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RESEARCH ARTICLE

PTH regulation of c-Jun terminal kinase and p38 MAPK cascades in intestinal cells from young and aged rats

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Abstract In the present study, we examined the role of Parathyroid hormone (PTH) on the c-Jun N-terminal kinase (JNK) 1/2 and p38 mitogen-activated protein kinase (MAPK) members of the MAPK family as it relates to ageing by measuring hormone-induced changes in their activity in enterocytes isolated from young (3 month old) and aged (24 month old) rats. Our results show that PTH induces a transient activation of JNK 1/2, peaking at 1 min (+threefold). The hormone also stimulates JNK 1/2 tyrosine phosphorylation, in a dose-dependent fashion, this effect being maximal at 10 nM. PTH-induced JNK 1/2 phosphorylation was suppressed by its selective inhibitor SP600125. Moreover, hormone-dependent activation of JNK 1/2 was dependent on calcium, since pretreatment of cells with BAPTA-AM or EGTA blocked PTH effects. With ageing, the response to PTH was significantly reduced. JNK basal protein expression was not different in the enterocytes from young and aged rats, however, basal protein phosphorylation increased with ageing. PTH did not stimulate, within 1–10 min, the basal activity and phosphorylation of p38 MAPK in rat intestinal cells. The hormone increased enterocyte DNA synthesis; the response was dose-dependent and decreased (-40%) with ageing. In agreement with the mitogenic role of the MAPK cascades, this effect was blocked by specific inhibitors of extracellular signal-regulated protein kinase (ERK) 1/2 and JNK 1/2. The results obtained in this work expand our knowledge on the mechanism of action of PTH in duodenal cells.

Keywords PTH · Enterocytes · JNK · p38 MAPK · Proliferation · Ageing

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Introduction

Parathyroid hormone (PTH) is a major regulator of calcium and phosphate metabolism. The peptide composed of 84-aminoacids, is the main secreted and circulating form of bioactive PTH with bone and kidney as main target organs (Rosenblatt et al. 1989). Through its effects on the renal 1α-hydroxylase, PTH stimulates the synthesis of the hormonally active form of vitamin D, 1,25$(OH)_2$-vitamin D_3, which in turn functions at the level of the duodenum, increasing the absorption of dietary calcium (Garabedian et al. 1972; Horiuchi et al. 1976). In intestinal cells, as well as in its main target tissues, PTH interacts with a G-protein-coupled membrane-bound receptor (Abou-Samra et al. 1989; Gentili et al. 2003a, b).

Signal transduction of PTH through the plasma membrane of intestinal cells involves both stimulation of adenylyl cyclase (AC) with cAMP production (Picotto et al. 1997) and phospholipase C-dependent hydrolysis of membrane-associated phosphatidylinositol 4,5-bisphosphate, leading to generation of inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG) (Massheimer et al. 2000). After second messenger formation, cAMP activates protein kinase A (PKA), IP_3 releases calcium from intracellular stores and DAG causes activation of protein kinase C (PKC), and in turn activation of calcium channels (Massheimer et al. 2000; Gentili et al. 2003a, b).

Emerging evidence indicates that PTH can regulate the activity of mitogen-activated protein kinases (MAPK) in a cell-specific and G protein type-dependent manner (Homme et al. 2004; Gesty-Palmer et al. 2006; Mahon et al. 2006). In rat enterocytes, PTH rapidly activates in a dose-dependent fashion the growth-related proteins extracellular signal-regulated protein kinase (ERK) 1 and 2 (Gentili and Russo de Boland 2000). Furthermore, c-Src kinase, PI3K, the AC/cAMP/PKA pathway and Ca^{2+} play a role upstream in the signalling pathway leading to ERK 1/2 activation by PTH in rat intestinal cells (Gentili et al. 2001, 2002). With ageing, the ERK response of rat enterocytes to PTH is significantly reduced (Gentili and Russo de Boland 2000). However, alterations of the direct effects of PTH on duodenal cell signalling with aging remain incompletely understood.

Mitogen-activated protein kinases are a family of Ser/Thr protein kinases that are activated by phosphorylation in response to a wide array of extracellular stimuli (Seger and Krebs 1995; Schaeffer and Weber 1999). The MAPK superfamily consists of three main protein kinase families: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases (JNKs) and the p38 family of kinases. These enzymes are regulated by a characteristic phosphorelay system in which a series of three proteins kinases phosphorylate and activate one another. Each is proving to have major roles in the regulation of intracellular metabolism and gene expression and integral actions in many areas including growth and development, disease, apoptosis and cellular responses to external stresses (Chang and Karin 2001).

To further elucidate the MAPK pathways stimulated by PTH in rat intestinal cells, we have investigated whether the hormone activates JNK 1/2 and p38 MAPK and examined the influence of ageing on these early signals elicited by PTH. We also investigated their relative contribution to hormonal regulation of intestinal cell proliferation.

Material and methods

Chemicals

Synthetic rat PTH (1–34) and Immobilon P (Polyvinylidene difluoride, PVDF) membranes were from Sigma-Aldrich Co. (St. Louis, MO, USA). Rabbit polyclonal anti-phospho JNK1/2, rabbit anti-JNK1/2, rabbit anti-phospho p38 MAPK and anti-p38 α antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). JNK and p38 MAPK assay kits were from Cell Signaling Technology Inc. (Beverly, MA, USA). Compounds SB 202190 and PD 98059 were from Tocris Cookson Inc. (Ellisville, MO, USA). BAPTA/AM (1,2-bis(*o*-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic tetra(acetoxymethyl)ester) was from Calbiochem (San Diego, CA, USA). Secondary antibody goat anti-rabbit horse-radish peroxidase-conjugated IgG and the Super Signal CL-HRP substrate system for enhanced chemiluminiscence (ECL) were obtained from Amersham Corp. (Arlington Heights, IL, USA). All other reagents were of analytical grade.

Animals

Three and 24-month-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 h light–12 h dark cycle. Animals were sacrificed by cervical dislocation. Animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals [1996 (7th ed.) Washington, DC: National Academy Press, Aka National Research Council Guide].

Isolation of duodenal cells

Duodenal cells were isolated as previously described (Massheimer et al. 1994). The method employed yields preparations containing only highly absorptive epithelial cells that are devoid of cells from the upper villus or crypt (Weiser 1973). The duodenum was excised, washed and trimmed of adhering tissue. The intestine was slit lengthwise, 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_2H-PO_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_2-PO_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 155*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.

In vitro treatments

Isolated duodenal cells were pre-equilibrated in solution D for 10 min and then exposed to PTH (10^{-8} M) for short-intervals (0.5–10 min). After treatment, enterocytes were 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 phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 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 Bradford (1976).

SDS-PAGE and immunoblotting

Immunoprecipitated proteins (or lysate proteins) dissolved in Laemmli sample buffer were separated on SDS-polyacrylamide (10%) gels (Laemmli 1970) and electrotransferred to PVDF membranes. The membranes were blocked for 1 h at room temperature in TBST (50 mM Tris–HCl pH 7.4, 200 mM NaCl, 0.1% Tween 20 containing 5% dry milk). Anti-phospho JNK1/2, and anti-phospho p38 MAPK were allowed to react with the membrane overnight at 4°C. Next, the membranes were washed three times in TBST, incubated with a 1:10,000 dilution of peroxidase-conjugated anti-rabbit secondary antibody for 1 h at room temperature and washed three additional times with TBST. The membranes were then visualized using an enhanced chemiluminescent technique, according to the manufacturers instructions. Images were obtained with a model GS-700 Imaging Densitometer from Bio-Rad (Hercules, CA, USA) by scanning at 600 dpi and printing at the same resolution. Bands were quantified using the Molecular Analyst program (Bio-Rad).

JNK activity assay

The in vitro activity of JNK was measured using a stress-activated protein kinase (SAPK)/JNK assay kit (Cell Signaling Technology, Beverly, MA, USA) according to the instruction from the manufacturer. Briefly, cell lysates prepared as described above were incubated with GST-c-Jun (1–89) fusion protein bound to glutathione agarose beads overnight. The immunoprecipitated JNK was washed thoroughly and resuspended in kinase buffer containing ATP. The reaction mixture was incubated at 30°C for 30 min and terminated by adding SDS sample buffer. Phosphorylation of GST-c-Jun on Ser63 was analysed by immunoblotting using specific phospho-c-Jun antibody.

p38 MAPK activity assay

The activity of p38 MAPK was analysed by using a p38 MAPK assay kit. Phosphorylated p38 was immunoprecipitated with a p38-phospho-specific antibody from 200 μg of lysate; this antibody specifically recognized phosphorylated p38 and did not cross-react with phosphorylated JNK or ERK1/2. The immune complex was washed thoroughly and resuspended in kinase buffer contain-

ing ATP and 1 μg of recombinant activating transcription factor-2 (ATF-2) as a p38 MAPK substrate. The reaction was incubated at 30°C for 30 min and terminated by adding SDS sample buffer. The kinase reaction was analysed by Western blotting with a phospho-specific anti-ATF-2 antibody (Banerjee et al. 2002).

Thymidine incorporation

The rate of thymidine incorporation into DNA was determined by adding 2 μCi [^{3}H]thymidine (20 Ci/mmol)/ml solution D to enterocytes followed by incubation for 2 h at 37°C under O_2/CO_2 (95%/5%). The cells were then centrifuged and washed four times with solution D. DNA and proteins were precipitated with ice-cold 12% trichloroacetic acid, dissolved in 1 N NaOH and the radioactivity was counted in a liquid scintillation counter.

Statistical evaluation

Statistical significance of the data was evaluated using Student's *t*-test (Snedecor and Cochran 1967) and probability values below 0.05 ($p < 0.05$) were considered significant. Results are expressed as means ± standard deviation (SD) from the indicated set of experiments.

Results

In this study, we investigated, for the first time, whether PTH modulates JNK and p38 MAPK in intestinal cells and the influence of ageing on this putative regulatory action of the hormone. We first tested the effects of PTH on JNK activity and phosphorylation in enterocytes from young (3 months) and aged (24 months) rats. For this purpose, cells were exposed for 30 s to 5 min to 10 nM PTH. The activity of JNK was assayed in vitro with GST-c-Jun (1–89) fusion protein as substrate. As shown in Fig. 1a, control cells showed low or undetectable levels of activated JNK; PTH caused a time-dependent, but non-linear, increase in JNK activity in young animals, which peaked at 1 min (two-fold) and remained elevated up to 5 min. Preincubation of enterocytes with SP600125 (20 μM), a highly specific inhibitor of JNK (Bennett et al. 2001), significantly blocked PTH-dependent increase of JNK activity (Fig. 1b). To monitor JNK phosphorylation, cell lysates were subjected to SDS-PAGE and then immunoblotted with anti-active JNK1/2 polyclonal antisera. These antibodies recognize only the dual phosphorylated active forms of JNK 1/2. As shown in Fig. 2a, PTH in a fast way increased JNK 1/2 phosphorylation in young enterocytes, the kinetics for this change in phosphorylation being similar to those found for the

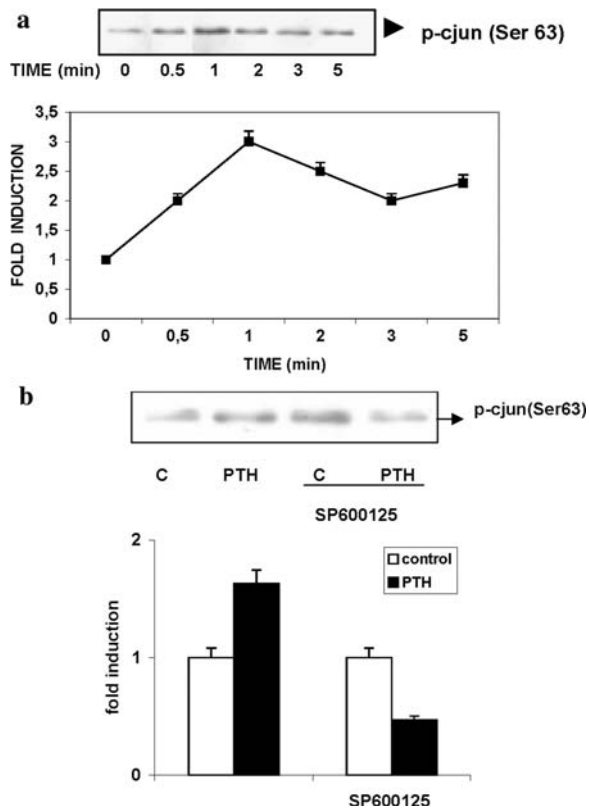

Fig. 1 PTH increases JNK activity in rat intestinal cells. a Enterocytes isolated from young (3 months) rats were treated with 10 nM PTH for 0.5–5 min. Cell lysates were incubated with GST-c-Jun (1–89) fusion protein overnight. The immunoprecipitated JNK was assayed for its kinase activity, as described under Methods. b Enterocytes were preincubated 10 min in the presence or absence of JNK specific pharmacological inhibitor SP600125 (20 μM), followed by exposure to 10 nM PTH for 1 min. Phosphorylation of GST-c-Jun was analysed by Western blotting using specific phospho-c-Jun (Ser63) antibody. Representative immunoblots and their quantification by scanning densitometry from three independent experiments ± SD are shown

increase in kinase activity. The effect of PTH was dose-dependent, with maximal JNK 1/2 phosphorylation achieved between 10 and 100 nM (Fig. 2b).

The role of calcium in the signalling cascade leading to the activation of JNK by PTH in intestinal cells has not yet been characterized. We then examined the effects of the intracellular

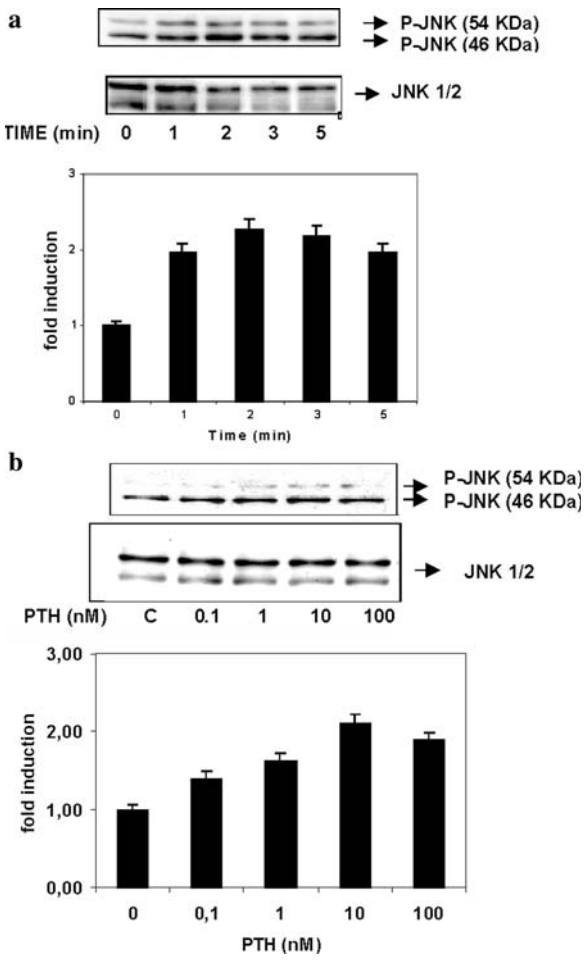

Fig. 2 Time-course and dose-response of changes in JNK1/2 phosphorylation in response to PTH. Enterocytes isolated from young (3 months) rats were treated with 10 nM PTH 1–5 min (a) or with several doses of PTH (0.1–100 nM) for 2 min (b). Cell lysates were subjected to SDS-PAGE electroforesis and blotted with anti-phospho-JNK1/2 antibody (P-JNK). To measure the amount of total protein loaded, the membrane was reprobed with antibodies against unphosphorylated JNK1/2. Representative immunoblots and their quantification by scanning densitometry from three independent experiments ± SD are shown

Ca^{2+} chelator 1,2-bis(*o*-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid, sodium (BAPTA-AM, 5 μM) and the extracellular Ca^{2+} chelator EGTA (0.5 mM) on hormone-induced JNK1/2 phosphorylation. As presented above, an optimal increase in enterocyte JNK1/2 phosphorylation was observed once the cells were stimulated with 10^{-8} M PTH for 2 min (Fig. 2a). We thus used these conditions to examine the effects of calcium chelation. As shown in Fig. 3, both BAPTA-AM and EGTA inhibited PTH-induced phosphorylation of JNK 1/2 in young enterocytes, suggesting a role of calcium as upstream mediator of PTH-dependent activation of the JNK pathway. Treatment with EGTA alone gave values greater than basal; this may be possibly due to the fact that when cells are depleted from extracellular Ca^{2+}, they liberate the cation from intracellular stores and thus activate the JNK cascade.

As shown in Fig. 4a, in enterocytes from 24-month-old rats the stimulation of phosphorylation of JNK 1/2 by PTH was greatly reduced (+0.5-fold) as compared to that from young rats (+2 fold) at the peak of activation. The amount of basal protein expression determined by Western blot analysis for JNK1/2 was not different in the enterocytes from young and aged rats (Fig. 4b), but basal JNK phosphorylation was higher in old animals and may explain the significant decline in PTH-dependent signalling in cells from old animals.

In this study, we also analysed p38 MAPK activation in response to PTH in rat intestinal cells. We tested the effects of the hormone on p38 MAPK activity and phosphorylation in enterocytes from young (3 months) rats. Cells were exposed for 1–10 min to 10^{-8} M PTH. The activity of p38 kinase was assayed in vitro with ATF-2 as substrate after immunoprecipitating the enzyme from cell lysates. The phosphorylation of p38 MAPK was assessed by subjecting whole cell lysates to SDS-PAGE and then immunoblotted with an antibody that reacts with the phosphorylated form of all p38 MAPK isoforms (α, β, γ, and δ). Treatment with PTH did not modify the basal activity (Fig. 5a) and phosphorylation (Fig. 5b) of p38 MAPK in young enterocytes.

In view of the role of MAP kinases in the regulation of cellular growth, studies were

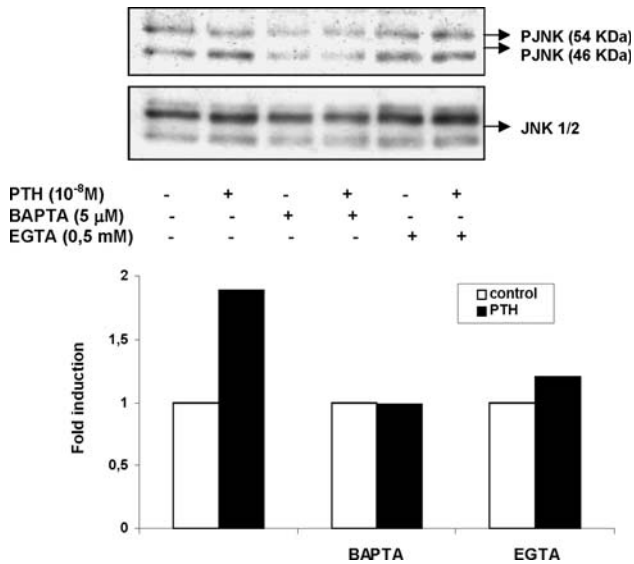

Fig. 3 Calcium dependence of PTH effects on JNK1/2 phosphorylation. Enterocytes isolated from young (3 months) rats were preincubated 10 min in the presence or absence of EGTA (0.5 mM) or BAPTA-AM (5 μM) followed by exposure to 10 nM PTH for 2 min. Cell lysates were subjected to SDS-PAGE electrophoresis and blotted with anti-phospho-JNK1/2 antibody (P-JNK). The immunoblots and their quantification by scanning densitometry are representative of three independent experiments

carried out to test whether PTH exerted a mitogenic action on duodenal cells through the observed mechanism of stimulation of MAPKs. Accordingly, Fig. 6 shows that PTH increased enterocyte DNA synthesis in a dose-dependent manner, with higher stimulation seen at 10^{-9} M (+30%), effect that was reduced with ageing (+18%,10^{-9} M). PTH-dependent increase in DNA synthesis was effectively suppressed by U0126 (10 μM), a specific inhibitor of ERK1/2 activation by the dual MAPK kinase MEK and by JNK1/2 inhibitor SP600125 (20 μM), while p38 MAPK inhibitor SB202190 (20 μM) did not affect hormone induced DNA synthesis in

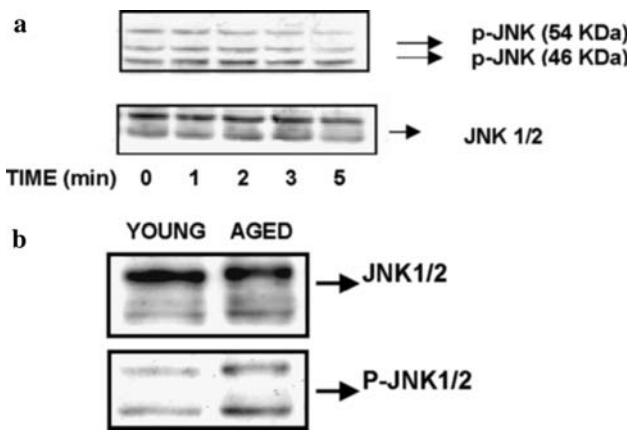

Fig. 4 A Stimulation of JNK1/2 phosphorylation by PTH is impaired with ageing. Enterocytes isolated from aged (24 months) rats were treated with 10 nM PTH 1–5 min and cell lysates were immunoblotted with anti-phospho-JNK1/2 antibody (P-JNK) as described in the legend to Fig. 2. b JNK1/2 basal protein and phosphorylation levels in enterocytes from young and aged rats. Cells lysates from enterocytes isolated from young (3 months) and aged (24 months) rats were immunoblotted with anti-JNK1/2 or anti-phospho-JNK1/2 antibodies (P-JNK). The same experiments were repeated three times and representative Western blots are shown

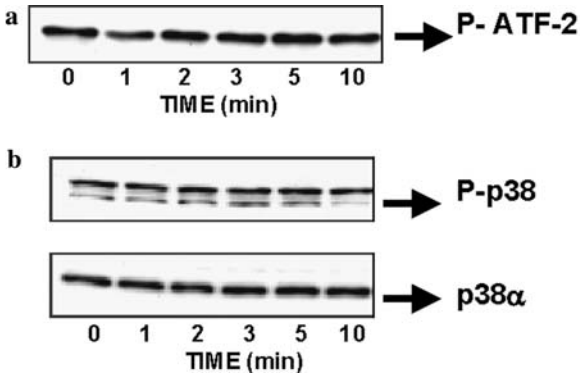

Fig. 5 Time-course of changes in p38 MAPK activity and phosphorylation in response to PTH. Enterocytes isolated from young (3 months) rats were treated with 10 nM PTH for 0–10 min. a Cell lysates were immunoprecipitated with anti-phospho-p38 MAPK antibody, followed by p38 MAPK kinase assay, using ATF-2 as a substrate, as described under Methods. b Cell lysates were subject to SDS-PAGE electroforesis and blotted with anti-phospho-p38 MAPK antibody (P-p38). To measure the amount of total protein loaded, the membrane was reprobed with antibodies against unphosphorylated p38α-MAPK. The immunoblots are representative of three independent experiments

young enterocytes, but partially suppressed it in cells from old animals (Fig. 7).

Discussion

The results of the present study are the first to demonstrate that PTH rapidly but transiently activates c-Jun NH2-terminal kinase in rat intestinal cells. JNK, also known as SAPK, represents one subgroup of MAPKs that is activated primarily by inflammatory cytokines and environmental stresses (Kyriakis and Avruch 1996). For the activation, JNK requires the dual phosphorylation of threonine and tyrosine residues located in a Thr-Pro-Tyr motif between kinase subdomains VII and VIII (Cobb and Goldsmith 1995). This phosphorylation is catalyzed by the dual specific kinases MKK4 (also known as SEK1 or MEK4) and MKK7 (SEK2), while MKK4 has a preference for the Tyr residue and MKK7 for the Thr residue (Weston and Davis 2002). In our study, enterocyte exposure to PTH increased the phosphorylation of 46 and 54 kDa JNK in a dose-dependent manner, with parallel increases in the kinase activity. Pretreatment of enterocytes with JNK specific inhibitor SP600125 suppressed PTH-induced JNK 1/2 activity. PTH regulation of MAPK cascades seems to vary depending on cell type. Opposite to intestinal cells, treatment of osteoblastic cells with PTH results in the inhibition of JNK activity from high-basal levels through activation of the PKA signalling cascade (Doggett et al. 2002) and via induction of MKP-1 synthesis (Homme et al. 2004).

One of the earliest PTH signals is an elevation in intracellular Ca^{2+}. In this regard, we have found that PTH elicits a rapid IP_3-mediated mobilization of Ca^{2+} from a thapsigargin-sensitive

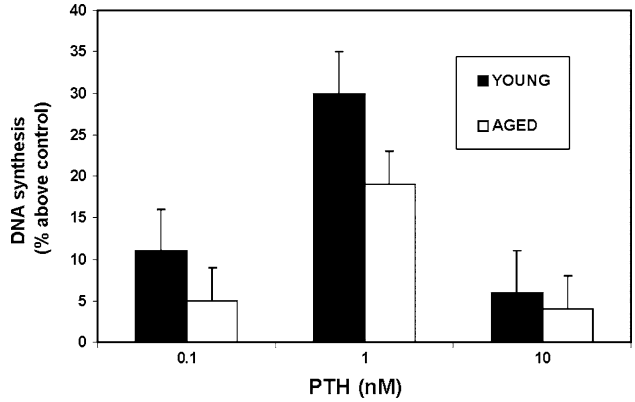

Fig. 6 PTH-induced DNA synthesis in rat enterocytes decreases with ageing. Enterocytes isolated from young (3 months) and aged (24 months) rats were incubated for 2 h with or without PTH (0.1–10 nM). DNA synthesis was measured by [^{3}H]-thymidine incorporation as described in Methods. Results are the average of three independent experiments ± SD

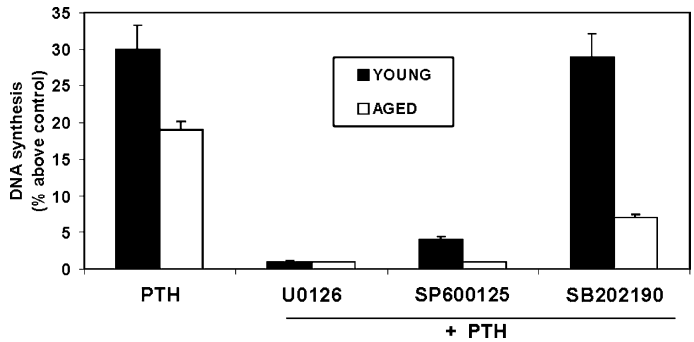

Fig. 7 Effect of inhibitors of MAP kinases on PTH-induced DNA synthesis. Enterocytes were incubated for 2 h with or without 1 nM PTH in the presence or absence of ERK inhibitor U0126 (10 μM), JNK inhibitor SP600125 (20 μM) or p38 MAPK inhibitor SB202190 (0.2 μM). DNA synthesis was measured by [^{3}H]-thymidine incorporation as described in Methods. Results are the average of three independent experiments ± SD

store and a sustained phase due to Ca^{2+} influx through voltage-dependent Ca^{2+}-channels (Massheimer et al. 2000; Gentili et al. 2003a, b). The relationship of the rise in intracellular Ca^{2+} by PTH leading to JNK1/2 activation is not defined. Therefore, in the present study, we also evaluated the participation of Ca^{2+} on PTH-dependent JNK 1/2 phosphorylation. Our results indicate that the Ca^{2+} signal precedes the activation of the kinase. The suppression of the PTH response by BAPTA and EGTA, strongly suggests that the stimulatory effect is specific for Ca^{2+}, consistent with a role for Ca^{2+} as an upstream activator of JNK 1/2 in enterocytes. Activation of JNK dependent on intracellular calcium signalling has been reported in other cell types (Inanami et al. 1999; Wylie et al. 1999; Xue et al. 2005). There is evidence that JNK phosphorylation and c-Jun expression occurs partly via extracellular Ca^{2+} influx through voltage-gated Ca^{2+} channels (Shimokawa et al. 2004). Furthermore, cooperative effects between G$\beta\gamma$-mediated signalling and increased intracellular Ca^{2+} levels have been shown to represent a robust mechanism for the stimulation of JNK by Gq-coupled receptors (Chan and Wong 2004).

There is considerable evidence indicating that hormonal regulation of signal transduction diminishes with age. In rat duodenal cells, we have demostrated that PTH-signalling is impaired with age. The hormone increased enterocyte $^{45}Ca^{2+}$ influx, the absolute levels of cAMP and AC activity to a greater extent in aged than in young rats. Moreover, the transient and biphasic production of IP_3 and DAG generated by PTH in these cells, is severely altered in old animals (Massheimer et al. 2000). Although the relative levels of p42 and p44 ERK do not change with age, the PTH-dependent ERK1/2 phosphorylation is significantly lower in enterocytes of aged rats compared with those of young animals (Gentili et al. 2001).

As aging progresses, the basal levels of activity of the stress-activated pathways increase, thereby becoming a basic factor in the development of a state of chronic stress in aged tissues and the ability of these stress-activated signalling pathways to respond is altered in aged tissues (Papaconstantinou 1994). In the present work, we found an age-related decline in JNK1/2 phosphorylation by PTH, that may be related to higher basal JNK phosphorylation in old animals. In line with our results, it has been shown that basal activities of JNKs increase with age in the brain (Suh 2001). In addition, *rac1*, a JNK activator involved in stress response, is up-regulated at old age (Lee et al. 1999). Moreover, age-related increases in JNK basal activities in vivo have been reported (Xiao and Majumdar 2000). The increase in basal activities of JNKs could be due to the well-documented increase in genotoxic stress during aging (Vijg 2000). Interestingly, in rat hepatocytes, no age-related differences in JNK activity in response to EGF treatment were reported (Liu et al. 1996).

Little is known of the functions of p38 MAPK in intestinal epithelial cells. Recent studies have

found that p38 MAPK activity appears to be involved in matrix adhesion and wound-induced signalling (Dieckgraefe et al. 1997; Goke et al. 1998; Yu et al. 2000), whereas the p38α isoform was identified as an important player in driving enterocyte differentiation (Houde et al. 2001). The results of the present investigation indicate that PTH does not affect the phosphorylation of p38 MAPK in rat enterocytes, at least at early time intervals. Of interest, Incubation of hypertrophic chondrocytes with PTH induces a PKC-dependent inhibition of p38 kinase activity (Zhen et al. 2001). So far, four p38 MAPK isoforms have been cloned and characterized, including p38α, p38β, p38γ and p38δ (Ono and Han 2000). The p38 upstream activators include MKK3 and MKK6. Its downstream effectors consist of kinases such as MK2 (MAPK-activating protein kinase 2) and PRAK (p38-related/activated protein kinase) and transcription factors including ATF-2 and MEF2 (myocyte enhancement factor 2) (Ono and Han 2000). In addition to these effectors, p38 can also signal through cross-talk with JNK (Nemoto et al. 1998; Chen et al. 2000) and ERKs (Xia et al. 1995; Oh et al. 2000).

Of physiological significance, in agreement with previous results (Gentili et al. 2001) and with the mitogenic role of the MAPK cascades, PTH increased enterocyte DNA synthesis, and to a greatest extent in young that in old animals.

The increased DNA synthesis in enterocytes from young animals is a major contributor of [^{3}H] thymidine incorporation in DNA during its replication, however, there is also a contribution to [^{3}H] thymidine incorporation due to DNA repair which could be less in enterocytes from old animals and this might have contributed to these results. Alterations in the regulation of DNA synthesis and cell division represent some of the most important functional manifestations of aging. A number of studies have demonstrated that the proliferative capacity of cells declines with aging (Macieira-Coelho 1988, 2003).

The effect of PTH was blocked by specific inhibitors of JNK and ERK in both, young and aged enterocytes, while p38 MAPK pharmacological inhibition also partially suppressed DNA synthesis in aged animals. In accordance with our results, it was reported that genetic inactivation of the stress signalling kinase, MKK7, a direct activator of JNKs in mice, results in embryonic lethality and impaired proliferation of hepatocytes (Wada et al. 2004).

In conclusion, our results provide evidence that expand our knowledge on the mechanism of action of PTH in duodenal cells, revealing that the hormone induces a rapid but transient activation of JNK1/2 cascade by a Ca^{2+}-dependent mechanism, which is linked to PTH regulation of intestinal cell proliferation, and that this mechanism is impaired with ageing. In addition, p-38 MAPK activity was not altered by PTH treatment of intestinal cells.

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