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Up-regulation of FGF23 release by aldosterone

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ABSTRACT

The fibroblast growth factor (FGF23) plasma level is high in cardiac and renal failure and is associated with poor clinical prognosis of these disorders. Both diseases are paralleled by hyperaldosteronism. Excessive FGF23 levels and hyperaldosteronism are further observed in Klotho-deficient mice. The present study explored a putative aldosterone sensitivity of Fgf23 transcription and secretion the putative involvement of the aldosterone sensitive serum & glucocorticoid inducible kinase SGK1, SGK1 sensitive transcription factor NFκB and store operated Ca^{2+} entry (SOCE). Serum FGF23 levels were determined by ELISA in mice following sham treatment or exposure to deoxycorticosterone acetate (DOCA) or salt depletion. In osteoblastic UMR106 cells transcript levels were quantified by qRT-PCR, cytosolic Ca^{2+} concentration utilizing Fura-2-fluorescence, and SOCE from Ca^{2+} entry following store depletion by thapsigargin. As a result, DOCA treatment and salt depletion of mice elevated the serum C-terminal FGF23 concentration. In UMR106 cells aldosterone enhanced and spironolactone decreased SOCE. Aldosterone further increased Fgf23 transcript levels in UMR106 cells, an effect reversed by mineralocorticoid receptor blockers spironolactone and eplerenone, SGK1 inhibitor EMD638683, NFκB-inhibitor withaferin A, and Ca^{2+} channel blocker YM58483. In conclusion, Fgf23 expression is up-regulated by aldosterone, an effect sensitive to SGK1, NFκB and store-operated Ca^{2+} entry.

1. Introduction

Regulation of calcium phosphate metabolism involves FGF23 (fibroblast growth factor 23), which is mainly released from bone cells [1]. FGF23 decreases formation and fosters inactivation of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) by down-regulating renal 1α hydroxylase (Cyp27b1) and up-regulating 25-hydroxyvitamin D 24-hydroxylase (Cyp24a1) [2]. FGF23 thus lowers serum levels of $1,25(OH)_2D_3$ [3,4], which stimulates renal and intestinal phosphate and calcium transport [5,6]. FGF23 deficiency is followed by increase of plasma $1,25(OH)_2D_3$ levels, hyperphosphatemia, and hypercalcemia leading to vascular calcification, rapid aging and a substantial decrease of the life span [2]. Vascular calcification is stimulated by hyperphosphatemia [7], a predictor of mortality [8] and by aldosterone [9], which similarly impacts on life span [9].

Excessive plasma FGF23 levels are observed and are associated with accelerated disease progression, morbidity and/or mortality in several clinical disorders including cardiac failure [10,11], acute renal failure [12], chronic kidney disease [11,13,14], diabetic nephropathy [15] and hepatic failure [16]. The pathophysiological significance of enhanced FGF23 formation, has, however, remained ill-defined [17,18] and little is known about mechanisms accounting for the up-regulation of FGF23 release in these clinical disorders.

Most recently, Fgf23 transcription has been shown to be stimulated by an increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) secondary to upregulation of store operated Ca^{2+} entry (SOCE) [19], which is triggered by depletion of intracellular Ca^{2+} stores [20]. SOCE is accomplished by the calcium release-activated channel (CRAC) moiety Orai1 (CRACM1) [21], which is activated by stromal interaction molecule 1 (STIM1) [22]. Expression and activity of Orai1 are up-regulated by the transcription factor NFκB [23].

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Regulators of NFκB include mineralocorticoids [24,25]. In patients with non-ischemic cardiac disease and early chronic kidney disease high FGF23 plasma levels are associated with high plasma aldosterone concentrations [11].

The present study explored whether aldosterone modifies FGF23 release and, if so, whether the effect involves NFκB and Ca^{2+} entry.

2. Materials and methods

2.1. Animal experiments

The animal experiments were conducted according to the German law for the welfare of animals and were approved by the state of Baden-Württemberg (Regierungspräsidium Tübingen). Fifty μl blood was collected from male and female C57BL/6 mice (8–20 weeks old). Then, mice were sham-treated or treated with a single s.c. injection of deoxycorticosterone acetate (DOCA; 100 mg/kg b.w.; Sigma, Schnelldorf, Germany) or spironolactone (75 mg/kg b.w.; Sigma). Twelve hours after the injection, another 50 μl blood was collected. For the effect of low salt diet on FGF23 release, male and female mice (2–13 months old) were fed a control diet or a low salt diet (containing <0.2% Na^+ and Cl^-; Altromin, Lage, Germany) for a total of 7–14 days and subsequently 50 μl blood were collected. Serum C-terminal FGF23 was determined with an ELISA kit (Immutopics, San Clemente, USA). Serum aldosterone and corticosterone were quantified by means of kits from IBL (Hamburg, Germany) according to the manufacturers' protocol.

2.2. Cell culture

UMR106 rat osteosarcoma cells were cultured in DMEM high glucose medium supplemented with 10% FCS and 1% penicillin/streptomycin under standard culture conditions. Cells were pretreated with 100 nM $1,25(OH)_2D_3$ (Sigma) for 42 h. Then, 100 nM aldosterone (Sigma) was added without or with 10 μM spironolactone (Sigma), 10 μM eplerenone (Sigma), 100 nM YM58483 (TOCRIS), 500 nM withaferin A (TOCRIS), or 50 μM SGK1 inhibitor EMD638683 (Merck Darmstadt, Germany) for 6 h.

For the calcium measurements and the quantification of Orai1 transcripts, cells were treated with 100 nM aldosterone (Sigma) or 10 μM spironolactone (Sigma) for 24 h in serum-free medium.

2.3. Quantification of mRNA expression

For the mRNA expression analysis in UMR106 cells, the final volume of the qRT-PCR reaction mixture was 20 μl and contained: 2 μl cDNA, 1 μM of each primer, 10 μl GoTaq Master Mix (Promega), and sterile water up to 20 μl. PCR conditions were 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 58 °C for 30 s. Quantitative RT-PCR was performed on a BioRad iCycler iQTM Real-Time PCR Detection System (Bio-Rad Laboratories, Munich, Germany).

For the determination of Fgf23 transcripts in mouse bone, bone was homogenized in liquid nitrogen using mortar and pestle. Total mRNA from bone was extracted with TRIzol (Invitrogen, Switzerland) followed by purification with RNeasy Mini Kit (Qiagen, Switzerland) according to the manufacturer's protocol. DNAse digestion was performed using the RNase-free DNAase Set (Qiagen, Switzerland). Total RNA extractions were analyzed for quality, purity, and concentration using the NanoDrop ND-1000 spectrophotometer (Wilmington, Germany). RNA samples were diluted to a final concentration of 100 ng/μl and cDNA was prepared using the TaqMan Reverse Transcriptase Reagent Kit (Applied Biosystems, Roche, Foster City, CA). In brief, in a reaction volume of 40 μl, 300 ng of RNA was used as template and mixed with the following final concentrations of RT buffer (1x): $MgCl_2$ (5.5 mmol/l), random hexamers (2.5 μmol/l), dNTP mix (500 μmol/l each), RNase inhibitor (0.4 U/μl), multiscribe reverse transcriptase (1.25 U/μl), and RNAse-free water. Reverse transcription was performed with thermocycling conditions of 25 °C for 10 min, 48 °C for 30 min, and 95 °C for 5 min on a thermocycler (Biometra, Göttingen, Germany). Semi-quantitative real-time PCR (RT-PCR) was performed on the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Primers were chosen to spanning intron–exon boundaries to exclude genomic DNA contamination. Probes were labeled with the reporter dye FAM at the 5′-end and the quencher dye TAMRA at the 3′-end (Microsynth, Balgach, Switzerland). Real-time PCR reactions were performed using KAPA PROBE FAST qPCR Kit (Kapa Biosystems, USA).

The following primers were used:

Rat Tbp (TATA box-binding protein):
forward (5′-3′): ACTCCTGCCACACCAGCC
reverse (5′-3′): GGTCAAGTTTACAGCCAAGATTCA
Rat Fgf23
forward (5′-3′): TGGCCATGTAGACGGAACAC
reverse (5′-3′): GGCCCCTATTATCACTACGGAG
Rat Orai1
forward (5′-3′): CGTCCACAACCTCAACTCC
reverse (5′-3′): AACTGTCGGTCCGTCTTAT
Mouse Fgf23
forward (5′-3′): TCGAAGGTTCCTTTGTATGGAT
reverse (5′-3′): AGTGATGCTTCTGCGACAAGT

Calculated mRNA expression levels were normalized to the expression levels of Tbp (in rat derived cell lines) or HPRT/18S (in mice) of the same cDNA sample. Relative quantification of gene expression was performed as indicated in the Figure legend.

2.4. Measurement of intracellular Ca^{2+}

To determine the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), UMR106 cells were loaded with Fura-2/AM (2 μM, Molecular Probes, Göttingen, Germany) for 15 min at 37 °C. Fluorescence measurements were carried out with an inverted phase-contrast microscope (Axiovert 100, Zeiss, Oberkochen, Germany). Cells were excited alternatively at 340 or 380 nm and the light was deflected by a dichroic mirror into either the objective (Fluar 40 × / 1.30 oil, Zeiss, Oberkochen, Germany) or a camera (Proxitronic, Bensheim, Germany). Emitted fluorescence intensity was recorded at 505 nm. Data acquisition was accomplished by using specialized computer software (Metafluor, Universal Imaging Downingtown, USA). As a measure for the increase in the cytosolic Ca^{2+} concentration, the slope and peak of the changes in the 340/380 nm ratio were determined in each experiment.

To determine SOCE, intracellular Ca^{2+} was measured before and after removal of extracellular Ca^{2+} (and addition of 0.5 mM EDTA), followed by addition of thapsigargin (1 μM) and subsequent re-addition of extracellular Ca^{2+} to Ringer solution, composed of (in mM): 125 NaCl, 5 KCl, 1.2 $MgSO_4$, 32.2 HEPES (NaOH/pH 7.4), 2 Na_2HPO_4, 0 or 2 $CaCl_2$ and 0.5 or 0 EGTA, respectively, and 5 glucose.

2.5. Immunofluorescence

UMR106 cells treated with 100 nM aldosterone were cultured on 4-well chamber slides (Thermo scientific), washed, and fixed with 4% paraformaldehyde for 30 min at room temperature. For blocking unspecific binding, UMR106 cells were incubated with 3% Albumin Fraction V (Carl Roth, Karlsruhe, Germany), 5% normal goat serum (Sigma, Schnelldorf, Germany), and 0.5% Triton in PBS

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