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PTH stimulates PLCβ and PLCγ isoenzymes in rat enterocytes: influence of ageing

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Abstract

We previously reported that in rat duodenal cells (enterocytes), parathyroid hormone (PTH [1–34]: PTH) stimulates the hydrolysis of polyphosphoinositides by phospholipase C (PLC), generating the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) and that this mechanism is severely altered in old animals. In the present study, we show that PTH [1–34]-dependent IP_3 release in young rats was blocked to a great extent by an antibody against guanine nucleotide binding protein Gαq/11, indicating that the hormone activates a β isoform of PLC coupled to the α subunit of Gq/11. In addition, PTH rapidly (within 30 s, with maximal effects at 1 min) stimulated tyrosine phosphorylation of PLCγ in a dose-dependent fashion (10^{-10}–10^{-7} M). The hormone response was specific as PTH [7–34] was without effects. The tyrosine kinase inhibitors, genistein (100 μM) and herbimycin (2 μM), suppressed PTH-dependent PLCγ tyrosine phosphorylation. Stimulation of PLCγ tyrosine phosphorylation by PTH [1–34] greatly decreased with ageing. PP1 (10 μM), a specific inhibitor of the Src family of tyrosine kinases, completely abolished PLCγ phosphorylation. The hormone-induced Src tyrosine dephosphorylation, a major mechanism of Src activation, an effect that was blunted in old animals. These results indicate that in rat enterocytes PTH generates IP_3 mainly through G-protein-coupled PLCβ and stimulates PLCγ phosphorylation via the nonreceptor tyrosine kinase Src. Impairment of PTH activation of both PLC isoforms upon ageing may result in abnormal hormone regulation of cell Ca^{2+} and proliferation in the duodenum.

Keywords: PTH; Rat enterocytes; IP_3; PLCβ; PLCγ; Ageing

1. Introduction

Parathyroid hormone (PTH) is a major regulator of calcium and phosphate metabolism. The peptide composed of 84 amino acids is the main secreted and circulating form of bioactive PTH [1]. PTH and its tumoral analog PTH-related protein (PTHrP) [2] share and interact with a membrane-bound receptor. Expression of the PTH/PTHrP receptor was observed in the classical target tissues, bone, and kidney, but was also found in many other tissues [3], including the gastrointestinal tract [4,5]. PTH elicits many of its physiological effects by activating distinct guanine nucleotide binding G-protein-coupled signaling cascades that lead to synthesis of cyclic AMP (cAMP) [6] and hydrolysis of membrane-associated phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C (PI-PLC) that generates inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [7]. After second messenger formation, cAMP activates protein kinase A (PKA) [8], IP_3 releases calcium from intracellular stores [9] and DAG causes translocation of protein kinase C (PKC) from the cytosol to the cell membrane [10].

Although the intestine is not considered a classical target tissue for PTH, we have recently demonstrated in rat duodenal enterocytes direct effects of PTH on extracellular Ca^{2+} influx and Ca^{2+} release from inner stores that involve the activation of the adenylyl cyclase/cAMP and PLC/IP_3/DAG second messenger pathways [11,12].

Ageing is associated with increased circulating PTH levels [13] and decreased serum vitamin D metabolites [14], intestinal calcium absorption [15], and bone density [16]. There is considerable evidence indicating that hormonal regulation of signal transduction diminishes during ageing. An age-related decline in PTH-stimulated adenylyl cyclase in both rat kidney slices and cell membranes [17,18]

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has been shown. In bone cells, altered PTH-dependent production of cAMP has been observed upon ageing [19–21]. In rat enterocytes, we have obtained evidence that PTH signaling is also affected with age. In old animals, only the adenylyl cyclase/cAMP messenger system is operative whereas the PLC-mediated early generation of IP_3 and DAG induced by PTH is severely impaired [12].

The present study was designed to identify the PLC isoforms activated by PTH in rat enterocytes and to investigate their involvement in age-related changes in hormone signal transduction.

2. Materials and methods

2.1. Materials

Synthetic rat PTH (rPTH [1–34]), leupeptin, aprotinin, and Immobilon P (polyvinylidene difluoride, PVDF) membranes were from Sigma (St. Louis, MO, USA). The IP_3 radioreceptor kit was purchased from New England Nuclear (Chicago, IL, USA). G-protein antibodies were generously provided by Dr. Günter Schultz (Institut für Pharmakologie, der Freien Universität, Berlin). Anti-PLCγ, anti-phosphotyrosine, and anti-Src antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other reagents were of analytical grade.

2.2. Animals

Young (3 months old) and aged (20–24 months old) male Wistar rats were fed with standard rat food (1.2% Ca; 1.0% phosphorus), given water ad libitum, and maintained on a 12:12-h light–dark cycle. Animals were sacrificed by cervical dislocation.

2.3. Isolation of duodenal cells

Duodenal cells from young and aged rats were isolated essentially as previously described [22]. The method employed yields preparations that contain only highly absorptive epithelial cells and are devoid of cells from the upper villus or crypt [23,24]. The duodenum was excised, washed, and trimmed of adhering tissue. The intestine was slit lengthwise and cut into small segments (2 cm length) and placed into Solution A: 96 mM NaCl, 1.5 mM KCl, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 27 mM Na citrate, pH 7.3, for 10 min at 37°C. The solution was discarded and replaced with Solution B (isolation medium): 154 mM NaCl, 10 mM NaH_2PO_4, 1.5 mM EDTA, 0.5 mM dithiothreitol (DTT), 5.6 mM glucose, pH 7.3, for 15 min at 37°C with vigorous shaking. The cells were sedimented by centrifugation at $750 \times g$ for 10 min, washed twice with 154 mM NaCl, 10 mM NaH_2PO_4, 5.6 mM glucose, pH 7.4, and resuspended in the incubation medium (Solution D): 154 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 1 mM $MgCl_2$, 10 mM NaMOPS, pH 7.4, 5.6 mM glucose, 0.5% BSA, 1 mM $CaCl_2$, 2.5 mM glutamine. All the above steps were performed under a 95% O_2–5% CO_2 atmosphere and using oxygenated solutions. The enterocytes were used between 20 and 60 min after their isolation. Cell viability was assessed by trypan blue exclusion in dispersed cell preparations; 85–90% of the cells were viable for at least 150 min. Phase-contrast microscopy of preparations revealed no morphological differences between enterocytes isolated from young and old rats as in previous studies [25].

2.4. In vitro treatments

Isolated duodenal cells were preequilibrated in Solution D for 20 min and then exposed for short intervals (30 s to 5 min) to different doses of PTH [1–34] (10^{-10}–10^{-7} M) or vehicle (H_2O) alone.

2.5. Cellular fractionation

Scrapped duodenal mucosae of young and aged rats were homogenized for 40 s in 20 mM Tris–HCl, pH 7.4, 0.33 M sucrose, 1 mM EGTA, 0.7 mM $CaCl_2$, 20 mM NaF, 0.5 nM phenylmethylsulfonyl fluoride (PMSF), 1 mM DTT, 2 μg/ml leupeptin, and 2 μg/ml aprotinin with a glass homogenizer. The homogenate was centrifuged at $12{,}000 \times g$ for 15 min in a Sorvall refrigerated centrifuge and the pellet obtained was discarded [26]. The protein content of the postmitochondrial fraction was measured according to Lowry et al. [27].

2.6. Measurement of inositol trisphosphate

The postmitochondrial fraction was exposed to PTH (10^{-9} M) or vehicle. Anti-Gαq/11 antibody (1:250) was added to the samples together with 30 mM $LiCl_3$ and incubated for 10 min on ice, followed by hormone treatment. Immediately thereafter, aliquots were transferred to ice-cold trichloracetic acid (TCA, 20% final concentration), mixed thoroughly, left for 15 min on ice, and centrifuged at $1000 \times g$ during 10 min at 4°C. TCA was extracted four times with 5 vol. of water-saturated diethylether. The extract (final pH 4.5) was used for measurement of IP_3 with a commercial radioreceptor kit [28].

2.7. Immunoprecipitation

Enterocytes were treated with PTH and then lysed in 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 2 mM EGTA, 25 mM NaF, 0.2 mM sodium orthovanadate, 1 mM PMSF, 2 μg /ml leupeptin, 2 μg /ml pepstatin, 2 μg /ml aprotinin, 0.25% sodium deoxycholate, and 1% NP40. Insoluble material was pelleted in a microcentrifuge at 14,000 rpm for 10 min. The protein content of the clear lysates was determined according to Lowry et al. [27]. Aliquots (500–700 μg protein) were incubated overnight at 4°C with anti-PLCγ antibody,

followed by precipitation of the complexes with protein A conjugated with Sepharose. The immune complexes were washed five times with cold immunoprecipitation buffer (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 0.2 mM PMSF, 0.2 mM sodium orthovanadate, 1% Triton X-100, and 1% NP40) and then subjected to Western blot analysis.

2.8. Western blot analysis

Proteins were separated by one-dimensional SDS-PAGE [29]. Briefly, samples were mixed with 2 × Laemmli sample buffer (250 mM Tris–HCl pH 6.8, 8% SDS, 40% glycerol, 20% 2-mercaptoethanol, and 0.02% bromophenol blue) and heated for 5 min at 95°C. Proteins (25 μg) were subjected to electrophoresis on 10% SDS-polyacrylamide minigels and then transferred to Immobilon P (PVDF) membranes. The membranes were immersed in TBS buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl) containing 5% skim milk for 2 h to block nonspecific binding. The membranes were then twice washed (5 min) with TBS-T (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.05% Tween 20), followed by one 10-min wash with TBS-T. Anti-phosphotyrosine antibody was diluted to 1: 250 in TBS-T and was allowed to react with the membrane overnight at 4°C. After washing with TBS-T, the membranes were incubated with 1 μg/ml of peroxidase-labeled goat anti-rabbit IgG antibody or goat anti-mouse IgG antibody in TBS-T for 1 h at room temperature. After two washes with TBS-T, the membrane was visualized by using an enhanced chemiluminescent technique (ECL, Amersham), according to the manufacturer's instructions. Images from Immobilon P membranes were obtained with a Hewlett Packard IIp scanner at 300 dpi and printed at the same resolution. The relative amounts of immune complexes were compared by the Band Leader Program written by Ma'ayan Aharaoni (copyright 1993, taken from Internet).

2.9. Statistical evaluation

The significance of the results was evaluated by Student's *t* test [30].

3. Results

To identify the PI-PLC isozymes involved in PTH-induced polyphosphoinositide hydrolysis, we first evaluated the involvement of a G-protein-coupled PLC. To that end, we employed antibodies directed against the α subunit of the transducing protein Gq/11 and measured IP_3 release in duodenum isolated from 3- and 24-month-old rats with a radioreceptor assay. As shown in Fig. 1, in agreement with our previous observations [12] treatment of duodenal cells from young rats with 10^{-9} M PTH for 30 s significantly increased IP_3 release (+71%), the hormone effects being

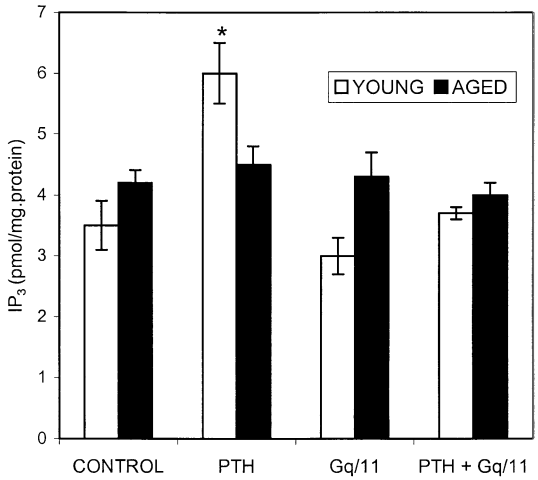

Fig. 1. Effects of anti-Gα q antibody on PTH-induced IP_3 production in rat duodenum. Duodenal mucosae postmitochondrial fractions isolated from young (3 months) and aged (24 months) rats were preincubated on ice for 10 min in the presence of anti-Gαq (1:250) antibody followed by exposure to 10^{-9} M PTH [1–34] for 30 s. IP_3 was extracted and measured as described in the Methods section. Results are the average ± S.D. of three independent experiments performed in triplicate. * $P < .005$ with respect to the control.

blunted in old animals. Preincubation with anti-Gαq/11 antibody diminished the PTH-mediated increase in IP_3 generation in young enterocytes to a great extent (+21% respect to the control pretreated with anti-Gαq/11), suggesting the involvement of the α subunit of Gq/11 in PTH stimulation of a PLCβ isozyme.

To determine whether PLCγ has a role in PTH signaling, and since the enzyme is regulated by tyrosine phosphorylation [31], we investigated the effects of PTH on the tyrosine phosphorylation of this isozyme. To evaluate the time-dependence of the hormone effects on PLCγ tyrosine phosphorylation, proteins in lysates from enterocytes treated with to 10^{-9} M PTH [1–34] for 30 s to 5 min were immunoprecipitated with anti-PLCγ, separated by SDS-PAGE, and immunoblotted with anti-phosphotyrosine antibody. As shown in Fig. 2A, we found that treatment of young rat duodenal cells with PTH markedly increased PLCγ-tyrosine phosphorylation. The effect was already significant at 30 s (+70%), reached highest stimulation at 1 min (+180%) and returned to near basal tyrosine phosphorylation levels by 5 min. In cells from aged animals, the action of the hormone was greatly diminished (38% and 70% at 30 s and 1 min, respectively) (Fig. 2B). When the order of antibody addition was reversed and anti-phosphotyrosine immunoprecipitates were probed with anti-PLCγ antibody, a similar time-course of PLCγ tyrosine phosphorylation was detected (not shown). To verify that the observed changes in tyrosine phosphorylation did not reflect differences in the amounts of PLCγ precipitated by the anti-PLCγ antibody, in above experiments immunoblots were stripped and reprobed with anti-PLCγ antibody. Equal amounts of PLCγ were shown to be

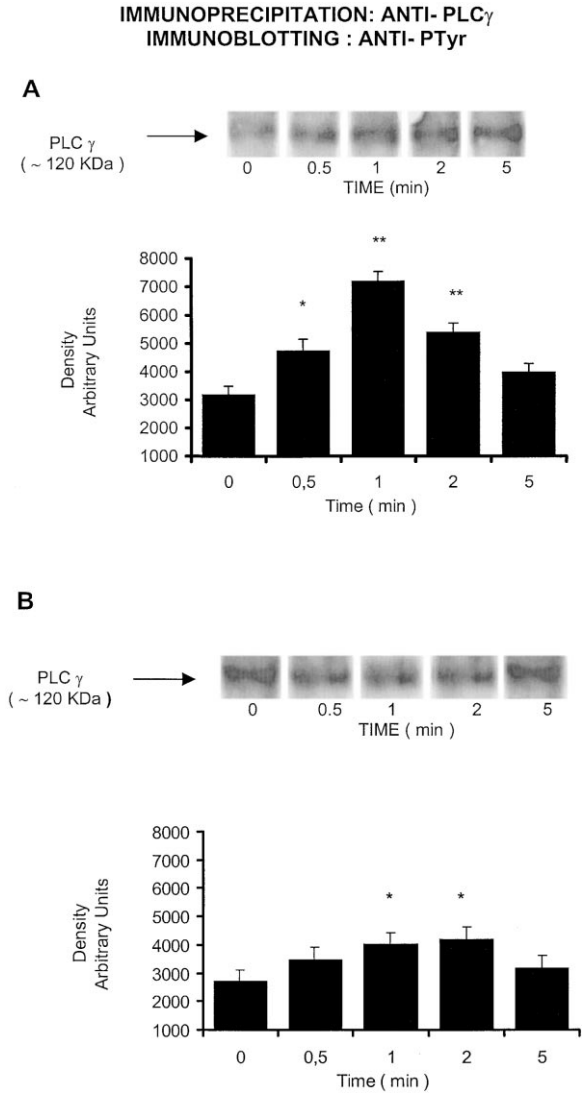

Fig. 2. Time course of PTH-induced PLCγ phosphorylation. Enterocytes isolated from young (3 months) and aged (24 months) rats were treated with 10^{-9} M PTH [1–34] for 30 s to 5 min. After cell lysis and immunoprecipitation with anti-PLCγ antibody, immunoprecipitated proteins were separated by SDS-PAGE followed by Western blotting with anti-phosphotyrosine as described under the Methods section. Representative immunoblots from young (A) and aged (B) rats. Quantification by scanning volumetric densitometry of blots from three independent experiments; averages ± S.D. are given (lower panels). (A) $*P<.035$, $**P<.01$ and (B) $*P<.05$.

precipitated by the antibody at each of the time points (data not given). In addition, dose–response studies with young enterocytes evidenced comparable effectiveness of 10^{-9}–10^{-10} M PTH to increase PLCγ phosphorylation, whereas increasing the concentration of the hormone to 10^{-8}–10^{-7} M decreased its potency after 1 min of treatment (Fig. 3A). Again, the PTH response in aged rats was markedly lower than in young animals at all hormone levels tested (Fig. 3B).

Pretreatment of young and aged enterocytes with the tyrosine kinase inhibitors genistein (100 μM) and herbimycin (2 μM) completely prevented PTH-induced phosphorylation of PLCγ (Fig. 4). At the concentrations employed or higher (up to 370 μM) genistein has been previously shown not to alter cAMP-dependent kinase (PKA), PKC, and phosphorylase kinase in other cell types [32–34]. Moreover, daidzein, an inactive analog of genistein, at concentrations as high as 100 μM did not block the increase in PLCγ phosphorylation caused by PTH (not shown). Herbimycin inhibits protein tyrosine kinases with higher selectivity than genistein [35,36].

The effect of PTH on PLCγ phosphorylation in duodenal cells from young rats was further characterized. As can be seen in Fig. 5, in contrast to PTH [1–34], treatment with the

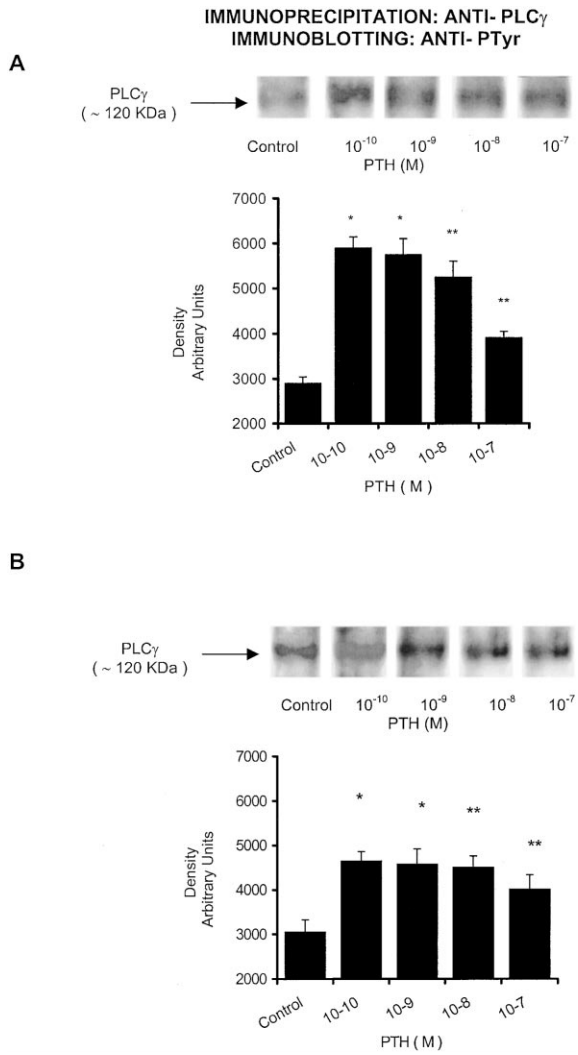

Fig. 3. PTH stimulates PLCγ phosphorylation in a dose-dependent fashion. Enterocytes isolated from young (3 months) and aged (24 months) rats were exposed for 1 min to 10^{-7}–10^{-10} M PTH [1–34]. The cells were then lysed and immunoprecipitated with anti-PLCγ antibody and protein A–Sepharose. The immunoprecipitates were analyzed by SDS-PAGE followed by anti-phosphotyrosine immunoblotting as described under the Methods section. A representative immunoblot from three independent experiments and its quantification is shown. (A) Young and (B) aged enterocytes. (A) $*P<.01$, $**P<.025$ and (B) $*P<.025$, $**P<.05$.

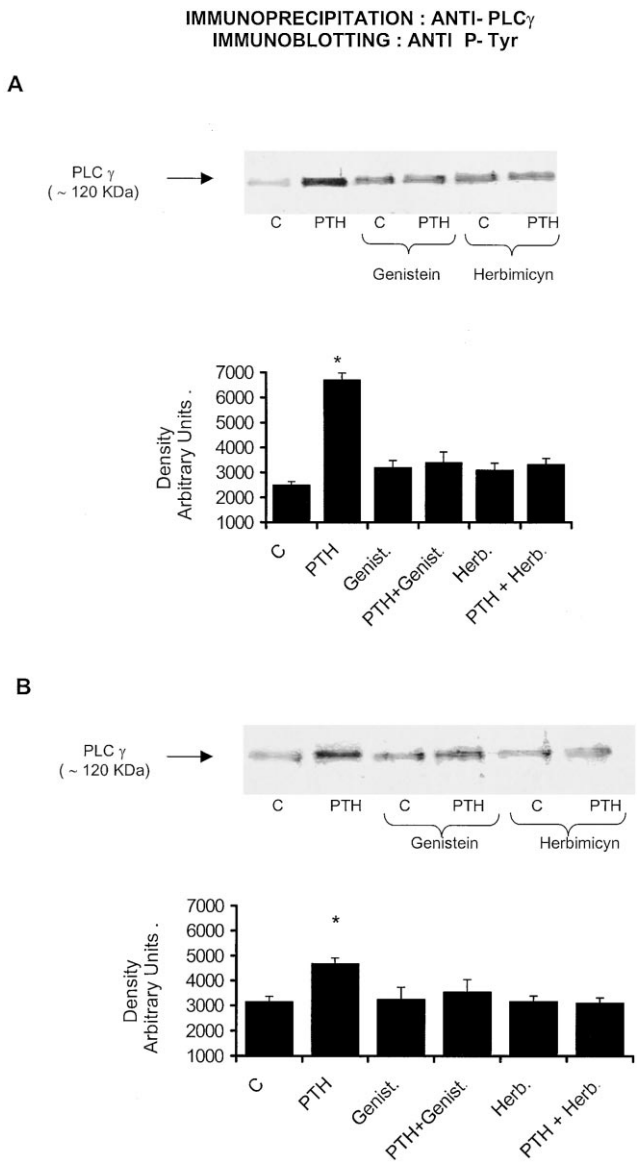

Fig. 4. PTH-induced PLCγ phosphorylation is suppressed by genistein and herbimycin. Enterocytes isolated from young (3 months) and aged (24 months) rats were exposed for 1 min to 10^{-9} M PTH [1–34], in the absence or presence of genistein (100 μM) or herbimycin (50 μM). Cell lysates were immunoprecipitated and immunoblotted as described in the legend of Fig. 2. A representative immunoblot from three independent experiments and its quantification is shown. (A) Young and (B) aged enterocytes. * $P < .01$.

NH_2-terminal-shortened fragment of the hormone, PTH [7–34], was ineffective in increasing PLCγ phosphorylation above basal levels. Stimulation of PLCγ by tyrosine phosphorylation [31] involves the cytosolic tyrosine kinase Src in other cell types [37,38]. To evaluate whether Src is part of the PTH signaling mechanism in rat enterocytes, we first investigated the effect of the Src family tyrosine kinase-selective inhibitor PP1 [39] on PLCγ phosphorylation induced by the hormone. To that end, cells were pretreated with 10 μM PP1, followed by exposure to 10^{-9} M PTH for 1 min. Under these conditions, the effects of the hormone on

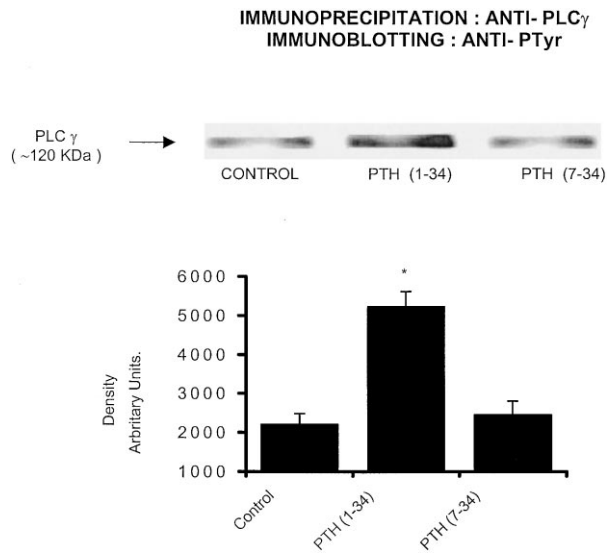

Fig. 5. Effect of PTH [1–34] and PTH [7–34] on PLCγ phosphorylation. Enterocytes isolated from young (3 months) rats were exposed for 1 min to 10^{-9} M PTH [1–34] and PTH [7–34]. Cell lysates were immunoprecipitated and immunoblotted as described in the legend of Fig. 2. A representative immunoblot from three independent experiments and its quantification is shown. * $P < .01$.

PLC phosphorylation were completely abolished (Fig. 6). We then investigated hormone-dependent decrease in tyrosine phosphorylation of Src, an established major mechanism of Src activation [40,41]. For this purpose, the enzyme from lysates of cells exposed to different concentrations of PTH (10^{-10}–10^{-8} M) for 1 min was immunoprecipitated with a highly specific anti-Src monoclonal antibody fol-

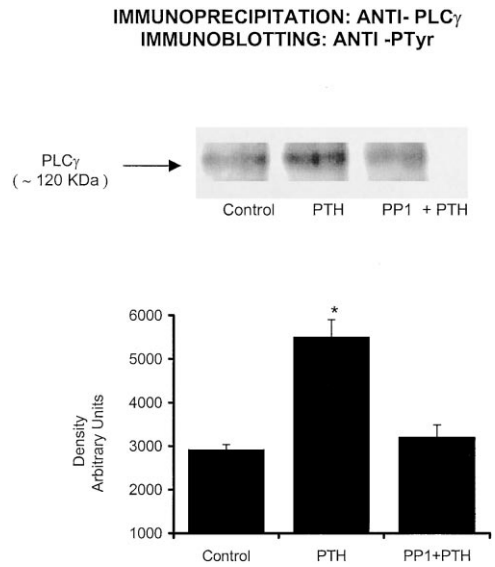

Fig. 6. PTH-dependent PLCγ phosphorylation is suppressed by the specific Src inhibitor PP1. Enterocytes isolated from young (3 months) rats were exposed for 1 min to 10^{-9} M PTH [1–34], in the absence or presence of PP1 (10 μM). Cell lysates were immunoprecipitated and immunoblotted as described in the legend of Fig. 2. A representative immunoblot from three independent experiments and its quantification is shown. * $P < .01$.

lowed by immunoblotting with anti-phosphotyrosine antibody. As shown in Fig. 7A, Src tyrosine phosphorylation was significantly decreased at PTH concentrations of

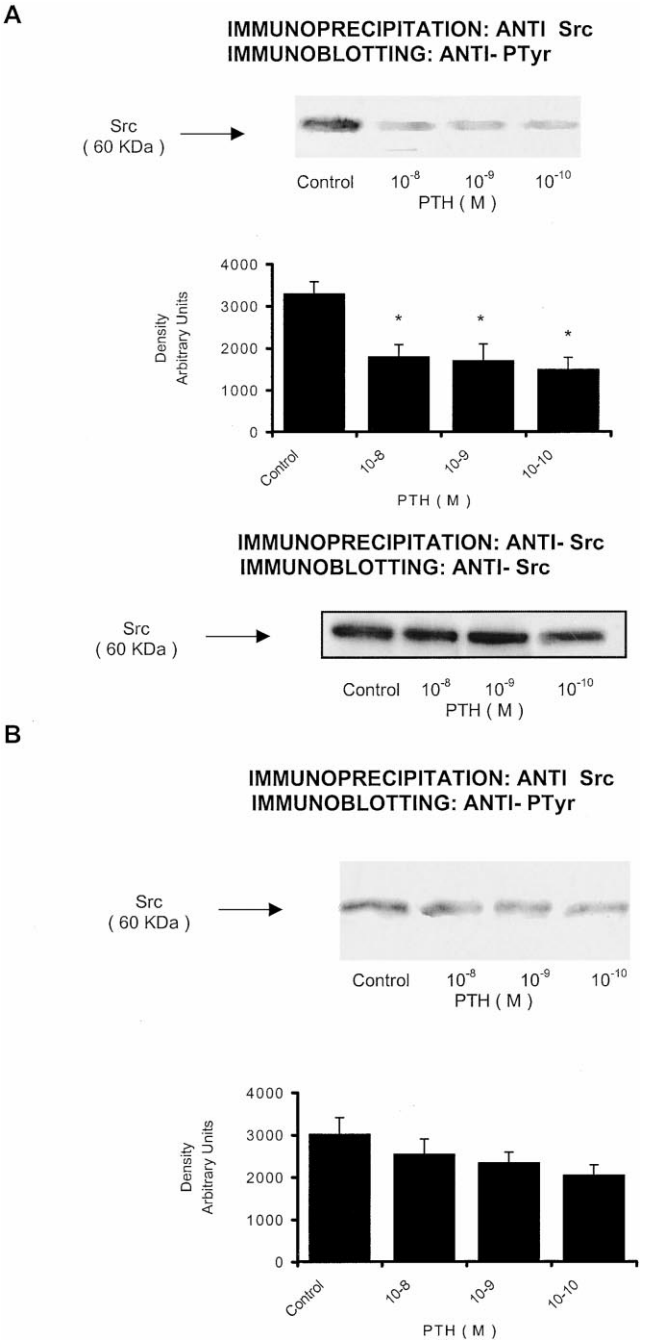

Fig. 7. Tyrosine-phosphorylated state of Src under PTH treatment. Enterocytes isolated from young (3 months) rats were exposed for 30 s to 10^{-10}–10^{-8} M PTH [1–34]. Cell lysates were obtained, Src was immunoprecipitated, and its tyrosine phosphorylation state was assayed by immunoblotting with anti-phosphotyrosine antibody as described under the Methods section. Representative immunoblots from three independent experiments. (A) Young and (B) aged enterocytes. Blotted membrane shown in panel (A) was reprobed with anti-Src antibody as indicated in the Methods section in order to evaluate equivalence of Src kinase content among the different experimental conditions. * $P < .01$.

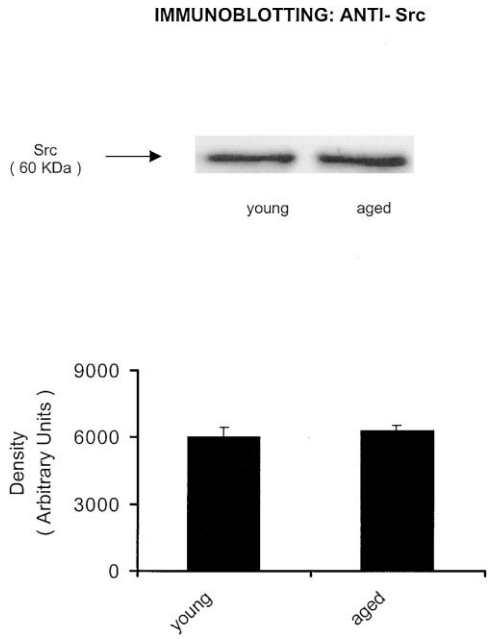

Fig. 8. Levels of Src in enterocytes from young and old rats. Cell lysates of enterocytes isolated from young (3 months) and aged (24 months were analyzed by SDS-PAGE followed by immunoblotting with anti-Src antibody as described under the Methods section. A representative immunoblot from three independent experiments and its quantification is shown.

10^{-10}–10^{-8} M (75–80% with respect to the control). Immunoblotting with anti-Src antibody confirmed that equivalent amounts of Src were present in samples from control and PTH-treated cells. These data together with those of Fig. 6 indicate that activation of Src by dephosphorylation is involved in PTH stimulation of PLCγ in young rat enterocytes. Fig. 7B reveals that this mechanism is altered during ageing explaining thereby the impairment of hormone-induced PLCγ tyrosine phosphorylation in old enterocytes shown before (Figs. 2–4). The possibility that a decrease in Src expression levels upon ageing may contribute to these differences was excluded as immunoblot analysis using an anti-Src antibody showed equal amounts of Src protein in young and old animals (Fig. 8).

4. Discussion

Mammalian phosphoinosite-specific PLC (PI-PLC) plays a key role in signal transduction by catalyzing hydrolysis of PIP_2 to yield the two second messengers IP_3 and DAG. Both products of PLC catalysis mediate release of intracellular calcium and activation of PKC. Ten mammalian PLC isozymes have been reported: PLCβ1–4, PLCγ1–2, and PLCδ1–4. These isoforms are distinguished by their mode of regulation. Association with heterotrimeric G-protein subunits stimulates the β-class of isozymes while the γ-class is regulated by tyrosine kinases. Regulation of the δ-class of isoforms has not been fully characterized [42].

We previously reported that in rat duodenum, PTH stimulates PI-PLC-mediated generation of IP_3 and DAG and that this mechanism is severely altered in old animals [12]. In the present study, we showed that the antibody anti-Gαq/11 blocks ca. 70% of PTH-dependent IP_3 release in duodenum from young rats, indicating that the hormone activates a β isoform of PLC coupled to the α subunit of Gq/11. Generally, receptor-induced stimulation of PLC has been shown to occur via PTX-insensitive Gαq/11 or via Gβγ from activated PTX-sensitive Gi [43] and, in agreement with our observation, PTH/PTHrP receptors have been shown to couple to Gαq family members in other cell types [44,45]. The efficiency of interaction between G-protein-coupled receptors and heterotrimeric G-proteins is greatly influenced by the absolute and relative amounts of these proteins in the plasma membrane [46]. In this regard, we recently reported reduced levels of Gαq/11 protein expression in enterocytes from 24-month-old rats as compared with 3-month-old animals [47]. In other cell types [48], diminished IP_3 production with ageing has also been related to a decrease of metabolically active phosphoinositides (PI, PIP, PIP_2) in the plasma membrane, which are precursors of IP_3 and DAG. Moreover, composition changes in the plasma membrane and cytoskeleton accompany the ageing process in human and experimental animals [49,50].

In addition to a PLCβ isoform, we found that PTH also stimulates, in a dose-dependent fashion, the phosphorylation of PLCγ in rat enterocytes. The hormone response was very rapid, with maximal effects achieved between 30 s and 1 min, and was suppressed by the tyrosine kinase inhibitors, genistein and herbimycin. Because PTH/PTHrP receptor has no intrinsic tyrosine kinase activity, hormone stimulation must activate one or more cytosolic tyrosine kinases. Suppression of PTH effects by PP1, a specific Src family tyrosine kinase inhibitor, indicates that the hormone stimulates PLCγ phosphorylation via the nonreceptor tyrosine kinase Src in duodenal cells. It is well established that phosphorylation of a conserved tyrosine residue (Y527) at the carboxy-terminal tail of the Src molecule negatively modulates its kinase activity by a mechanism that apparently implies intramolecular interaction of the carboxy-terminal-phosphorylated tyrosine with the SH2 domain of Src [40,41]. Therefore, a possible way by which PTH stimulates Src could be, at least in part, by altering the state of tyrosine phosphorylation of the enzyme. In agreement with our observations, changes in Src tyrosine phosphorylation and activity induced by PTH have been previously reported in osteoblastic cells [51]. The mode of signal transduction from the PTH receptor to nonreceptor Src tyrosine kinase is not known at present. Recent studies in COS-7 cells suggest that the βγ subunits of G-protein-coupled receptors mediate activation of Src family of nonreceptor tyrosine kinases [52]. Further studies are necessary to evaluate whether PTH employs a similar pathway in rat enterocytes.

Western analysis and immunoprecipitation demonstrated that the above described mechanism of PTH-induced PLCγ phosphorylation in duodenal cells is greatly decreased in old animals. Age-related alterations in signaling events related to protein tyrosine kinase activity and G-protein function have been less studied. Decreased protein tyrosine phosphorylation by FMLP in granulocytes and lymphocytes with ageing has been reported [53].

In summary, the present study demonstrates several previously unrecognized features of PTH signaling in rat enterocytes: generation of IP_3 through a G-protein-coupled PLCβ isoform and stimulation of PLCγ phosphorylation via activation of the cytosolic tyrosine kinase cSrc, effects that are severely altered by ageing. These alterations, coupled with G-protein changes found with ageing, may combine to produce an impairment of PLC activity with advanced age that may result in abnormal PTH regulation of cell Ca^{2+} and proliferation in the duodenum.

Acknowledgments

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