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Parathyroid hormone and the regulation of cell cycle in colon adenocarcinoma cells

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ABSTRACT

Parathyroid hormone (PTH) functions as a major mediator of bone remodeling and as an essential regulator of calcium homeostasis. In this study, we investigated the role of PTH in the regulation of the cell cycle in human colon adenocarcinoma Caco-2 cells. Flow cytometry analysis revealed that PTH (10^{-8} M, 12–24 h) treatment increases the number of cells in the G0/G1 phase and diminishes the number in both phases S and G2/M. In addition, analysis by Western blot showed that the hormone increases the expression of the inhibitory protein p27Kip1 and diminishes the expression of cyclin D1, cyclin D3 and CDK6. However, the amounts of CDK4, p21Cip1, p15INK4B and p16INK4A were not different in the absence or presence of PTH. Inhibitors of PKC (Ro-318220, bisindolylmaleimide and chelerythine), but not JNK (SP600125) and PP2A (okadaic acid and calyculin A), reversed PTH response in Caco-2 cells. Taken together, our results suggest that PTH induces G0/G1 phase arrest of Caco-2 intestinal cells and changes the expression of proteins involved in cell cycle regulation via the PKC signaling pathway.

1. Introduction

Proliferation of eukaryotic cells depends on progression through the cell cycle, and cell cycle control is achieved through the actions of a family of cyclin-dependent protein kinases (CDKs) and cyclins that initiate phosphorylation events to allow progression through checkpoints in the cell cycle. In mammalian cells, progression through the G1 phase requires, early in G1, the activity of the cyclin D-dependent kinases CDK4 and/or CDK6 and, later in G1, the cyclin E-dependent kinase CDK2. G1 kinases can be regulated by changes in cyclin levels or CDK activity and by a CDK-activating kinase (CAK) [1]. Two families of CDK inhibitors (CDKIs) are critical mediators of anti-proliferative signals that arrest the cell cycle. Cip/Kip family includes p21Cip1 (also known as WAF1, Sdi1, and CAP20), p27Kip1, and p57Kip2 whereas INK4 family includes p16INK4A, p15INK4B, p18INK4C, and p19INK4D. These inhibitors negatively regulate G1 phase progression by forming complexes with CDKs and thus preventing S phase entry [2,3]. In vitro, p21Cip1, p27Kip1, and p57Kip2 inhibit a wide variety of cyclin-CDK complexes, including cyclin D-CDK4/6 and cyclin A/E-CDK2. Ink4 inhibitors are specific for CDK4 and CDK6 and interfere with cyclin D binding to these kinases.

Parathyroid hormone (PTH) is a major mediator of bone remodeling and an essential regulator of calcium homeostasis. Very small decrements in serum calcium levels induce the secretion of PTH from the parathyroid glands initiating a rapid response to raise serum calcium levels by acting directly on kidney and bone or indirectly on intestine (via $1,25(OH)_2$ vitamin D_3) facilitating calcium absorption [4,5]. Mammalian PTH is an 84-amino acid single-chain polypeptide, although only the first 34 amino acids are required for most biological effects [6,7]. The PTH receptor (PTH1R) is highly expressed in bone and kidney, but is found also in a variety of tissues not regarded as classical PTH target tissues, including intestinal cells [8–10]. PTH binds to its receptor and activates at least two signal transduction systems: the cAMP-dependent protein kinase (PKA) [11,12] and the phospholipase C-activated calcium/protein kinase C (Ca^{2+}/PKC) pathways [11,13].

Depending on the experimental conditions, PTH also can inhibit or promote proliferation and induces alterations in cell cycle regulation [14,15].

Using the human colon cell line Caco-2 we previously obtained evidence that PTH diminishes the number of viable cells, induces morphological changes typical of apoptotic cells [10] and activates several pro-apoptotic factors [16]. However, the role of PTH in the proliferation of Caco-2 cells remains understood. The present study was designed to explore if PTH regulates the cell cycle progression in these colon cancer cells and to investigate the mechanisms that are involved in this process.

2. Materials and methods

2.1. Materials

Human PTH (1–34) was obtained from Calbiochem (San Diego, CA, USA). High glucose Dubelcco's modified Eagle's medium (DMEM) was from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum

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(FBS) was from Natocord (Córdoba, Argentina). Antibodies were from the following sources: anti-cyclin D1, anti-cyclin D3, anti-CDK4, anti-CDK6, anti-p27Kip1, anti-p21Cip1, anti-p15Ink4B, anti-p16Ink4A, were from Cell Signaling Technology (Beverly, MA, USA). Goat anti-rabbit peroxidase-conjugated secondary antibody and goat anti-mouse peroxidase-conjugated secondary antibody were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-actin antibody, bisindolylmaleimide (BIM), chelerythine, okadaic acid sodium and calyculin A were from Sigma (Sigma Chemical Co. St. Louis, MO, USA). Ro-31-8220 was from Calbiochem (San Diego, CA, USA). SP600125 was obtained from Tocris Cookson Inc. (Ellisville, MO, USA). RNase Cocktail™ Enzime Mix was from Applied Biosystems (Carlsbad, California). Propidium iodide (PI) was from Invitrogen (Carlsbad, California). Protein size markers were from Amersham Biosciences (Piscataway, NJ, USA), and PVDF (Immobilon polyvinylidene difluoride) membranes and ECL chemiluminescence detection kit were from Amersham (Little Chalfont, Buckinghamshire, England). All other reagents used were of analytical grade.

2.2. Cell culture and treatment

The human colon cell line Caco-2 (from the American Tissue Culture Bank (Bethesda, USA)) was cultured at 37 °C in DMEM containing 10% FBS, 1% non-essential acids, 100 UI/ml penicillin, 100 mg/ml streptomycin and 50 mg/ml gentamycin in a humid atmosphere of 5% CO2 in air. Cultures were passaged every 2 days with fresh medium. Experimental cultures were grown to 50–70% confluence in serum-containing medium, and then cells were serum deprived 24 h before the addition of PTH (10^{-8} M) in DMEM containing 2% FBS for 3, 6, 14 or 24 h. Where indicated, cells were pretreated for 30 min with Ro-31-8220, bisindolylmaleimide (BIM), chelerythine, okadaic acid sodium, calyculin A or SP600125. The inhibitors were present during subsequent exposure to the hormone.

2.3. Cell cycle analysis

Cell cycle distribution was analyzed by flow cytometry. Cells incubated with or without PTH for 12 or 24 h were trypsinized, washed once with PBS, and fixed in 70% ethanol for at least 1 h at −20 °C. Fixed cells were washed with PBS and incubated with propidium iodide (PI) staining solution (69 μM IP, 38 mM sodium citrate and 0.7 mg/ml ribonuclease A, pH 7.4) for 30 min at 37 °C in the dark. The stained cells were analyzed by FACSCalibur flow cytometer (Becton Dickinson). The program used for the acquisition and analysis of the samples was the CellQuest.

2.4. Western blot analysis

Caco-2 cells were washed with PBS buffer plus 25 mM NaF and 1 mM Na_3VO_4, and lysed in buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 3 mM KCl, 1 mM EDTA, 1% Tween-20, 1% Nonidet P-40, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 25 mM NaF and 1 mM Na_3VO_4. The lysates were incubated on ice for 10 min, vortexed for 45 s and maintained on ice for another 10 min. After centrifugation at 14,000×*g* and 4 °C during 15 min the supernatant was collected and proteins were quantified by the Bradford method [17]. Lysate proteins dissolved in 6× Laemmli sample buffer were separated (25 μg/lane) using SDS-polyacrylamide gels (10% or 15% acrylamide) and electrotransfered to PVDF membranes. After blocking with 5% non-fat milk in TBST buffer (50 mM Tris pH 7.2–7.4, 200 mM NaCl, 0.1% Tween-20), the membranes were incubated overnight with the appropriate dilution of primary antibody in TBST with 1% non-fat milk. After washing, membranes were incubated with the appropriate dilution of horseradish peroxidase-conjugated secondary antibody in TBST with 1% non-fat milk. Finally, the blots were developed by ECL with the use of Kodak BioMax Light film and digitalized with a GS-700 Imaging Densitomer (Bio-Rad, Hercules, CA, USA).

2.5. Stripping and reprobing membranes

The complete removal of primary and secondary antibodies from the membranes was achieved by incubating the membranes in stripping buffer (62.5 mM Tris–HCl pH 6.8, 2% SDS and 50 mM β-mercaptoethanol) at 55 °C for 30 min with agitation. Then, membranes were washed for 10 min in TBST (1% Tween-20) and blocked, as indicated above, for 1 h at room temperature. After that, membranes were ready to reprobe with the corresponding antibodies.

2.6. Statistical analysis

The statistical significance of the data was evaluated using Student's *t* test, and probability values below 0.050 ($p<0.050$) were considered significant. Quantitative data are expressed as means ± SD from the indicated set of experiments.

3. Results

Initial experiments were performed to investigate the effect of PTH on the progression of the cell cycle in Caco-2 cells. To that end, cells were incubated with or without PTH (10^{-8} M) for 12 and 24 h and the percentages of cells in the G0/G1, S, and G2/M phases were determined by flow cytometric analysis of propidium iodide stained cells as described in Materials and methods. As shown in Fig. 1 the hormone increased the percentage of cells in G0/G1 from 55.9% to 66.9% ($p<0.025$) and 67.5% to 73.9% ($p<0.01$) at 12 and 24 h, respectively, which was accompanied by a corresponding reduction in the percentage of cells in S phase from 33.1% to 20.7% ($p<0.01$) and 17.4% to 15% ($p<0.025$) at 12 and 24 h, respectively. These data suggest that PTH induces cell cycle arrest at G0/G1 phase in Caco-2 cells.

We next analyzed whether the cell cycle arrest at G0/G1 phase by PTH was related to changes in the expression of cell cycle-regulatory proteins that are essential for cell cycle progression from G1 to S phase. To that end, Caco-2 cells were treated with or without PTH (10^{-8} M) for 3–24 h followed by immunoblot analysis using specific antibodies. As demonstrated in Fig. 2, PTH markedly diminished the expression of cyclin D1, cyclin D3 and CDK6, which are responsible for cell cycle progression in the early G1 phase, whereas CDK4 levels were unaffected by PTH treatment. In order to evaluate the equivalence of protein content among the different experimental conditions, blotted membranes were re-probed with anti-β-actin antibody. To further clarify the mechanism of PTH-induced G0/G1 cell cycle arrest and, since cell cycle progression is also controlled by specific CDK inhibitors (INK4 and Cip/Kip family), we evaluated the expression of p16INK4A, p15INK4B, p27Kip1 and p21Cip1 after PTH treatment for 3 to 24 h. In Fig. 3A and B, Western blot analysis show that the hormone increased the expression of the inhibitory protein p27Kip1. However, the amounts of p21Cip1, p15INK4B and p16INK4A were not different in the absence or presence of PTH. Taken together, these results reveal that PTH (10^{-8} M) arrests the cell cycle by up-regulation of p27Kip1 expression and down-regulation of cyclin D1, cyclin D3 and CDK6 expressions. The expressions of these cell cycle-regulatory proteins were not affected when Caco-2 cells were treated with concentrations of PTH lower and higher than 10^{-8} M (data not shown).

It has been demonstrated that signaling through c-jun N-terminal kinases (JNKs) regulates cell cycle control and the induction of apoptosis [18,19]. Previously, Grösch S. et al. [20] showed that S-flurbiprofen, an unselective cyclooxygenase inhibitor, and its inactive enantiomer inhibit the proliferation of Caco-2 cells through G1-cell cycle blockade associated with an activation of JNK, an increase of the DNA binding activity of the transcription factor AP-1 and down-regulation of cyclin D1 expression. Therefore, to gain insight into the

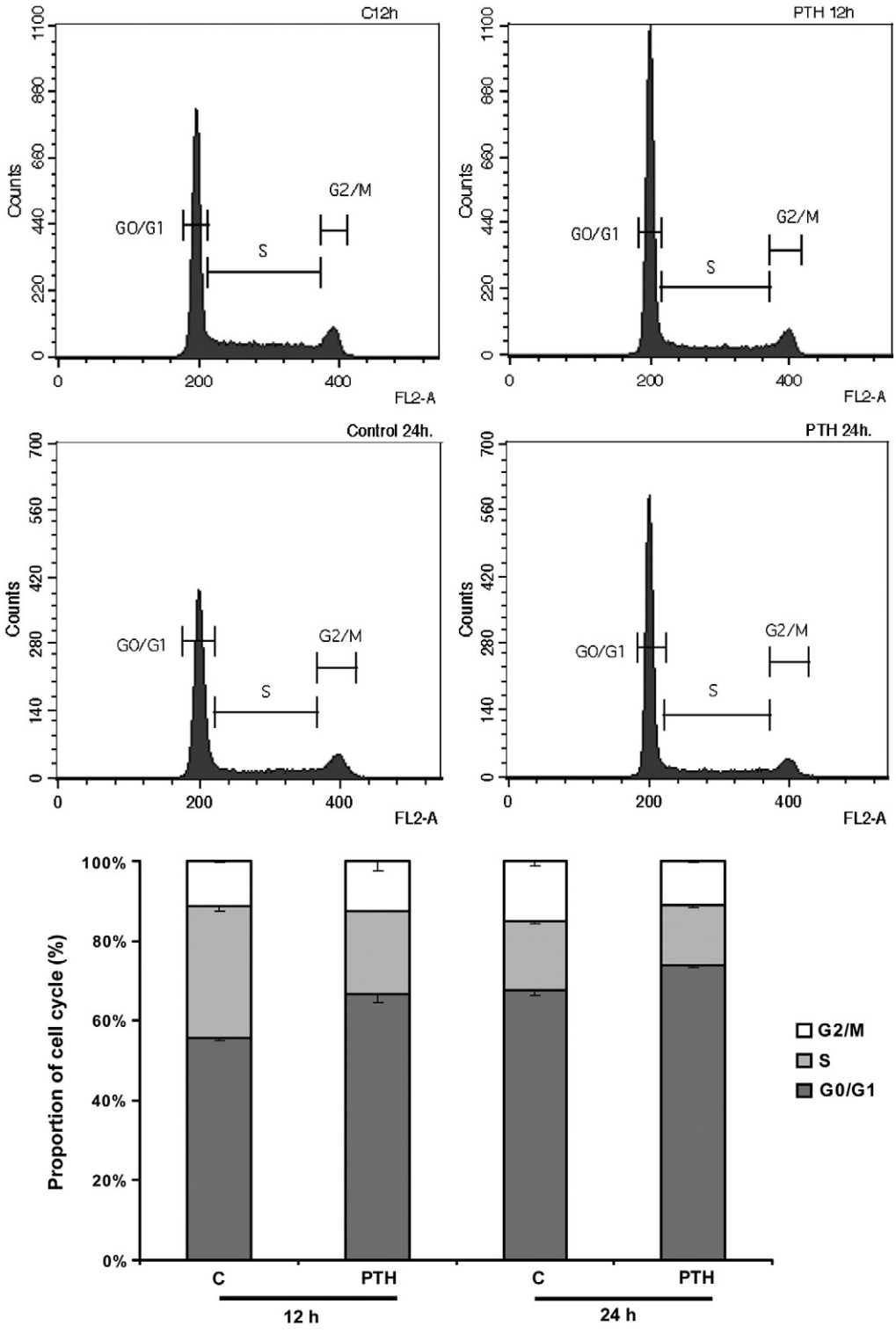

Fig. 1. Effect of PTH on cell cycle progression. Caco-2 cells were incubated in serum-free DMEM for 24 h and then treated with or without PTH (10^{-8} M, in DMEM 2% FBS) for 12 and 24 h. Cells were then stained with propidium iodide and the distribution of cells in the cell cycle was analyzed by flow cytometry of DNA content. Representative cytometric profiles and percentage of each phase are shown. The program CellQuest was used for acquisition and analysis the FACS scans. Data represents mean ± SD of three independent experiments. G0/G1 = cells in G0/G1 cell cycle phases; S = cells in S cell cycle phase; G2/M = cells in G2/M cell cycle phases.

signaling events that link PTH to the cell cycle regulatory machinery, Caco-2 cells were pre-incubated with JNK inhibitor SP600125 (20 μM) for 30 min and then treated with PTH (10^{-8} M, 6 h). As shown in Fig. 4, the inhibition of JNK activity did not reverse hormone response, suggesting that the effect of PTH on the expression of cyclin D1, cyclin D3 and CDK6 is independent of the JNK pathway.

It has been shown that protein phosphatase 2A (PP2A) mediates the down-regulation of cyclin D1 in the intestinal crypt cells IEC-18 [21]. Since we previously reported that PTH induced PP2A activation in Caco-2 cells [22], we also investigated the role of PP2A in the regulation of PTH-induced cell cycle arrest. To that end, cells were pre-treated with the phosphatase inhibitors okadaic acid (OA) at a

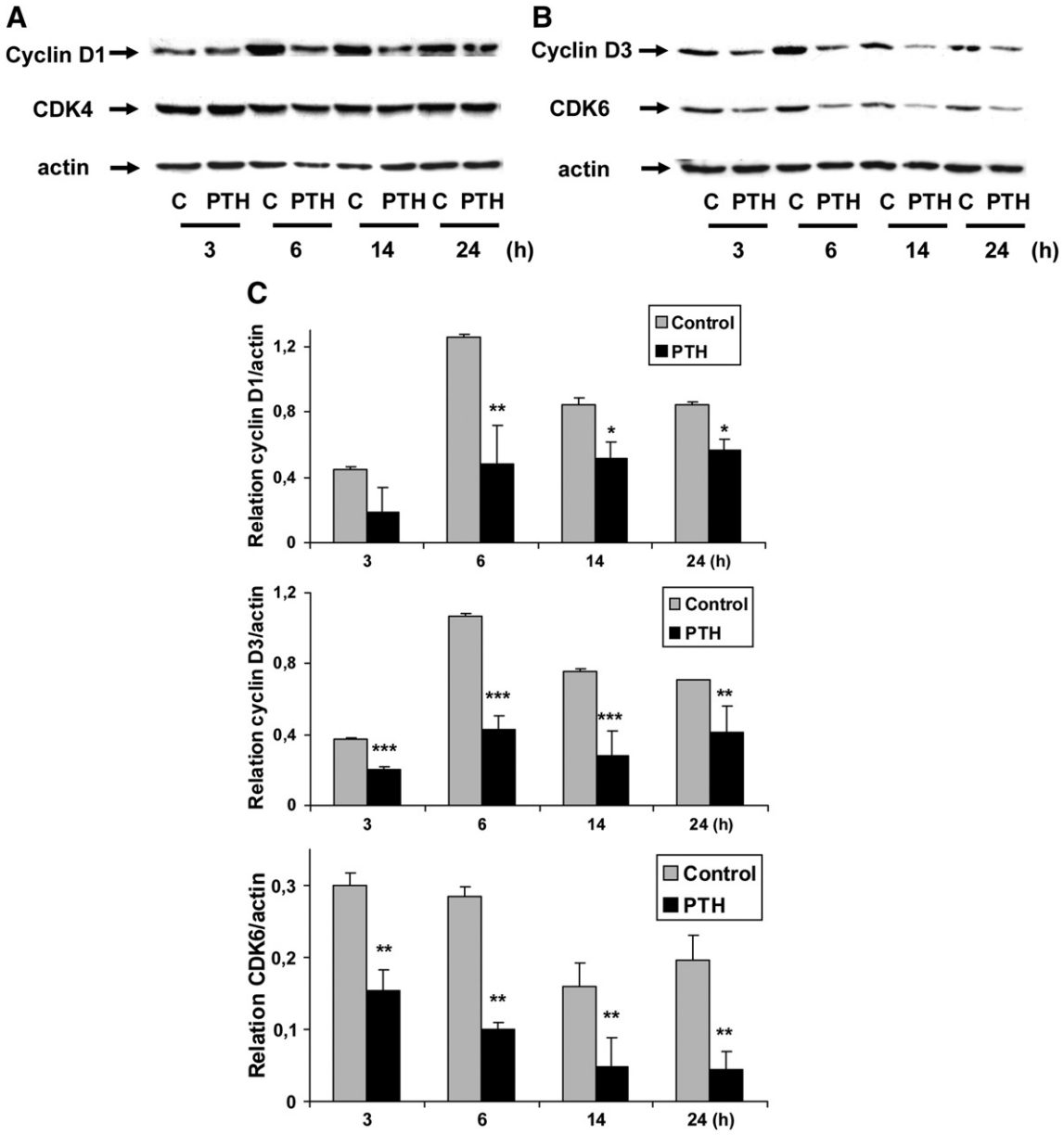

Fig. 2. PTH diminishes the expression of cyclin D1, cyclin D3 and CDK6. Caco-2 cells were incubated in serum-free DMEM for 24 h and then treated with or without PTH (10^{-8} M, in DMEM 2% FBS) for 3, 6, 14 and 24 h. Proteins from lysates were prepared as described in Materials and methods, separated on 15% SDS-PAGE, and immunoblotting using (A) anti-cyclin D1 or anti-CDK4 antibodies; (B) anti-cyclin D3 or anti-CDK6 antibodies. In order to evaluate the equivalence of protein content among the different experimental conditions, blotted membranes were reprobed with anti-β-actin antibody. (C) Densitometric analyses were performed on the anti-cyclin D1, anti-cyclin D3 and anti-CDK6 immunoblots from three independent experiments; means ± SD are given. *P<0.05, **P<0.025, ***P<0.01 with respect to the corresponding control.

dose that inhibit PP2A activity (1 nM) or with calyculin A (3 nM) and then incubated with PTH (10^{-8} M, 6 h). Inhibition of PP2A did not reverse hormone-mediated down-regulation of cyclin D1, cyclin D3 and CDK6, suggesting that the effect of PTH on the expression of these cell cycle regulatory proteins is independent of the PP2A pathway (Fig. 4 A and B).

PKC is a member of serine/threonine kinase whose isoforms have been shown to be involved in a number of cellular processes. Several studies implicate PKC isozymes in either positive or negative regulation of G1-S progression, via alterations in the expression of cyclins and/or CDK inhibitors and modulation of the activity of specific cyclin-CDK complexes [23,24]. In attempt to investigate whether the PKC pathway is involved in PTH-induced cell cycle arrest, PKC inhibitors Ro-31-8220, (200 nM); BIM (5 μM) and chelerythrine, (2 μM), were added before the cells were treated with PTH followed by immunoblot analysis using specific antibodies. As shown in Fig. 5, PKC inhibitors prevented PTH-induced downregulation of cyclin D1, cyclin D3 and CDK6. Moreover, p27Kip1 protein level significantly decreased when Caco-2 cells were incubated with Ro-31-8220, BIM or chelerythrine followed by PTH treatment for 24 h (Fig. 6).

These findings indicate that PTH regulation of cyclin D1, cyclin D3, CDK6 and p27Kip1 are PKC dependent in Caco-2 cells.

4. Discussion

The results of the present investigation provide, to our knowledge, the first direct evidence demonstrating that PTH induces G0/G1 cell cycle arrest in Caco-2 cells. Many reports have shown that PTH inhibits or promotes cell cycle progression, depending on the experimental conditions and cell type. PTH stimulates the progression

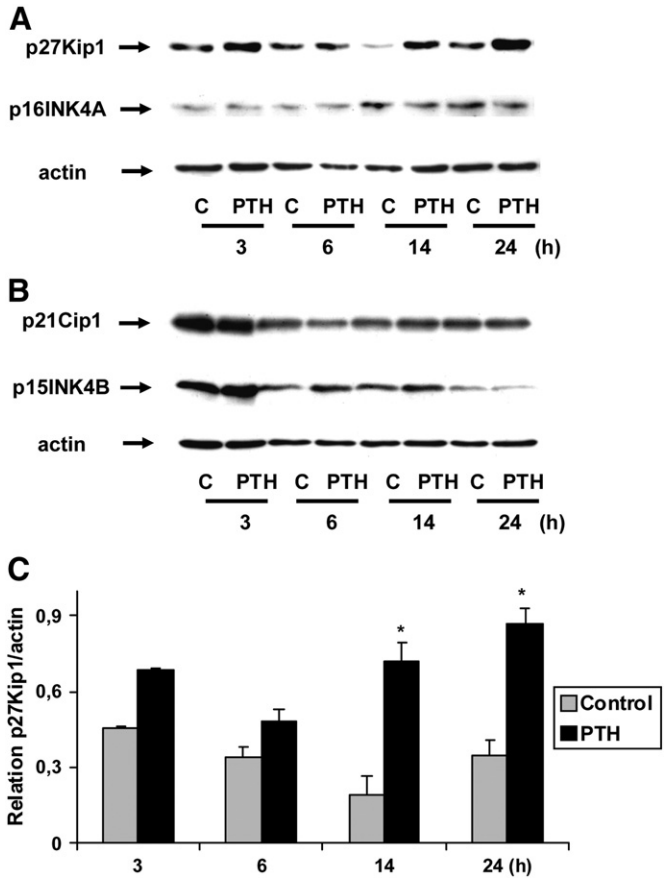

Fig. 3. PTH increases p27Kip1 expression. Caco-2 cells were treated as described in the legend of Fig. 2. Immunoblots were performed using (A) anti-p27Kip1 or anti-p16INK4A antibodies; (B) anti-p21Cip1 or anti-p15INK4B antibodies. In order to evaluate the equivalence of protein content among the different experimental conditions, blotted membranes were reprobed with anti-β-actin antibody. (C) Densitometric analyses were performed on the anti-p27Kip1 immunoblots from three independent experiments; means ± SD are given. *$P<0.025$ with respect to the corresponding control.

of the cell cycle increasing CDK1 (cyclin-dependent kinase 1) expression and its activity through the actions of the E2F transcription factor in the human osteosarcoma cell line TE-85 [25]. The hormone also induces the expression of cyclin D1 in early osteoblastic cells [14] and, in chondrocytes and chondrosarcoma cells, an activating mutation of the PTH receptor (PTH1R) causes an increase in cyclin D1 and cyclin A expression [26]. However, PTH is known to inhibit cell cycle progression in the UMR-106 cell line, a relatively differentiated osteoblastic osteogenic sarcoma line, by increasing p27Kip1 levels and inhibiting CDK2 activity, which are mediated through the protein kinase A pathway [27]. In addition, Qin L. et al. [15], showed that PTH inhibits the growth of osteoblastic cell lines, arresting them in G1 phase by inducing the expression of both, MAPK phosphatase 1 (MKP-1) and p21Cip1 and by decreasing cyclin D1 expression. Previous studies in our laboratory, using the human colon cell line Caco-2, demonstrated that PTH decreases the number of viable cells [10]. However, at the moment the effects that PTH exerts on the cell cycle in these cells were unknown. Therefore, we first analyzed the DNA contents by flow cytometry in Caco-2 cells, and we found that PTH increases the number of cells in the G0/G1 phase and diminishes the number of cells in S phase.

It has been well documented that cell cycle is primarily regulated by complexes containing CDKs and cyclins, which are critical for progression of cell cycle and their inactivation leads to cell cycle arrest [28,29]. The complexes responsible for the progression of cells through the G1 phase of cell cycle and the initiation of DNA replication include cyclin D-CDK4/CDK6 and cyclin E-CDK2 [30]. Aberrant proliferation of cancer cells involves the deregulation of key G1 phase cell cycle regulators, and overexpression of cyclins and CDKs provides a selective growth advantage to tumor cells [31]. Therefore, targeting cyclin-CDK complexes that promote tumor progression is therapeutically relevant for the treatment of cancer. Our results showed that PTH treatment caused a decrease in the protein levels of CDK 6, cyclin D1, and cyclin D3, explaining the plausible role of these cell cycle regulatory proteins, in the PTH-induced G0/G1 arrest observed in Caco-2 intestinal cells.

p27Kip1 is a negative regulator of the cell cycle and a potential tumor suppressor gene as well as a promoter of apoptosis [32–34]. p27Kip1 expression, that is primarily increased in response to extracellular anti-mitogenic signals for cell cycle arrest [35–37], is strong in non-proliferating cells. Thus, this inhibitor plays important role in the regulation of both quiescence and G1 progression. Reduced expression of p27Kip1 is known as an independent prognostic marker in a large variety of cancers, such as breast, prostate, colon, and gastric carcinomas, and is associated with unfavorable prognosis [38]. Our data demonstrate that PTH increases the levels of p27Kip1 in Caco-2 cells, but not the levels of p21Cip1, p16/INK4A and p15/INK4B suggesting that PTH-induced G0/G1 arrest is mediated by p27Kip1. In agreement with our results, Onishi and Hruska [27] showed that, in UMR 106–01 osteoblastic cells, PTH inhibits the progression of cell cycle from G1 to S phase by increasing the expression of p27Kip.

Mitogen-activated protein kinases (MAPKs) are commonly divided into subfamilies that include the extra-cellular regulated protein kinases (ERKs), the c-jun N-terminal kinases (JNKs), the p38, and the Big MAP kinases (BMKs) [39]. JNKs are activated in response to inflammatory cytokines, environmental stresses, such as UV radiation, osmotic and heat shock, and DNA and protein synthesis inhibition [40]. JNK activation has been implicated in the inhibition of Caco-2 cell proliferation by R- and S-flurbiprofen [20], whereas sulforaphane and erucin, that induced G2/M arrest and cell death of these cells, activate ERK1/2 but had no effect on JNK and p38 activation [41]. The present study excludes, at least under our experimental conditions, the involvement of JNK on PTH-induced Caco-2 cell cycle arrest. Further studies are necessary to evaluate whether PTH employs p38 and ERK1/2 pathways to regulate cell cycle progression.

Protein phosphatase 2A (PP2A) is an abundant and ubiquitous Ser/Thr phosphatase with pleiotropic functions [42]. Kim S.W. et al. [43,44] showed that induction of cell cycle arrest by ceramide and by a synthetic trans-stilbene analog is regulated by PP2A-dependent Akt dephosphorylation in PC-3 prostate cancer cells. In the intestinal cell line IEC-18 it has been shown [21] that the down-regulation of cyclin D1 was mediated by PP2A. However, we found that PP2A did not participate in PTH regulation of cyclin D1 levels neither in the expression of cyclin D3 and CDK6.

PKC constitutes a family of serine-threonine kinases, which are classified into three major groups based on their structure and activation mechanisms, and contains 11 isoforms encoded by 10 genes: conventional (α, βI, βII, γ), novel (δ, ε, η, θ, μ), and atypical (ζ, λ) [45]. PKC plays a crucial role in signal transductions related with cell proliferation [46], apoptosis [47], cell differentiation [48], and hormone release [49]. Moreover, recent evidence found that PKCs can impact on the cell cycle in either a positive or negative way, depending on the cell type and isozyme specificity [50]. Indeed, PKCs have been shown to regulate the progression of cells from G1 to S phase as well as the transition from G2 to M phase, although the underlying molecular mechanisms remain unclear [23]. Alterations in the biochemical activity and expression of a number of PKCs have been implicated in the malignant transformation process of several organs, including the colon, in both humans and experimental animals. Studies have also shown that human colon tumors may

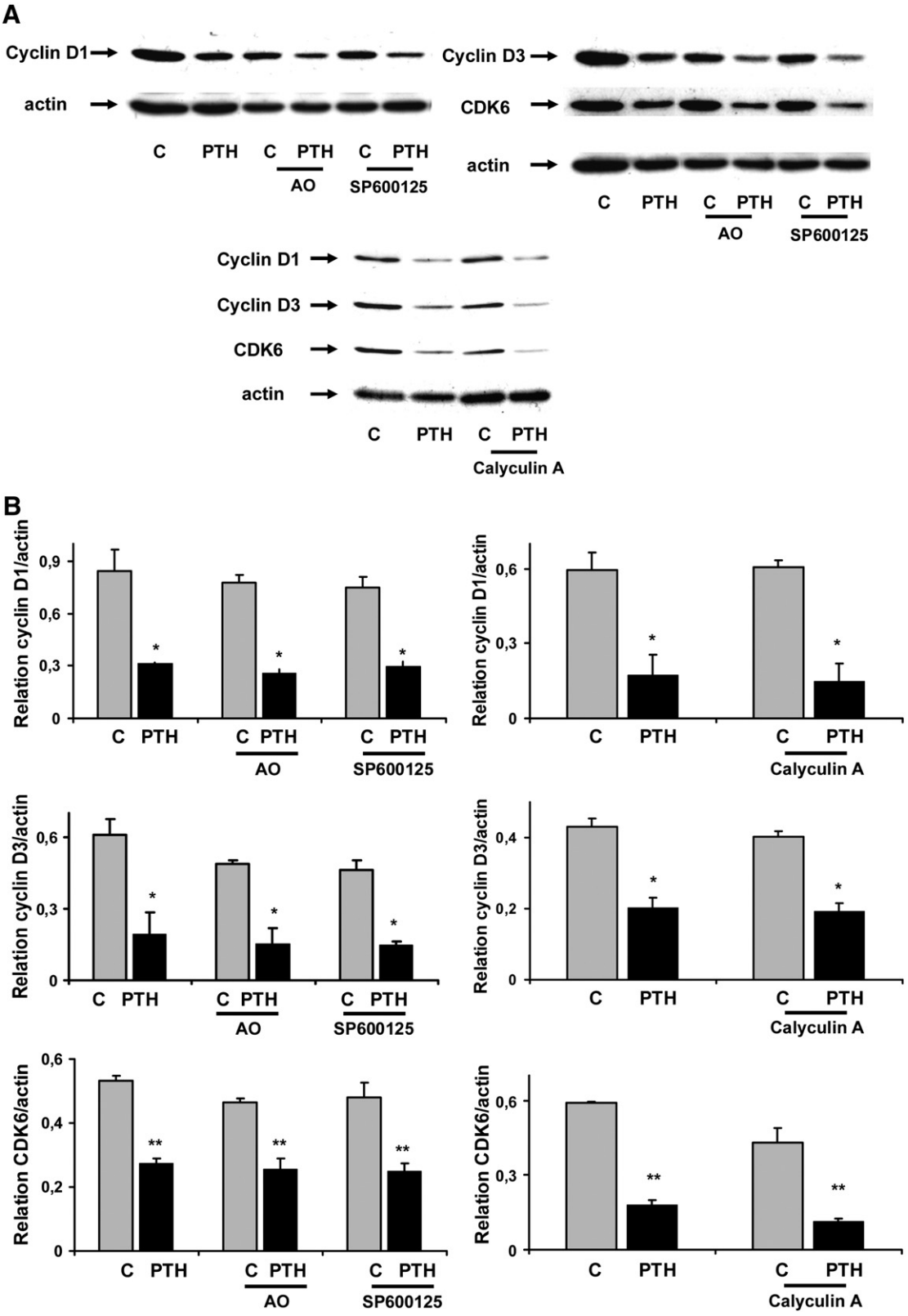

Fig. 4. Effects of SP600125, Okadaic Acid and Calyculin A on the expression of cell cycle regulatory proteins. Caco-2 cells were pre-incubated for 30 min with SP600125 (20 μM), an inhibitor of JNK, with Okadaic Acid (OA, 1 nM) or with Calyculin A (3 nM), inhibitors of PP2A, and then exposed to PTH (10^{-8} M, in DMEM 2% FBS) for 6 h followed by Western blot analysis of proteins from cell lysates using anti-cyclin D1 antibody, anti-cyclin D3 or anti-CDK6 antibodies. Blotted membranes were re-probed with anti-β-actin antibody. A representative immunoblot (A) and the quantification by scanning densitometry (B) of three independent experiments are shown; means ± SD are given. *$P<0.025$; **$P<0.01$ with respect to the control.

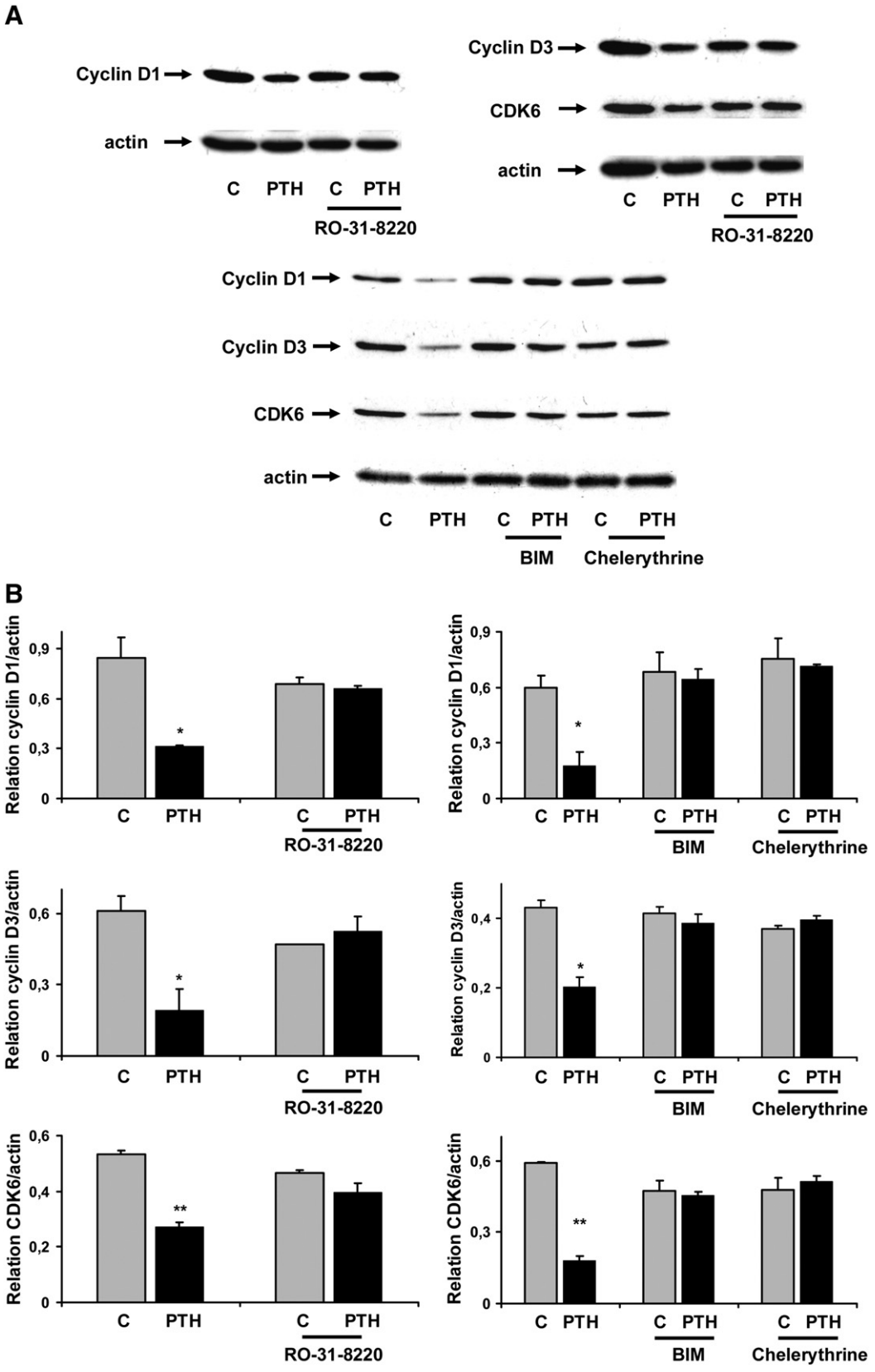

Fig. 5. Downregulation of cyclin D1, cyclin D3 and CDK6 by PTH is PKC-dependent. Caco-2 cells were pretreated for 30 min with PKC inhibitors, Ro-31-8220 (200 nM), BIM (5 μM) or chelerythine (2 μM) and then were exposed to PTH (10^{-8} M, in DMEM 2% FBS) for 6 h followed by Western blot analysis of protein from cell lysates using anti-cyclin D1 antibody, anti-cyclin D3 or anti-CDK6 antibodies. Blotted membranes were re-probed with anti-β-actin antibody. A representative immunoblot (A) and the quantification by scanning densitometry (B) of three independent experiments are shown; means ± SD are given. *$P<0.025$, **$P<0.01$ with respect to the corresponding control.

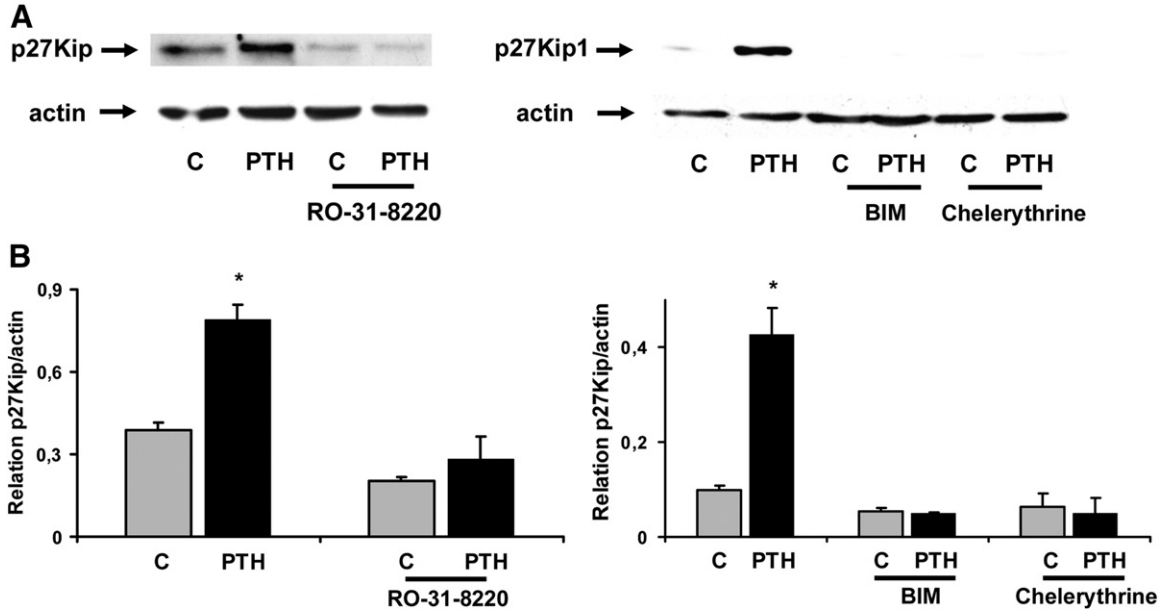

Fig. 6. Upregulation of p27Kip1 by PTH is PKC-dependent. Caco-2 cells were pretreated for 30 min with PKC inhibitors, Ro-31-8220 (200 nM), BIM (5 μM) or chelerythine (2 μM) followed by exposition to PTH (10^{-8} M, in DMEM 2% FBS) for 24 h. Proteins from cell lysates were immunoblotted with anti-p27Kip1 antibody. In order to evaluate the equivalence of protein content among the different experimental conditions, blotted membranes were reprobed with anti-β-actin antibody. A representative immunoblot (A) and the quantification by scanning densitometry (B) of three independent experiments are shown; means ± SD are given. *$P < 0.025$ with respect to the corresponding control.

have a decrease in the protein expression of several specific PKC isoforms, including PKC-α [51,52]. Moreover, PKC activation can initiate a specific program of molecular events associated with cell cycle withdrawal into G0 or a G0-like state in IEC-18 intestinal crypt cells by downregulation of D-type cyclins and induction of p21Cip1 and 27Kip1 [53].

This study shows that, in Caco-2 cells, the inhibition of PKC up-regulated the levels of cyclin D1, cyclin D3 and CDK6 but down-regulated p27Kip1 protein level indicating that PTH regulates the expression of these proteins through a PKC-dependent pathway. The precise series of molecular events underlying PKC-mediated cell cycle arrest remains to be investigated.

In conclusion, our results suggest that PTH changes the expression of proteins involved in cell cycle regulation by activating the PKC signaling pathway and induces G0/G1 phase arrest of human colon Caco-2 cells.

Acknowledgments

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