Biochemical and Biophysical Research Communications 461 (2015) 582–588

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Parathyroid hormone inhibition of Na^+/H^+ exchanger 3 transcription: Intracellular signaling pathways and transcription factor expression

Elida Adalgisa Neri, Camila Nogueira Alves Bezerra*, Gabriella Duarte Queiroz-Leite, Juliano Zequini Polidoro, Nancy Amaral Rebouças

Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

ARTICLE INFO

Article history:
Received 26 March 2015
Available online 16 April 2015

Keywords:
Proximal tubule
Transcriptional regulation
Sodium hydrogen exchanger
OKP cells

ABSTRACT

The main transport mechanism of reabsorption of sodium bicarbonate and fluid in the renal proximal tubules involves Na^+/H^+ exchanger 3 (NHE3), which is acutely and chronically downregulated by parathyroid hormone (PTH). Although PTH is known to exert an inhibitory effect on NHE3 expression and transcription, the molecular mechanisms involved remain unclear. Here, we demonstrated that, in opossum kidney proximal tubule (OKP) cells, PTH-induced inhibition of *Nhe3* gene promoter occurs even in the core promoter that controls expression of the reporter gene. We found that inhibition of the protein kinase A (PKA) and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways transformed PTH from an inhibitor of promoter activity into an activator of that same activity, as did point mutations in the EGR1, Sp1, and Sp3 binding consensus elements in the promoter. In nuclear extracts of PTH-treated OKP cells, we also observed increased expression of EGR1 mRNA and of some Sp3 isoforms. Electrophoretic mobility shift assay showed a supershift of the −61 to −42-bp probe with an anti-EGR1 antibody in PTH-treated cells, suggesting that EGR1 binding is relevant for the inhibitory activity of PTH. We conclude that PTH-induced inhibition of NHE3 transcription is related to higher EGR1 expression; to EGR1 binding to the proximal and core promoters; and to PKA and JAK/STAT pathway activation. This mechanism might be responsible, at least in part, for lower NHE3 expression and sodium reabsorption in renal proximal tubules in the presence of high PTH levels.

1. Introduction

The Na^+/H^+ exchanger proteins constitute a family of electroneutral exchangers, present in the plasma membrane of cells and in other intracellular organelles, that are particularly relevant for the maintenance of renal volume and acid–base homeostasis. In renal proximal tubules, the Na^+/H^+ exchanger 3 (NHE3) is the primary transporter of transcellular sodium reabsorption in the apical membrane and, consequently, of reabsorption of bicarbonate, chloride, and water through the epithelium [1,2]. NHE3 is a highly regulated protein that is directly or indirectly influenced by a variety of factors [3–5].

Parathyroid hormone (PTH) is known to regulate phosphate transport in proximal tubules. Animal and cell culture studies have shown that PTH also inhibits NHE3 activity and expression, acutely and chronically [6–8]. In a previous study, we demonstrated that PTH chronically downregulates NHE3 in rats (over an eight-day period) and in opossum kidney proximal tubule (OKP) cells (after 6, 16, and 24 h), reducing total and apical protein levels, as well as NHE3 mRNA abundance [6,9]. PTH reduces the activity of the proximal NHE3 promoter and has been associated with a slight decrease in NHE3 mRNA half-life. Protein kinase A (PKA) inhibitors have been shown to abolish the effect of PTH on NHE3 expression [9,10].

A GC box is a promoter type found in more than 80% of all genes, typically containing binding sites for the transcription factors Sp1/Sp3 and EGR1 [11–13]; and the *Nhe3* promoter is GC box-rich. In a detailed analysis of this promoter, Neri et al. [14] showed that nucleotides between −44 and +33 bp are essential for efficient transcription of the reporter gene, and that Sp1/Sp3 and EGR1 binding sites are essential for *Nhe3* transcription. Those transcription factors have zinc finger DNA-binding domains, which bind with high affinity to CpG islands present in the promoter and contribute to regulating the transcription rate [15,16].

* Corresponding author. Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Av. Prof. Lineu Prestes, 1524, Sao Paulo, SP, 05508-000, Brazil. Fax: +55 11 3091 7285.
E-mail address: camilab@icb.usp.br (C.N.A. Bezerra).

http://dx.doi.org/10.1016/j.bbrc.2015.04.049
0006-291X/

Considering the relevance of the proximal *Nhe3* promoter for efficient transcription, we proposed to investigate the mechanisms involved in PTH-induced downregulation of NHE3. To that end, we analyzed the promoter activity of the progressively shorter 5′ flanking sequence (5′FS), the main transcription factors that bind the proximal promoter, and the signaling pathways related to PTH effects.

2. Materials and methods

2.1. Materials

We purchased Dulbecco's modified Eagle's medium (DMEM), heat-inactivated fetal bovine serum (FBS), sodium pyruvate, and penicillin-streptomycin from Life Technologies (Gaithersburg, MD); 1-34 bovine PTH from Bachem (Torrance, CA); and PKA inhibitor (KT5720) and STAT3 inhibitor (Static) from Tocris Bioscience (Ellisville, MO). We obtained anti-Sp3 antibodies (Sp3-Ab) and anti-glyceraldehyde-3-phosphate dehydrogenase antibodies from Santa Cruz Biotech (Dallas, TX); anti-Sp1 and anti-EGR1 rabbit polyclonal antibodies (Sp3-Ab and EGR1-Ab, respectively) from Abcam (Cambridge, MA); a monoclonal anti-actin antibody (JLA20) from Calbiochem (San Diego, CA); and horseradish peroxidase-conjugated secondary antibodies from Zymed (San Francisco, CA). We obtained Lipofectamine Plus Reagent and Platinum Taq DNA Polymerase from Invitrogen (Carlsbad, CA); a Dual Luciferase Assay System, pGL3-Basic vector, pRL-CMV vector, and restriction endonucleases from Promega (Madison, WI); and SYBR Green Core Reagents and other reagents employed for real-time quantitative reverse transcription polymerase chain reaction (real-time qRT-PCR), from Applied Biosystems (Foster City, CA). The OKP cells, derived from a clonal subline of the opossum kidney cell line [17], were kindly provided by Dr. O. W. Moe (University of Texas Southwestern Medical Center, Dallas, TX). Other reagents and chemicals were obtained from Sigma (St. Louis, MO).

2.2. Cell culture

OKP cells were maintained in 75-cm^2 tissue culture flasks in DMEM containing 10% heat-inactivated FBS, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 g/ml streptomycin. Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Cells were subcultured with Ca/Mg-free phosphate buffered saline and 0.25% trypsin/ethylenediaminetetraacetic acid. For RNA and protein extraction, cells were transferred to 6- or 24-well plates and serum-starved for 24 h after confluence. In all experiments, PTH was used at a final concentration of 10^{-7} M for 24 h.

2.3. Assembly of reporter gene constructs

We chose the segments of the proximal promoter containing binding sites for Sp1/Sp3 and EGR1. The −65 to +31-bp regulatory sequence of the of the gene encoding NHE3 was obtained by PCR amplification of the recombinant pGL3 vector containing the −2095 to +55-bp NHE3 promoter, with the forward and reverse primers 5′-AGCAGGTACCAAGGCCCCGCCCTG-3′ and 5′-AGCAAAGC TTCTCTGCGCTATCCGGAG-3′, respectively. We obtained the segment extending from −44 bp of the 5′FS to 31 bp of the first exon (lowercase) by annealing the corresponding synthetic sense and antisense oligonucleotides, resulting in the double-stranded sequence 5′-AGGGGGCAGCGGGATTAAAGGCCACCCGGGCAGGCCTG GGGACCGtgactgctgccagctccgggatagcgcagag-3′, which was inserted into the pGL3-basic reporter vector. We introduced specific point mutations using PCR with mutated oligonucleotides.

2.4. Luciferase reporter gene assay

Using Lipofectamine Plus Reagent, we transiently transfected OKP cells with the recombinant pGL3 vectors. After transfection, cells were treated or not with 10^{-7} M PTH for 24 h. When indicated, specific inhibitors were added to the culture medium 30 min before the addition of PTH.

2.5. Preparation of nuclear extracts and electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared with a Nuclear Extraction Kit (Chemicon, Billerica, MA). Using the wild-type probes 5′-^{32}P-CCCCGCCCTGGCGCGGGAGG-OH-3′ (−61 to −42 bp) and 5′-^{32}P-CGCGGGAGGGGCAGCGGGATTAAAGG-OH-3′ (−50 to −24 bp), we performed electrophoretic mobility shift assays (EMSA) as described previously (24) (Sp1/3 or EGR1 binding sites are in bold). The binding specificity of each probe was verified by competition EMSA with cold wild-type probes at 20-, 50-, 100-, 200-, and 300-fold molar excesses and with a cold mutated −50 to −24-bp probe at a 300-fold molar excess, added to the reaction mixture 20 min before the labeled probe. We performed antibody supershift assay by incubating 2 μg of Sp1-Ab, EGR1-Ab, or Sp3-Ab in binding reaction mixtures, overnight at 4 °C.

2.6. Immunoblotting

Nuclear proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), after which we performed immunoblotting, as previously described [9], with Sp1-Ab and EGR1-Ab. Protein band density was quantified with the ImageJ program, version 1.44p (National Institutes of Health, Bethesda, MD).

2.7. Cell RNA extraction and real-time qRT-PCR analysis

OKP cells were treated with vehicle or 10^{-7} M PTH for 24 h, and total RNA was isolated as described elsewhere [9]. Random primers were used for reverse transcription, and the following forward and reverse primers were used for real-time qRT-PCR: Sp3—5′-GGCGCCAATCTTGGGAAAAA-3′ and 5′-A GCGTTTTGAACATTCT GGACA-3′, respectively; EGR1—5′-CTGGAGAGAAACCCTTCGCC-3′ and 5′-ACAATAGGAGGTGGGTACTGGA-3′, respectively; and β-actin (housekeeping gene used as an internal control)—5′-GTGATCAC-CATTGGCAATGAGAG-3′ and 5′-CGGTATTGGCATACAAATCCTTACG-3′, respectively. The relative amplicon abundance was detected with SYBR Green, using the comparative threshold cycle method. The results are given as relative expression of Sp3 or EGR1 mRNA, normalized to the respective internal control.

2.8. Statistical analysis

Values are presented as means ± SE. Statistical significance was determined by ANOVA followed by Bonferroni post hoc test. Student's *t*-tests were used when indicated. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Proximal Nhe3 promoter activity

We previously demonstrated that at least 44 bp of the *Nhe3* 5′FS are necessary for efficient transcription of the luciferase reporter gene [14]. Although rat and human proximal *Nhe3* promoters are highly similar in the first 100 bp of the 5′FS [13], including binding

sites for some transcription factors, they diverge beyond −100 bp. As shown in Fig. 1, PTH caused a significant (≈30%) decrease in the promoting activity of segments spanning the −65 to +31-bp and −44 to +31-bp ranges, including nucleotide of the 5′FS and of the first exon ($P = 0.0019$ and $P = 0.0301$, respectively), which include the core promoter (defined as the −40 to +40-bp nucleotide sequence) and the proximal promoter (defined as the −200 to −40-bp nucleotide sequence) in the 5′FS, respectively [18].

3.2. Involvement of PKA and JAK/STAT signaling pathways

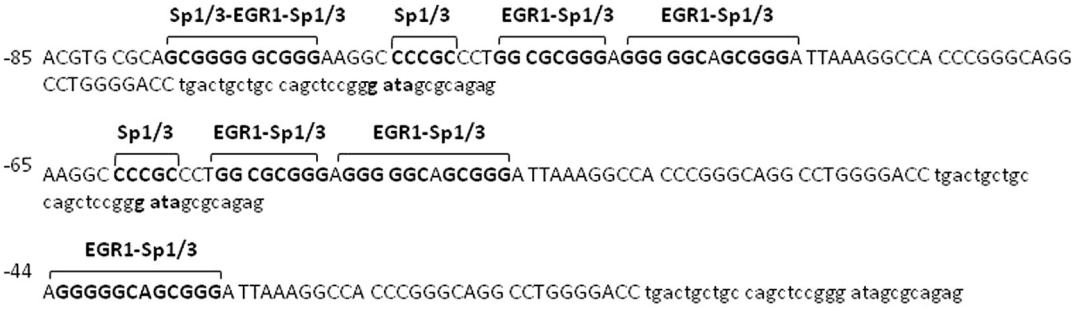
We have previously shown that PTH increases cAMP levels in OKP cells, and that the PKA signaling pathway is involved in PTH inhibition of *Nhe3* transcription [9]. Here, we observed that PTH does not inhibit the −44 to +31-bp *Nhe3* core promoter in OKP cells incubated for 30 min with 10^{-6} M of the PKA inhibitor KT5720 (Fig. 2A), suggesting that transcription modulators that interact with the transcription initiation complex recruited by PTH are regulated by PKA. Recent studies have shown that, in the cytoplasm and in the nucleus, there is crosstalk between the PKA and JAK/STAT signaling pathways [19,20]. To determine whether such crosstalk occurs in our model, transiently transfected cells with the −44 to +31-bp *Nhe3* promoter construct were incubated for 30 min with 5×10^{-5} M 1,2,3,4,5,6-hexabromocyclohexane (a JAK2 autophosphorylation inhibitor) or 10^{-5} M Static (a STAT3 inhibitor) and exposed to 10^{-7} M PTH for 24 h. The results suggest that the JAK/STAT pathway is involved in PTH inhibition of *Nhe3* promoter activity, the PTH-induced reduction of promoter activity being completely abolished—PTH in fact becoming an activator of that activity—in the presence of the JAK2 and STAT3 inhibitors (Fig. 2B). In the absence of PTH, neither inhibitor affected promoter activity.

3.3. Effects of mutations at EGR1/Sp1/Sp3 binding sites in the proximal Nhe3 promoter

We designed proximal *Nhe3* promoter constructs in which nucleotides of consensus binding sites for EGR1 and Sp1/Sp3 were mutated: G_{-72} to A_{-72} and G_{-70} to A_{-70}; $CG_{-58\ to\ -57}$ to $AA_{-58\ to\ -57}$ and $CG_{-50\ to\ -49}$ to $AA_{-50\ to\ -49}$; and $GC_{-36\ to\ -35}$ to $AA_{-36\ to\ -35}$. The point mutations in the −80 to +31-bp, −60 to +31-bp, and −40 to +31-bp promoter regions abrogated the inhibitory effect of PTH (Fig. 2C). Mutations in the −80 to +31-bp and −60 to +31-bp regulatory regions resulted in PTH-induced activation, rather than inhibition, of transcription in the promoter regions.

3.4. Sp3/EGR1 nuclear expression

The complex immunoblot pattern of Sp3 is due to alternatively spliced mRNA, together with post-translational modification of the proteins by addition of small ubiquitin-like modifier (SUMO), a process known as SUMOylation [21]. The Sp3 immunoblot pattern of OKP cells is similar to that of human cells, presenting long and short isoforms (Sp3li and Sp3si, respectively). PTH increased the abundance of Sp3li-1; probably SUMOylated Sp3si3 or Sp3si4, Sp3si-3, and extra bands detected by SDS-PAGE; and reduced Sp3li-2 and Sp3si-4 (Fig. 3A and B). PTH did not alter Sp3 mRNA



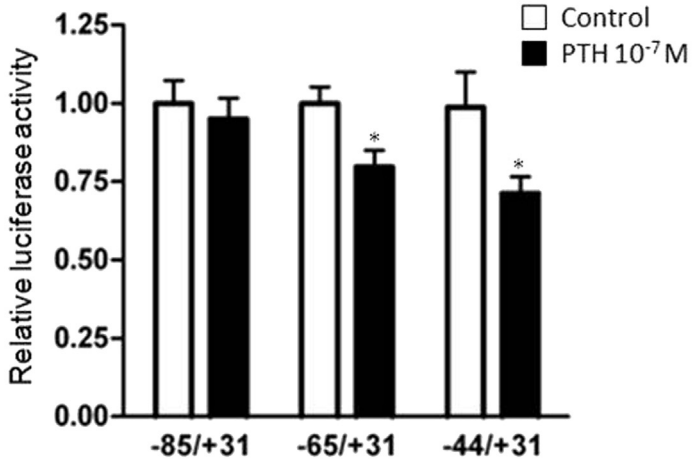


Fig. 1. PTH effects on the proximal NHE3 promoter ($n = 6$). The inhibitory effect of PTH was observed in the −65 to +31-bp and −44 to +31-bp segments of the promoter region (−65/+31 and −44/+31, respectively) but not in its −85 to +31-bp segment (−85/+31). $^{*}P < 0.05$ vs. the control cells transfected with the same recombinant pGL3 vectors, as determined by Student's *t*-test.

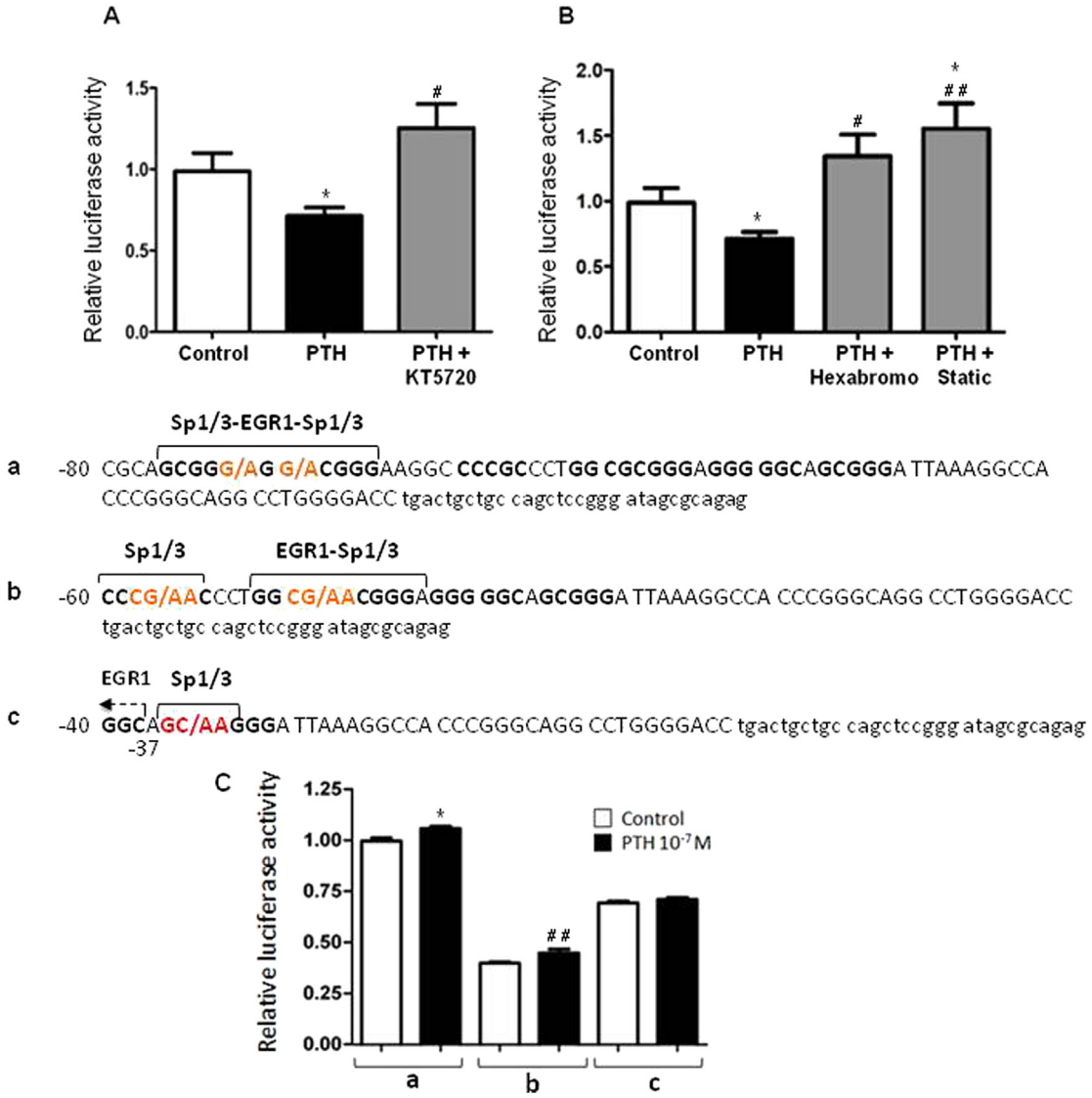


Fig. 2. (A) The inhibitory effect of PTH on the −44 to +31-bp segment of the proximal NHE3 promoter was transformed into an activating effect by the PKA inhibitor KT5720 (n = 6), as well as (B) by the inhibitor of JAK2 autophosphorylation − hexabromocyclohexane (Hexabromo) (n = 6) − and the STAT3 inhibitor Static (n = 6). ANOVA, $^{*}P < 0.05$ treated vs. control and $^{\#}P < 0.05$ inhibitor vs. PTH and $^{\#\#}P < 0.01$ inhibitor vs. PTH. (C) Effect of double-point mutations on consensus binding sites for transcription factors in the proximal NHE3 promoter (n = 6). Mutations in Sp1/3 and Sp1/3-EGR1 binding elements located upstream of nucleotide −37, within the −80 to +31-bp (a), −60 to +31-bp (b), and −40 to +31-bp (c) segments, abolished/inverted the inhibitory effect of PTH on the promoter. $^{*}P < 0.05$ and $^{\#\#}P < 0.01$, as determined by Student's *t*-test.

abundance (data not shown). Although EGR1-protein expression was unchanged after treatment with PTH (Fig. 3C and D), we detected a significant PTH-induced increase in EGR1 mRNA abundance (Fig. 3E).

3.5. EGR1 binding to the proximal NHE3 promoter

The EMSA employing the probe corresponding to −61 to −42-bp segment of the proximal NHE3 promoter, which includes potential binding sites for Sp1/Sp3 and EGR1, is depicted in Fig. 4. In PTH-treated cells, EGR1-Ab induced a supershift, suggesting increased EGR1 binding to its consensus binding element at the promoter in the presence of PTH. In addition, Sp3-Ab apparently induced a slight displacement of the labeled probe.

We also tested the −50 to −24-bp probe, which includes a complete Sp1/Sp3 binding site but only a partial EGR1 binding site. We observed reduced protein binding to the −50 to −24-bp probe in presence of PTH, albeit with no supershift (data not shown).

4. Discussion

We previously reported that persistently high PTH levels lead to a reduction in NHE3 expression [6], as confirmed by Wang et al. [22]. In another study, we showed that chronic PTH treatment downregulates NHE3 promoter activity and reduces mRNA stability, both effects inhibiting NHE3 expression [9]. Here, we showed that, in OKP cells, PTH has an inhibitory effect on the −65 to +31-bp and −44 to +31 bp nucleotide sequences (proximal and core promoters, respectively, of the *Nhe3* gene), indicating direct interference with the transcription machinery. PTH-induced inhibition of NHE3 expression has previously been reported to be sensitive to PKA inhibitors [9], which is in agreement with our present findings. However, we found that the effect was also sensitive to JAK/STAT inhibitors. The relationship between the PKA and JAK/STAT signaling pathways has been described in intracellular signaling triggered by some membrane receptors, such as corticotropin receptors in adrenal cells, and alpha-adrenergic receptors in

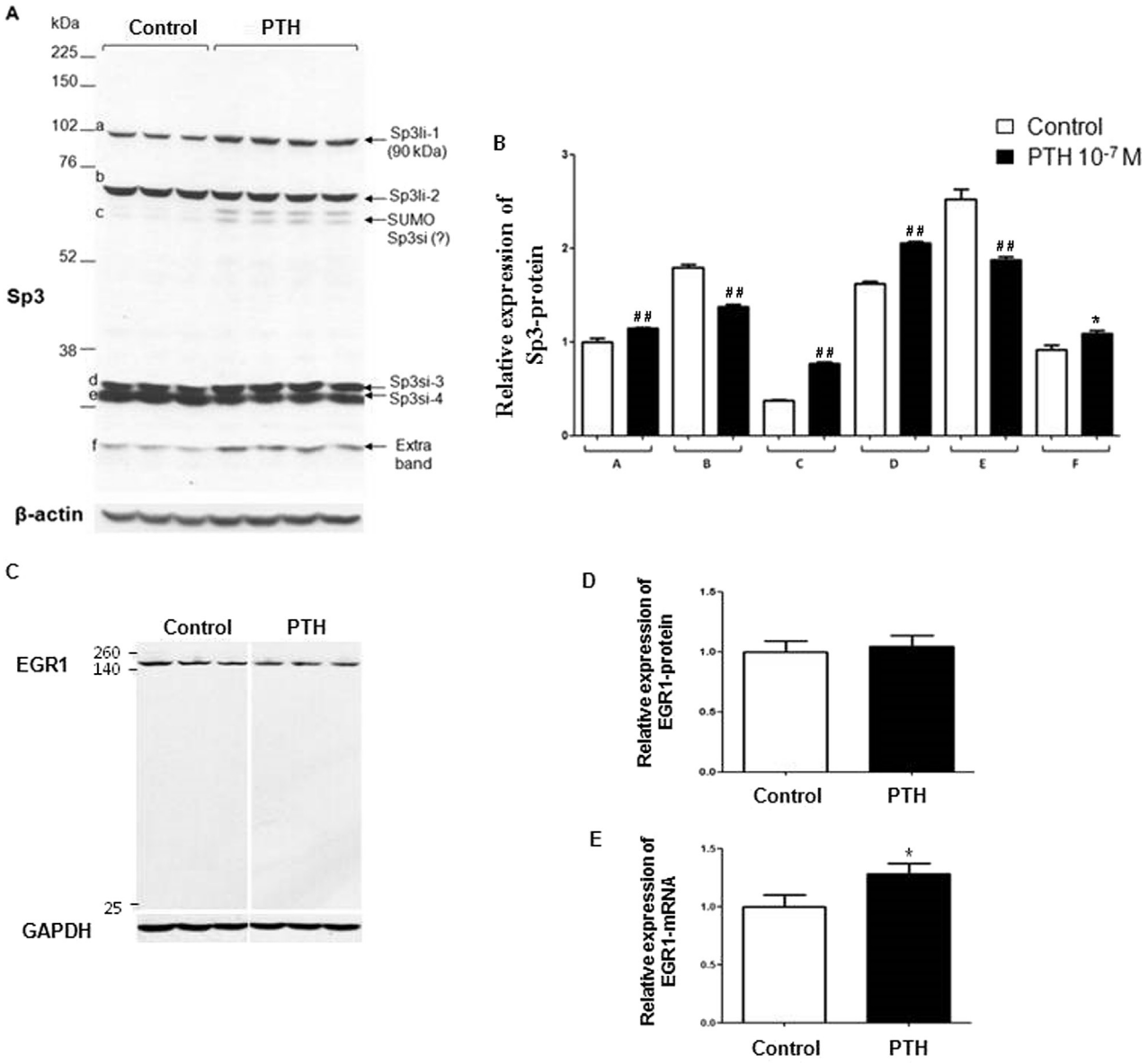


Fig. 3. (A) Western blot analysis of Sp3 expression in PTH-treated cells (n = 4) vs. control cells (n = 3); PTH induced changes in Sp3 expression, which could be due to differential expression of long and short isoforms (Sp3li and Sp3si, respectively) derived from splicing variants or SUMOylation. (B) Densitometry of bands. ANOVA, $^{*}P < 0.01$; $^{\#}P < 0.05$. (C) Western blot analysis of EGR1 expression in PTH-treated cells ($n = 3$). (D) Densitometry of bands. (E) Real-time qRT-PCR analysis of EGR1 mRNA in PTH-treated cells ($n = 7$). GAPDH = glyceraldehyde-3-phosphate dehydrogenase. Student's t-test, $^{*}P < 0.05$.

adipocytes [19,20]. JAK-tyrosine kinase is a key molecule that can initiate multiple signal transduction pathways by inducing phosphorylation of cytokine receptors, including glycoprotein 130 (gp130), the common receptor subunit of the interleukin-6 superfamily, to which various signaling molecules are recruited, including STAT [23]. These multiple signal transduction pathways closely regulate the expression of several genes, including EGR1. It is noteworthy that gp130 is required for PTH-induced osteoblast differentiation [24], suggesting that gp130 is related to PTH activation of JAK/STAT in the renal proximal tubules, which merits investigation.

In rats, the proximal and core promoters of the gene encoding NHE3 encompass consensus binding sites for EGR1 and Sp1/Sp3, to which OKP transcription factors can bind [14–16]. It is known that Sp1/Sp3 and EGR1 are involved in the regulation of NHE3 transcription by multiple agents [25–27]. Point mutations in consensus sequences where these transcription factors can bind, located upstream of −37 bp, abrogate the inhibitory effect of PTH, indicating that these sequences are important for PTH-induced down-regulation of NHE3 gene transcription.

We observed PTH-induced changes in the relative expression of splicing isoforms of Sp3 and possibly in Sp3 SUMOylation, which was not explored here. Although PTH did not change the relative abundance of Sp3 mRNA, it increased that of EGR1 mRNA. Expression of Sp1 (unphosphorylated and phosphorylated), was unchanged by PTH (data not shown). Although Sp1 is mainly considered an activator of transcription, various studies have demonstrated efficient repression of Sp1-mediated transcription by Sp3 in promoters containing adjacent multiple Sp1/Sp3 binding sites [28–30].

Our EMSA and antibody supershift assay results indicate that PTH induces increased EGR1 binding to the −61 to −42-bp DNA

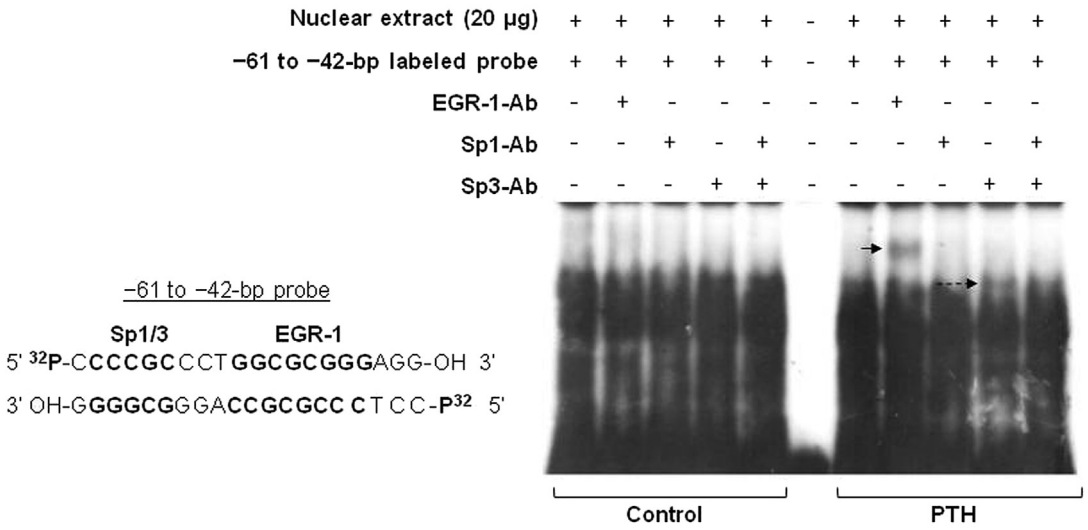


Fig. 4. EMSA and antibody supershift assay with a −61 to −42-bp probe ($n = 3$). A supershift was observed in the presence of EGR1-Ab (solid arrows). Sp3-Ab also induced slight displacement of a band (dashed arrow).

segment, in which there is one EGR1 consensus element. We also observed PTH-induced changes in the relative expression of different isoforms of Sp3, which apparently also binds to this region, contributing to the PTH effect on NHE3 gene promoter activity. Sp3 can act as activator or inhibitor of transcription depending on the promoter context [31]. Our data suggest increased SUMOylation of small isoforms of Sp3. SUMOylated Sp3 can recruit inhibitors of transcription to the promoter, as reported by Ross et al. [32]. Other mechanisms of repression have been associated with Sp3, including phosphatase recruitment, which seems important for Sp3 regulation of the local balance between kinase and phosphatase activities [33]. By regulating phosphorylation of proteins that coordinate RNA polymerase II pausing, such as negative elongation factor, Sp3 might modulate the transition from pausing to productive elongation, an important mechanism for fine-tuning gene expression.

Taken together, our results indicate that PTH-induced inhibition of NHE3 expression is related to a complex intracellular signaling involving PKA and JAK/STAT activation, which changes the Sp3 isoform expression profile and increases EGR1 binding to the core and proximal promoters. The PTH-mobilized transcription factors, due to the proximity of their binding sites to the *Nhe3* gene transcription start site, might interfere with the assembling of the basal transcription machinery or with the transition of RNA polymerase II to the productive elongation phase.

Conflict of interest

None.

Acknowledgments

This study was supported by the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, São Paulo Research Foundation; Grant Nos. 2008/58287-5 and 2013/23087-4) and the Brazilian *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development; Grant Nos. 301198/2010-1).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.049.

References

[1] J.N. Lorenz, P.J. Schultheis, T. Traynor, et al., Micropuncture analysis of single-nephron function in NHE3-deficient mice, Am. J. Physiol. 277 (1999) F447–F453.
[2] J.Y. Choi, M. Shah, M.G. Lee, et al., Novel amiloride-sensitive sodium-dependent proton secretion in the mouse proximal convoluted tubule, J. Clin. Invest. 105 (2000) 1141–1146.
[3] I.A. Bobulescu, O.W. Moe, Luminal Na(+)/H (+) exchange in the proximal tubule, Pflugers Arch. 458 (2009) 5–21.
[4] M. Donowitz, X. Li, Regulatory binding partners and complexes of NHE3, Physiol. Rev. 87 (2007) 825–872.
[5] H. Hayashi, K. Szaszi, S. Grinstein, Multiple modes of regulation of Na^+/H^+ exchangers, Ann. N Y Acad. Sci. 976 (2002) 248–258.
[6] A.C. Girardi, S.M. Titan, G. Malnic, N.A. Reboucas, Chronic effect of parathyroid hormone on NHE3 expression in rat renal proximal tubules, Kidney Int. 58 (2000) 1623–1631.
[7] C. Helmle-Kolb, M.H. Montrose, H. Murer, Parathyroid hormone regulation of Na^+/H^+ exchange in opossum kidney cells: polarity and mechanisms, Pflugers Arch. 416 (1990) 615–623.
[8] A.M. Kahn, G.M. Dolson, M.K. Hise, et al., Parathyroid hormone and dibutyryl cAMP inhibit Na^+/H^+ exchange in renal brush border vesicles, Am. J. Physiol. 248 (1985) F212–F218.
[9] C.N. Bezerra, A.C. Girardi, L.R. Carraro-Lacroix, N.A. Reboucas, Mechanisms underlying the long-term regulation of NHE3 by parathyroid hormone, Am. J. Physiol. Ren. Physiol. 294 (2008) F1232–F1237.
[10] A. Azarani, D. Goltzman, J. Orlowski, Parathyroid hormone and parathyroid hormone-related peptide inhibit the apical Na^+/H^+ exchanger NHE-3 isoform in renal cells (OK) via a dual signaling cascade involving protein kinase A and C, J. Biol. Chem. 270 (1995) 20004–20010.
[11] A. Cano, Characterization of the rat NHE3 promoter, Am. J. Physiol. 271 (1996) F629–F636.
[12] R.A. Kandasamy, J. Orlowski, Genomic organization and glucocorticoid transcriptional activation of the rat Na^+/H^+ exchanger NHE3 gene, J. Biol. Chem. 271 (1996) 10551–10559.
[13] J. Malakooti, V.C. Memark, P.K. Dudeja, K. Ramaswamy, Molecular cloning and functional analysis of the human Na(+)/H(+) exchanger NHE3 promoter, Am. J. Physiol. Gastrointest. Liver Physiol. 282 (2002) G491–G500.
[14] E.A. Neri, C.N. Bezerra, N.A. Reboucas, Essential regulatory elements for NHE3 gene transcription in renal proximal tubule cells, Braz. J. Med. Biol. Res. 44 (2011) 514–523.
[15] M.R. Amin, J. Malakooti, R. Sandoval, et al., IFN-gamma and TNF-alpha regulate human NHE3 gene expression by modulating the Sp family transcription factors in human intestinal epithelial cell line C2BBe1, Am. J. Physiol. 291 (2006) C887–C896.

[16] P.R. Kiela, J. LeSueur, J.F. Collins, F.K. Ghishan, Transcriptional regulation of the rat NHE3 gene. Functional interactions between GATA-5 and Sp family transcription factors, J. Biol. Chem. 278 (2003) 5659–5668.
[17] J.A. Cole, L.R. Forte, W.J. Krause, et al., Clonal sublines that are morphologically and functionally distinct from parental OK cells, Am. J. Physiol. 256 (1989) F672–F679.
[18] M. Baumann, J. Pontiller, W. Ernst, Structure and basal transcription complex of RNA polymerase II core promoters in the mammalian genome: an overview, Mol. Biotechnol. 45 (2010) 241–247.
[19] A.M. Lefrancois-Martinez, A. Blondet-Trichard, N. Binart, P. Val, et al., Transcriptional control of adrenal steroidogenesis: novel connection between Janus kinase (JAK) 2 protein and protein kinase A (PKA) through stabilization of cAMP response element-binding protein (CREB) transcription factor, J. Biol. Chem. 286 (2011) 32976–32985.
[20] S. Westphal, N. Perwitz, K.A. Iwen, et al., Expression of ATRAP in adipocytes and negative regulation by beta-adrenergic stimulation of JAK/STAT, Horm. Metab. Res. 40 (2008) 165–171.
[21] A. Sapetschnig, F. Koch, G. Rischitor, et al., Complexity of translational controlled transcription factor Sp3 isoform expression, J. Biol. Chem. 279 (2004) 42095–42105.
[22] W. Wang, C. Li, T.H. Kwon, et al., Reduced expression of renal Na^+ transporters in rats with PTH-induced hypercalcemia, Am. J. Physiol. 286 (2004) F534–F545.
[23] T. Hirano, Interleukin 6 and its receptor: ten years later, Int. Rev. Immunol. 16 (1998) 249–284.
[24] T. Standal, R.W. Johnson, N.E. McGregor, et al., gp130 in late osteoblasts and osteocytes is required for PTH-induced osteoblast differentiation, J. Endocrinol. 223 (2014) 181–190.
[25] J. Malakooti, R. Sandoval, M.R. Amin, et al., Transcriptional stimulation of the human NHE3 promoter activity by PMA: PKC independence and involvement of the transcription factor EGR1, Biochem. J. 396 (2006) 327–336.
[26] H.W. Su, S.W. Wang, F.K. Ghishan, et al., Cell confluency-induced Stat3 activation regulates NHE3 expression by recruiting Sp1 and Sp3 to the proximal NHE3 promoter region during epithelial dome formation, Am. J. Physiol. 296 (2009) C13–C24.
[27] P.R. Kiela, E.R. Hines, J.F. Collins, F.K. Ghishan, Regulation of the rat NHE3 gene promoter by sodium butyrate, Am. J. Physiol. 281 (2001) G947–G956.
[28] G. Suske, The Sp-family of transcription factors, Gene 238 (1999) 291–300.
[29] M.J. Birnbaum, A.J. van Wijnen, P.R. Odgren, et al., Sp1 trans-activation of cell cycle regulated promoters is selectively repressed by Sp3, Biochemistry 34 (1995) 16503–16508.
[30] B. Majello, L.P. De, G. Hagen, et al., Different members of the Sp1 multigene family exert opposite transcriptional regulation of the long terminal repeat of HIV-1, Nucleic Acids Res. 22 (1994) 4914–4921.
[31] B. Yu, P.K. Datta, S. Bagchi, Stability of the Sp3-DNA complex is promoter-specific: Sp3 efficiently competes with Sp1 for binding to promoters containing multiple Sp-sites, Nucleic Acids Res. 31 (2003) 5368–5376.
[32] S. Ross, J.L. Best, L.I. Zon, G. Gill, SUMO-1 modification represses Sp3 transcriptional activation and modulates its subnuclear localization, Mol. Cell. 10 (2002) 831–842.
[33] A. Valin, J. Ouyang, G. Gill, Transcription factor Sp3 represses expression of p21CIP1 via inhibition of productive elongation by RNA polymerase II, Mol. Cell. Biol. 33 (2013) 1582–1593.