 775–779.
[24] N.A. Ameen, B. Martensson, L. Bourguinon, C. Marino, J. Isenberg, G. E. McLaughlin, CFTR channel insertion to the apical surface in rat duodenal villus epithelial cells is upregulated by VIP in vivo, J. Cell Sci. 112 (Pt 6) (1999) 887–894.
[25] L. Fan, M.R. Wiederkehr, R. Collazo, H. Wang, L.A. Crowder, O.W. Moe, Dual mechanisms of regulation of Na/H exchanger NHE-3 by parathyroid hormone in rat kidney, J. Biol. Chem. 274 (1999) 11289–11295.
[26] C. Gentili, S. Morelli, R. Boland, A.R. de Boland, Parathyroid hormone activation of map kinase in rat duodenal cells is mediated by 3',5'-cyclic AMP and Ca^{2+}, Biochim. Biophys. Acta 1540 (2001) 201–212.
[27] C.P. Ribeiro, L.J. Mandel, Parathyroid hormone inhibits proximal tubule Na^+-K^+-ATPase activity, Am. J. Physiol. 262 (1992) F209–F216.
[28] G. Hattersley, T. Dean, B.A. Corbin, H. Bahar, T.J. Gardella, Binding selectivity of abaloparatide for PTH-type-1-receptor conformations and effects on downstream signaling, Endocrinology 157 (2016) 141–149.
[29] M. Bastepe, S. Turan, Q. He, Heterotrimeric G proteins in the control of parathyroid hormone actions, J. Mol. Endocrinol. 58 (2017) R203–R224.
[30] I. Kurtz, Q. Zhu, Proximal renal tubular acidosis mediated by mutations in NBCe1-A: unraveling the transporter's structure-functional properties, Front. Physiol. 4 (2013) 350, https://doi.org/10.3389/fphys.2013.00350.