Original Paper

OSR1-Sensitive Renal Tubular Phosphate Reabsorption

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Key Words

Na⁺ • Phosphate cotransporter • FGF23 • Bone density • Proximal tubule • Oxidative stress-response kinase

Abstract

Background: The oxidative stress-responsive kinase 1 (OSR1) participates in the WNK-(with no K) kinase dependent regulation of renal salt excretion and blood pressure. Little is known, however, about the role of OSR1 in the regulation of further renal transport systems. The present study analyzed the effect of OSR1 on NaPiIIa, the major renal tubular phosphate transporter. **Methods:** Immunohistochemistry and confocal microscopy were employed to determine renal localization of OSR1 and NaPiIIa. To elucidate the effect of OSR on NaPiIIa activity, cRNA encoding NaPiIIa was injected into *Xenopus* oocytes with or without additional injection of cRNA encoding OSR1, and phosphate transport was estimated from phosphate-induced currents determined with dual electrode voltage clamp. To elucidate the *in vivo* significance of OSR1 serum phosphate and hormone concentrations as well as urinary phosphate output of mice carrying one allele of WNK-resistant OSR1 (*osr1^{tg/+}*) were compared to the respective values of wild type mice (*osr1^{+/+}*). **Results:** NaPiIIa and OSR1 were both expressed in proximal renal tubule cells. Coexpression of OSR1 significantly up-regulated phosphate-induced currents in NaPiIIa-expressing *Xenopus* oocytes. Despite decreased serum phosphate concentration urinary phosphate excretion was significantly increased and NaPiIIa protein abundance in the brush border membrane significantly reduced in *osr1^{tg/+}* mice as compared to *osr1^{+/+}* mice. Serum PTH and calcitriol levels were similar in *osr1^{tg/+}* mice and in *osr1^{+/+}* mice, serum FGF23 concentration was, however, significantly higher in *osr1^{tg/+}* mice than in *osr1^{+/+}* mice. **Conclusions:** OSR1 is expressed in proximal renal tubules and participates in the regulation of FGF23 release and renal tubular phosphate transport.

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Introduction

The oxidative stress-responsive kinase 1 (OSR1) participates in the signaling regulating transport during oxidative and osmotic stress [1-6]. OSR1 is activated by the osmosensitive WNK (with no K) kinase isoforms WNK1 and WNK4 [3, 7, 8] and up-regulates the thiazide-sensitive Na⁺-Cl⁻ cotransporter (NCC) and the furosemide-sensitive Na⁺,K⁺,2Cl⁻ cotransporters (NKCC1 and NKCC2), thus contributing to the regulation of cell volume, transepithelial transport, renal salt excretion, and GABA neurotransmission [1-3, 9-14]. Presumably due to their impact on renal tubular salt transport WNK1 and WNK4 [2, 15-18] as well as OSR1 [7, 19-22] participate in the regulation of blood pressure.

Nothing is known about a role of OSR1 in other nephron segments and in the regulation of other renal tubular transport systems. The present study thus explored whether OSR1 influences the activity of the Na⁺-coupled phosphate transporter NaPiIIa (SLC34A1), which is the most important carrier accomplishing renal tubular phosphate transport across the apical brush border membrane of proximal renal tubules [23-25]. Renal tubular phosphate reabsorption is tightly regulated by dietary phosphate intake, acid-base status, parathyroid hormone, 1,25-(OH)₂ vitamin D₃, FGF23, insulin and insulin-like growth factor IGF1 [26-32]. Signaling known to regulate NaPiIIa includes the protein kinases A and C, ERK1/2, Klotho and the PI3K/PKB/GSK3 kinase cascade [33-40].

The present study addressed the putative role of OSR1 in the regulation of NaPiIIa. Coexpression of OSR1 enhanced phosphate-induced currents reflecting electrogenic phosphate transport in NaPiIIa-expressing *Xenopus* oocytes. Immunohistochemistry and confocal microscopy revealed expression of both, OSR1 and NaPiIIa, in proximal renal tubules. To explore the *in vivo* significance of OSR1-sensitive phosphate transport, experiments were performed in mice carrying a mutation of OSR1 resistant to WNK-mediated activation (*osr1^{tg/+}*) and in the respective wild type mice (*osr1^{+/+}*). The mutation compromises OSR1 activity [19]. Urinary phosphate excretion was indeed higher and serum phosphate concentration lower in *osr1^{tg/+}* mice than in *osr1^{+/+}* mice. Thus, the observations point to a role of OSR1 in the regulation of renal tubular phosphate transport.

Materials and Methods

In vitro expression

For generation of cRNA, constructs were used encoding wild type human NaPiIIa [41] and human OSR1. The cRNA was generated as described previously [42]. The constructs encoding wild type and catalytically-inactive ^{D164A}OSR1 were kindly provided by Dario Alessi (University of Dundee, UK). For electrophysiology, *Xenopus* oocytes were prepared as previously described [43, 44]. Fifteen ng of NaPiIIa cRNA were injected on the first day and 10 ng OSR1 cRNA on the second day into *Xenopus* oocytes. All experiments were performed at room temperature 3 days after the second injection. Two electrode voltage-clamp recordings were performed at a holding potential of -50 mV. The data were filtered at 10 Hz and recorded with a Digidata A/D-D/A converter and Chart V.4.2 software for data acquisition and analysis (Axon Instruments). The control solution (superfusate / ND96) contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂ and 5 mM HEPES, pH 7.4. One mM phosphate was added to induce NaPiIIa-dependent currents. The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s.

Animals

All animal experiments were conducted according to the German law for the welfare of animals and were approved by local authorities. Blood was drawn, urine collected or tissue isolated from sex- and age-matched 3-8-month-old heterozygous OSR1 knockin mice (*osr1^{tg/+}*) and respective wild type mice (*osr1^{+/+}*), kindly provided by Dario Alessi. As described earlier [45], in the knockin mice the T-loop Thr residue in OSR1 (Thr185) was mutated to Ala to prevent activation by WNK isoforms. Homozygous OSR1 knockin mice (*osr1^{tg/tg}*) are not viable [45]. Mice had free access to control diet (Ssniff, Soest, Germany) containing 7000 mg/kg phosphorus or to phosphate-depleted diet (Altromin, Lage, Germany) containing 131 mg/kg phosphate and to tap drinking

water ad libitum. To obtain serum, mice were anaesthetized with diethylether (Roth, Karlsruhe, Germany) and blood was drawn into capillaries by puncturing the retrobulbar plexus.

To determine creatinine clearance, urinary flow rate and urinary excretion of phosphate, mice were placed individually in metabolic cages (Techniplast, Hohenpeissenberg, Germany) as described previously [46]. They were allowed a 2 days habituation period during which food and water intake, urinary flow rate and phosphate excretion were recorded every day to ascertain that mice were adapted to the new environment. Subsequently, 24 h collection of urine was performed for three consecutive days in order to obtain the urinary parameters. This procedure was repeated under low-phosphate diet. To assure quantitative urine collection, metabolic cages were siliconized, and urine was collected under water-saturated oil.

The phosphate concentration was determined colorimetrically utilizing a commercial diagnostic kit (Roche Diagnostics, Mannheim, Germany). The urinary concentration of Ca^{2+} was measured by flame photometry (Eppendorf, Hamburg, Germany). The creatinine concentration in urine was determined using the Jaffe reaction (Labor und Technik, Berlin, Germany). The serum intact parathyroid hormone concentration was measured using an ELISA kit (Immunotopics, San Clemente, USA). The assay sensitivity, i. e. the lowest concentration of mouse PTH which is measurable, is 4 pg/ml according to the manufacturer. The concentration of $1,25(\text{OH})_2\text{D}_3$ (IDS Diagnostics, Frankfurt/Main, Germany) and of FGF23 (Immunotopics) were also determined by ELISA.

Brush border membrane vesicles (BBMV) preparation and immunoblotting

BBMVs were prepared from mouse kidney cortex and outer medulla using the Mg^{2+} precipitation technique as described previously [47, 48]. The total protein concentration was measured using the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA, USA). BBMVs were stored at -80°C until further use. Five micrograms of renal brush border membrane proteins were solubilized in loading buffer containing dithiothreitol (DTT) and separated on 8% polyacrylamide gels. For immunoblotting, the proteins were transferred electrophoretically to polyvinylidene fluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA, USA). After blocking with 5% milk powder in Tris-buffered saline/0.1% Tween-20 for 60 min, the blots were incubated with the primary antibodies: rabbit polyclonal anti-NaPilla (1:6000) [49] and mouse monoclonal anti- β -actin antibody (42 kD; Sigma, St. Louis, MO; 1:5000) either for 2 h at room temperature or overnight at 4°C . Membranes were then incubated for 1 h at room temperature with secondary goat anti-rabbit or donkey anti-mouse antibodies 1:5000 linked to alkaline phosphatase (Promega, Mannheim, Germany) or to horseradish peroxidase (HRP) (Amersham, Freiburg, Germany). The protein signal was detected with the appropriate substrates (Millipore, Schwalbach, Germany) using the DIANA III-chemiluminescence detection system (Raytest, Straubenhardt, Germany). All images were analysed using the software Advanced Image Data Analyser AIDA, Raytest to calculate the protein of interest/ β -actin ratio.

Western blotting of whole kidney

To determine protein abundance in renal tissue, mice were anesthetized with diethylether. The kidneys were removed and immediately shock-frozen in liquid nitrogen. Renal tissue was homogenised with an electric homogenizer at 4°C in lysis buffer (54.6 mM HEPES; 2.69 mM $\text{Na}_4\text{P}_2\text{O}_7$; 360 mM NaCl; 10% [vol/vol] Glycerol; 1% [vol/vol] NP40) containing phosphatase and protease inhibitors (Complete mini, Roche, Mannheim, Germany). Homogenates were clarified by centrifugation at 20000 g for 20 min. Total protein (100 μg) was separated by SDS-PAGE (10% Tris-Glycine), transferred to nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany), blocked for 3 h in blocking buffer (5% fat-free milk in Tris-buffered saline (TBS) containing 0.1% Tween), and incubated overnight with an anti Klotho antibody (kindly provided by Akiko Saito from the Kyowa Hakko Kirin Co., Ltd., Japan) (1:1.000 in 5% fat free milk in TBS). After incubation with a horseradish peroxidase-conjugated anti-rat secondary antibody (Cell Signaling, Germany), the bands were visualized with enhanced chemiluminescence according to the manufacturer's instructions. Homogenates were also probed with a primary GAPDH (Cell Signaling) antibody as loading control. Densitometric analysis of Klotho and GAPDH was performed using Quantity One software (Bio-Rad Laboratories).

Immunohistochemistry

For immunofluorescence microscopic detection of NaPilla and OSR in proximal tubule immunolabeling was performed on cryostat or paraffin sections of 5 μm thickness, blocked with 5% milk powder in PBS,

Fig. 1. Distribution of the oxidative stress responsive kinase 1 (OSR1) in wild type (*osr1^{+/+}*) kidneys. A. Confocal images showing double-labeling of OSR1 (green signal) and NaPiIIa (red signals) in proximal tubules from wild type kidneys. Merged images demonstrate localization of OSR1 in close proximity of NaPiIIa in a subset of proximal tubules, whereas some proximal tubules express

either OSR1 or NaPiIIa. B. Higher resolution images demonstrating localization of OSR1 (green signal in the apical cellular compartment) in close proximity of NaPiIIa (red signal in the brushborder membrane) in proximal tubules from wild type kidney; nuclei are counterstained in blue on the merged image.

incubated for 2 h with primary antibodies (sheep anti-OSR antibody [1:500 dilution; University of Dundee] and rabbit anti-NaPiIIa [1:400 dilution; kind gift of J. Biber, Zurich]), followed by secondary antibodies (Cy3-coupled donkey-anti sheep IgG and Cy2-coupled swine-anti rabbit IgG [Dianova]). Specificity of the double-staining procedures was controlled by parallel incubation of consecutive sections, each incubated only with one single probe. Fluorescence signals were detected by confocal laser scanning microscopy (LSM 510 META; Carl Zeiss, Jena, Germany) with a Plan Neofluar 100 $\times$ /1.3 oil objective [50, 51].

Statistics

Data are provided as means $\pm$ SEM, *n* represents the number of independent experiments. All data were tested for significance using unpaired Student's *t*-test or ANOVA, as appropriate. Only results with *p* < 0.05 were considered statistically significant.

Results

Immunohistochemistry was employed to explore whether OSR1 is expressed in proximal renal tubules. As illustrated in Fig. 1, OSR1 protein could indeed be detected in the proximal nephron. Concomitant labelling of the proximal tubular phosphate transporter NaPiIIa revealed localization of both, the kinase and the carrier, in a subset of proximal tubule cells (Fig. 1).

To possibly disclose an effect of OSR1 on NaPiIIa, cRNA encoding NaPiIIa was injected into *Xenopus* oocytes with or without cRNA encoding OSR1 and electrogenic phosphate transport determined by the dual-electrode voltage-clamp technique. Addition of phosphate (1 mM) to the bath induced only a small inward current in non-injected or water-injected *Xenopus* oocytes, indicating that *Xenopus* oocytes do not express endogenous electrogenic phosphate transport (Fig. 2). In *Xenopus* oocytes expressing NaPiIIa, phosphate induced an inward current (*I_p*) consistent with electrogenic entry of Na⁺ together with phosphate (Fig.

Fig. 2. Coexpression of OSR1 up-regulates electrogenic phosphate transport in NaPiIIa-expressing *Xenopus* oocytes. A. Representative original tracings showing phosphate (1 mM)-induced current (I_p) in *Xenopus* oocytes injected with water (a) as well as expressing NaPiIIa without (b) or with additional coexpression of wild type OSR1 (c) or inactive mutant D^{164A} OSR1 (d). B. Arithmetic means $\pm$ SEM ($n = 17$ -62) of phosphate (1 mM)-induced current (I_p) in *Xenopus* oocytes injected with water (H_2O), or expressing NaPiIIa without (2nd bar) or with additional coexpression of wild type OSR1 (3rd bar) or of the inactive mutant D^{164A} OSR1 (4th bar). *** ($p < 0.001$) indicates statistically significant difference from the absence of OSR1. ### ($p < 0.001$) indicates statistically significant difference between wild type OSR1 and mutant D^{164A} OSR1. C. Arithmetic means $\pm$ SEM ($n = 7$) of I_p as a function of phosphate concentration in *Xenopus* oocytes expressing NaPiIIa without (open circles) or with (closed circles) additional coexpression of wild type OSR1. ** ($p < 0.01$) indicates statistically significant difference from the absence of OSR1.

2A,B). As illustrated in Fig. 2A,B, I_p was significantly enhanced by additional coexpression of OSR1. In contrast, coexpression of the inactive mutant D^{164A} OSR1 did not significantly modify I_p in NaPiIIa-expressing *Xenopus* oocytes (Fig. 2A,B).

Fig. 2C displays a kinetic analysis of phosphate-induced current. In NaPiIIa-expressing *Xenopus* oocytes the maximal current was 21.7 ± 1.1 nA ($n = 7$). The phosphate concentration required for halfmaximal current (K_M) was 59.6 ± 20.1 μ M. The coexpression of OSR did not significantly modify apparent K_M (55.2 ± 9.4 μ M), but significantly increased the maximal current to 48.8 ± 1.2 nA ($n = 7$).

Fig. 3. Urinary phosphate excretion in *osr1*^{+/+} and *osr1*^{tg/+} mice. Arithmetic means $\pm$ SEM (n = 14-16) of urinary phosphate excretion in heterozygous OSR1 knockin mice (*osr1*^{tg/+}, black bars) and wild type mice (*osr1*^{+/+}, white bars) under normal diet (A) and following dietary phosphate depletion (B). * (p<0.05), ** (p<0.01) indicate significant difference from *osr1*^{+/+} mice, ### (p<0.001) indicates significant difference from normal diet.

Fig. 4. Serum phosphate concentration in *osr1*^{+/+} and *osr1*^{tg/+} mice. Arithmetic means $\pm$ SEM (n = 14-16) of the serum phosphate concentration in heterozygous OSR1 knockin mice (*osr1*^{tg/+}, black bars) and wild type mice (*osr1*^{+/+}, white bars) under normal diet (A) and following dietary phosphate depletion (B). * (p<0.05), ** (p<0.01) indicate significant difference from *osr1*^{+/+} mice, # (p<0.05), ## (p<0.01) indicate significant difference from normal diet.

In order to elucidate the *in vivo* significance of OSR1-sensitive renal tubular phosphate transport, experiments were performed in OSR1 knockin mice (*osr1*^{tg/+}), which were heterozygously carrying a WNK-insensitive T185A OSR1 mutant. The animals were compared to respective wild type mice (*osr1*^{+/+}). On a standard diet, urinary phosphate excretion was significantly higher in *osr1*^{tg/+} mice than in *osr1*^{+/+} mice (Fig. 3A). A low-phosphate diet decreased the urinary phosphate excretion in both, *osr1*^{tg/+} and *osr1*^{+/+} mice (Fig. 3B). During the low-phosphate-diet, however, urinary phosphate excretion was still significantly higher in *osr1*^{tg/+} mice than in *osr1*^{+/+} mice (Fig. 3B). Urinary calcium excretion tended to be lower in *osr1*^{tg/+} mice (0.24 ± 0.06 μ mol/24h/g BW, n = 8) than in *osr1*^{+/+} mice (0.17 ± 0.03 μ mol/24h/g BW, n = 10), a difference, however, not reaching statistical significance.

In theory, the phosphaturia of *osr1*^{tg/+} mice could have been due to an increased serum phosphate concentration. Conversely, phosphaturia due to decreased renal tubular phosphate transport should result in a decrease of serum phosphate concentration. Thus, serum phosphate concentration was determined. As illustrated in Fig. 4A, the serum phosphate concentration was significantly lower in *osr1*^{tg/+} mice than in *osr1*^{+/+} mice. Thus, the phosphaturia of *osr1*^{tg/+} mice was not due to hyperphosphatemia but obviously resulted in hypophosphatemia. Dietary phosphate depletion decreased the serum phosphate concentration in both, *osr1*^{tg/+} mice and in *osr1*^{+/+} mice and further enhanced the difference between the genotypes (Fig. 4B). Serum calcium levels were similar in *osr1*^{+/+} mice (2.38 ± 0.05 mM, n = 8) and *osr1*^{tg/+} mice (2.41 ± 0.04 mM, n = 10). To study whether the partial OSR1

Fig. 5. Protein abundance of renal sodium-dependent phosphate cotransporter NaPiIIa in the brush border membrane in kidneys from *osr1*^{+/+} and *osr1*^{tg/+} mice. A. Western blot for NaPiIIa. The membrane was stripped and re probed for β-actin to control for loading. Densitometry was performed and the ratio of the protein of interest over β-actin calculated. B. Bar graphs present data as arithmetic means ± SEM (n = 10). *** indicates significant difference from *osr1*^{+/+} mice (Student's t-test, p<0.001).

deficiency results in lower apical expression of NaPiIIa, BBMVs from the proximal tubule were retrieved and NaPiIIa protein expression determined. As shown in Fig. 5, BBMVs from *osr1*^{tg/+} mice indeed expressed NaPiIIa to a significantly lesser extent than BBMVs from *osr1*^{+/+} mice.

At least in theory, the decreased renal tubular phosphate transport could have resulted from altered hormone levels. Thus, the serum concentration of parathyroid hormone (PTH), calcitriol (1,25(OH)₂D₃), and fibroblast growth factor 23 (FGF23) were determined. As shown in Fig. 6, the serum concentrations of PTH (Fig. 6A) and of 1,25(OH)₂D₃ (Fig. 6B) were similar in *osr1*^{tg/+} mice and in *osr1*^{+/+} mice. The serum FGF23 concentration was, however, significantly higher in *osr1*^{tg/+} mice than in *osr1*^{+/+} mice (Fig. 6C). Dietary phosphate depletion resulted in a significant increase in the 1,25(OH)₂D₃ concentration of both, *osr1*^{tg/+} mice and *osr1*^{+/+} mice and tended to decrease the serum concentrations of PTH and FGF23, effects, however not reaching statistical significance (Fig. 6).

As FGF23 signaling depends on Klotho, additional experiments were performed to elucidate whether partial OSR deficiency modifies renal Klotho expression. To this end, renal Klotho protein was determined in *osr1*^{tg/+} mice and *osr1*^{+/+} mice (Fig. 7). As a result, no significant difference of Klotho protein abundance could be observed between *osr1*^{tg/+} mice and *osr1*^{+/+} mice.

Discussion

The present observations reveal that the oxidative stress-responsive kinase 1 (OSR1) is expressed in proximal renal tubules and stimulates renal tubular phosphate transport. To the best of our knowledge OSR1 expression in proximal renal tubules has not been shown before. We further demonstrate that WNK resistance of OSR1 led to phosphaturia. Accordingly, renal tubular phosphate reabsorption was decreased and thus renal phosphate excretion increased in OSR1 knockin mice carrying one allele of the WNK-insensitive T185A OSR1 mutant (*osr1*^{tg/+}) as compared to the respective wild type mice (*osr1*^{+/+}).

The electrophysiological recordings with the two-electrode voltage clamp technique in *Xenopus* oocytes heterologously expressing NaPiIIa with and without OSR1 pointed to a stimulatory effect of OSR1 on NaPiIIa activity. Along those lines, partial deficiency of OSR1 activity in *osr1*^{tg/+} mice led to lower NaPiIIa surface abundance in brush border membrane vesicles isolated from the proximal tubule. The observations in the oocytes suggest that OSR1 directly stimulates NaPiIIa and thus influences renal phosphate excretion. The phosphaturia may, however, reflect in part other mechanisms.

Fig. 6. Serum concentration of PTH, 1,25(OH)₂D₃, and FGF23 in *osr1*^{+/+} and *osr1*^{tg/+} mice. Arithmetic means ± SEM (n = 8-11) of serum PTH (A), 1,25(OH)₂D₃ (B), and FGF23 (C) in *osr1*^{+/+} mice (white bars) and *osr1*^{tg/+} mice (black bars) under normal diet (left bars) and following dietary phosphate depletion (right bars). * (p < 0.05) indicates significant difference from *osr1*^{+/+} mice, ### (p < 0.001) indicates significant difference from normal diet.

The phosphaturia of *osr1*^{tg/+} mice was not secondary to an increased serum phosphate concentration, which was actually decreased. In theory, the phosphaturia *osr1*^{tg/+} could result from an increased serum level of PTH, a hormone increasing urinary phosphate output by stimulation of internalization and subsequent degradation of NaPiIIa [30, 34]. The PTH serum concentration was, however, not significantly different between *osr1*^{tg/+} and

Fig. 7. Abundance of Klotho protein in the kidneys from *osr1*^{+/+} and *osr1*^{tg/+} mice. (A) Original Western blots and (B) arithmetic means $\pm$ SEM (n = 10) of the Klotho over GAPDH density ratio in *osr1*^{+/+} mice (white bar) and *osr1*^{tg/+} mice (black bar).

osr1^{+/+} mice. This observation does not rule out minor alterations of serum PTH levels. Renal tubular phosphate transport and serum phosphate concentration could further be modified by calcitriol (1,25(OH)₂D₃). The serum 1,25(OH)₂D₃ concentration was, however, again not significantly different between *osr1*^{tg/+} mice and *osr1*^{+/+} mice. The renal 1 α -hydroxylase and thus the formation of 1,25(OH)₂D₃ are stimulated by PTH [52] and by cellular phosphate depletion [53]. The present observations confirm the increase in serum 1,25(OH)₂D₃ following dietary phosphate depletion and confirm the decrease of urinary phosphate output in phosphate-depleted animals.

Our study further shows that the FGF23 serum concentration was higher in *osr1*^{tg/+} mice than in *osr1*^{+/+} mice. FGF23 is known to be up-regulated by 1,25(OH)₂D₃ and in turn to decrease the formation of 1,25(OH)₂D₃ [54, 55]. In view of the serum 1,25(OH)₂D₃ levels, the slightly but significantly enhanced FGF23 serum level in *osr1*^{tg/+} mice cannot be explained by 1,25(OH)₂D₃-dependent stimulation of FGF23 expression.

FGF23 is secreted by osteocytes and decreases renal tubular phosphate transport [56]. The increased serum FGF23 concentrations could thus contribute to the phosphaturia of the *osr1*^{tg/+} mice. Since FGF23 formation is stimulated by PTH, increased phosphate intake and hyperphosphatemia [57-59], the enhanced FGF23 serum level of *osr1*^{tg/+} mice cannot be explained by renal phosphate loss and hypophosphatemia. In theory, the enhanced FGF23 release in *osr1*^{tg/+} mice could be secondary to the effect of OSR1 on NaCl-co-transporter NCC. The carrier is expressed in osteoblasts, fosters bone differentiation and increases bone mineral density [23-25]. OSR1 activates and phosphorylates NCC [1-3, 9-14]. Alternatively, OSR1 could be effective through inhibition of renal NCC or by more directly modulating FGF23 release from osteocytes. Further studies are warranted addressing the mechanisms causing the elevated FGF23 plasma concentration in *osr1*^{tg/+} mice.

The effect of FGF23 on renal cells requires expression of Klotho, which thus similarly influences phosphate homeostasis [38]. The determination of total renal Klotho protein expression did, however not reveal any significant differences between *osr1*^{tg/+} mice and *osr1*^{+/+} mice.

At least in theory, OSR1 may be in part effective by regulating the thiazide-sensitive Na⁺-Cl⁻ cotransporter and the furosemide-sensitive Na⁺,K⁺,2Cl⁻ cotransporters (NKCC1 and

NKCC2) [13, 60]. Inhibition of NCC by thiazides is well known to cause phosphaturia [61] and anticalciuria [62]. Decreased stimulation of NCC in *osr1^{tg/+}* bone cells may further contribute to or even account for the enhanced FGF23 plasma concentrations [63].

The OSR1 sensitivity of renal phosphate excretion and plasma phosphate concentration may have clinical implications for bone mineralization on the one hand [64] and vascular calcification on the other [65, 66]. It is tempting to speculate that decreased OSR1 activity may foster demineralization of bone and enhanced OSR1 activity may predispose to phosphate retention and subsequent tissue calcification. However, calcification is an active process not only depending on plasma concentrations of Ca^{2+} and phosphate [67]. Thus, additional experimental effort is needed to elucidate the impact of OSR1 activity on mineral metabolism.

Conclusion

OSR1 participates in the regulation of FGF23 release and renal tubular phosphate transport. OSR1 stimulates NaPiIIa, and partial loss of OSR1 activity leads to phosphaturia and increased formation of FGF23. The present observations thus disclose a completely novel, powerful element in the regulation of mineral metabolism and a novel functional role of oxidative stress-responsive kinase 1.

Conflict of Interests

The authors declare that they have no competing financial interests to disclose.

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