Original Paper

Lithium- Sensitive Store-Operated Ca^{2+} Entry in the Regulation of FGF23 Release

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Key Words

Lithium • FGF23 • $1,25(\text{OH})_2\text{D}_3$ • SOCE • Calcium • 2-APB • Orai1 • STIM1

Abstract

Background/Aims: Lithium, a widely used drug for the treatment of mood disorders, has previously been shown to stimulate the release of fibroblast growth factor FGF23, a powerful regulator of $1,25(\text{OH})_2\text{D}_3$ formation and mineral metabolism. The cellular mechanisms involved have remained elusive. Lithium has been shown to modify Ca^{2+} signaling. In a wide variety of cells, Ca^{2+} entry is accomplished by the pore-forming Ca^{2+} channel subunit Orai1 and its regulator STIM, which stimulates Orai following Ca^{2+} depletion of intracellular stores. Transcription factors promoting Orai1 expression include NF- κ B. The present study thus explored whether the effect of lithium on FGF23 involves and requires Ca^{2+} entry. **Methods:** Experiments were performed in UMR106 osteoblastic cells and immortalized primary osteoblasts (IPO). FGF23 and Orai1 transcript levels were estimated from qRT-PCR, cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) from Fura2 fluorescence and store-operated Ca^{2+} entry (SOCE) from an increase in $[\text{Ca}^{2+}]_i$ following store depletion by inhibition of the sarcoendoplasmatic Ca^{2+} ATPase (SERCA) with thapsigargin (1 μM). **Results:** SOCE in UMR106 cells was enhanced by lithium treatment, an effect abrogated by Orai1 inhibitor 2-APB (50 μM). FGF23 transcript levels were increased by lithium and inhibited by Orai1 inhibitors 2-APB (50 μM) and YM58483 (100 nM) as well as NF- κ B inhibitors wogonin (100 μM) and withaferin A (500 nM). Moreover, Orai1 transcript levels were up-regulated by lithium, an effect attenuated by wogonin and withaferin A. **Conclusion:** Lithium stimulates FGF23 release at least in part by NF- κ B dependent up-regulation of Orai1 transcription and store operated Ca^{2+} entry.

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Introduction

FGF23 (fibroblast growth factor 23), a bone-derived regulator of calcium phosphate metabolism [1, 2] reduces the formation and enhances the inactivation of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) by down-regulation of renal 1 α hydroxylase (Cyp27b1) and up-regulation of 25-hydroxyvitamin D 24-hydroxylase (Cyp24) [3-6], respectively. FGF23 thus decreases the serum levels of 1,25(OH)₂D₃ [5-12], a powerful regulator of renal and intestinal phosphate and calcium transport [13-16]. FGF23 inhibits renal tubular phosphate reabsorption [2, 5, 7, 8, 10-12, 17] and fosters renal phosphate elimination [5, 7, 8, 10-12]. FGF23 deficiency results in greatly elevated serum phosphate, calcium, and 1,25(OH)₂D₃ levels, effects eventually resulting in rapid aging and a dramatic reduction of lifespan in part due to excessive vascular calcification [4, 12, 18-21].

For its renal effects, FGF23 requires Klotho as a co-receptor [22-24]. Mice lacking functional Klotho similarly suffer from multiple age-related disorders and decreased life span resulting from extensive soft tissue calcification [23, 25-39]. The effects of Klotho deficiency are also largely due to increased 1,25(OH)₂D₃ formation, enhanced renal tubular phosphate reabsorption and increased serum phosphate levels [40-42]. As a matter of fact, hyperphosphatemia fosters vascular calcification [43] and is considered a predictor of mortality [44].

FGF23 release is stimulated by 1,25(OH)₂D₃ [45, 46]. Further stimulators of FGF23 formation in osteoblasts include PHEX (phosphate-regulating gene with homology to endopeptidase) and DMP-1 (dentin matrix protein or cyclin D binding myb-like protein 1), sustained phosphate load even in the absence of hyperphosphatemia, increased extracellular Ca²⁺ concentration and parathyroid hormone [45].

Recent observations revealed that FGF23 release is stimulated by lithium [47], which is widely used in the treatment of bipolar disorders and Alzheimer's disease [48-50]. Cellular mechanisms sensitive to lithium include Ca²⁺ oscillations, which are triggered by release of Ca²⁺ from intracellular stores [51]. Store-operated Ca²⁺ entry [52] may be accomplished by the 4-transmembrane-spanning pore forming calcium release-activated channel (CRAC) moiety Orai1 (CRACM1), which mediates entry of extracellular Ca²⁺ [53-55], and stromal interaction molecule 1 (STIM1), an Orai1-regulating Ca²⁺ sensor predominantly expressed in the endoplasmatic reticulum (ER) [56-58].

The present study explored whether lithium modifies the cytosolic Ca²⁺ concentration in FGF23-producing UMR106 osteoblast-like cells [59] and whether cytosolic Ca²⁺ impacts on FGF23 formation in these cells.

Materials and Methods

Cell culture

UMR106 rat osteosarcoma cells were cultured in DMEM high glucose medium supplemented with 10% FCS and 1% penicillin/streptomycin under standard cell culturing conditions. Human immortalized primary periosteal cells (IPO) [60] were cultured in DMEM F-12 (1:1 mixture of DMEM and Ham's F-12, high glucose) containing Glutamax and 10% FCS and 1% penicillin/streptomycin/1% fungizide. The cells were pretreated for 24 h with 100 nM 1,25(OH)₂D₃ (Sigma, Schnellendorf, Germany) and subsequently with 2 mM LiCl (Lithium) (Calbiochem, Darmstadt, Germany) with or without 50 μ M Orai inhibitor 2-APB (TOCRIS, Bristol, UK), 100 nM Orai inhibitor YM58483 (TOCRIS), 500 nM NF κ B inhibitor withaferin A (TOCRIS), or 100 μ M NF κ B inhibitor wogonin (Sigma) for another 24 h.

Quantitative Real Time-PCR (qRT-PCR)

Total RNA was isolated with TriFast RNA extraction reagent (Peqlab Biotechnologie GmbH, Erlangen, Germany). mRNA was transcribed with SuperScript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany) using an oligo dT primer. Quantitative RT-PCR was performed on a BioRad iCycler iQTM Real-Time

PCR Detection System (Bio-Rad Laboratories, Munich, Germany) using the following primers:

Rat *Tbp* (*TATA box-binding protein*):

forward (5'-3'): ACTCCTGCCACACCAGCC

reverse (5'-3'): GGTCAGTTTACAGCCAAGATTCA

Rat *Fgf23*

forward (5'-3'): TGGCCATGTAGACGGAACAC

reverse (5'-3'): GGCCCCTATTATCACTACGGAG

Rat *Orai1*

forward (5'-3'): CGTCCACAACCTCAACTCC

reverse (5'-3'): AACTGTGGTCCGTCTTAT

Rat *NFκB p65*

forward (5'-3'): TTCCCTGAAGTGGAGCTAGGA

reverse (5'-3'): CAGTCGAGGAAGACACTGGA

Human *GAPDH*

forward (5'-3'): CAACGGATTGTGGTCGT

reverse (5'-3'): GACAAGCTTCCCGTTCTCAG

Human *Fgf23*

forward (5'-3'): TGCTGGCTCCGTAGTGATAA

reverse (5'-3'): TGATGCTTCGGTGACAGGTA

The final volume of the PCR reaction mixture was 20 µl and contained: 2 µl cDNA, 1 µM of each primer, 10 µl GoTaq qPCR Master Mix (Promega, Mannheim, Germany) and sterile water up to 20 µl. qPCR conditions were 95°C for 3 min, followed by 40 cycles of 95°C for 10 s and 58°C for 30 s. Calculated mRNA expression levels were normalized to the expression levels of *Tbp* of the same cDNA sample. Gene expression was quantified with the $\Delta\Delta C_t$ method.

Measurement of intracellular Ca^{2+}

To determine the cytosolic Ca^{2+} concentration, the cells were loaded with Fura-2/AM (2 µM, Molecular Probes, Goettingen, 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). The emitted fluorescence intensity was recorded at 505 nm and 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 prior to and following removal of extracellular Ca^{2+} (and addition of 0.5 mM EDTA), subsequent addition of thapsigargin (1 µM) and subsequent re-addition of extracellular Ca^{2+} to the Ringer solution, composed of (in mM): 125 NaCl, 5 KCl, 1.2 $MgSO_4$, 32.2 Hepes, 2 Na_2HPO_4 , 0 or 2 $CaCl_2$ and respectively 0.5 or 0 EGTA, and 5 glucose at pH 7.4 (NaOH).

Immunofluorescence

UMR106 cells treated without or with LiCl and cultured on 4-well chamber slides (Thermo scientific), were washed and fixed with 4% paraformaldehyde for 30 min at room temperature. For blocking unspecific binding, UMR106 cells were incubated with 3% Albumin Fraktion V (Carl Roth, Karlsruhe, Germany), 5% normal goat serum (Sigma, Schnelldorf, Germany), and 0.5% Triton in PBS (PAA, Cölbe, Germany) for 30 min at room temperature. Then, the cells were exposed to rabbit anti-p65 (1:1000, Genetex, Irvine, USA) at 4°C in a humidified chamber overnight. The cells were rinsed four times with PBS and incubated with DyLight® 488-conjugated goat anti-rabbit antibody (1:3000, BIOZOL, Eching, Germany) for 1 h at room temperature. After four washing steps the nuclei were stained with DRAQ-5 dye (1:400; BIOZOL) for 30 min at room temperature. The slides and coverslips were mounted with FluorSave™ Reagent (Calbiochem, Darmstadt, Germany). Images were taken on a LSM 5 EXCITER confocal laser-scanning microscope (Zeiss, Germany) with a water-immersion Plan-Neofluar 40×/1.3 NA differential interference contrast and analyzed with the instrument's software.

Statistics

Data are provided as means $\pm$ SEM, n represents the number of independent experiments. All data were tested for significance using unpaired Student t -test or ANOVA unless otherwise stated. Only results with $p < 0.05$ were considered statistically significant.

Results

In order to elucidate the mechanism underlying lithium-sensitive FGF23 release, experiments were performed in UMR106 osteoblast-like cells. As lithium is known to stimulate Ca^{2+} entry, the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was determined utilizing Fura2-fluorescence. The measurements were made following depletion of intracellular Ca^{2+} stores by inhibition of the sarcoendoplasmic Ca^{2+} ATPase (SERCA) with thapsigargin in the absence of extracellular Ca^{2+} , and the store-operated Ca^{2+} entry (SOCE) was estimated from the increase in $[\text{Ca}^{2+}]_i$ following re-addition of extracellular Ca^{2+} . As illustrated in Fig. 1, thapsigargin ($1 \mu\text{M}$) treatment in the absence of extracellular Ca^{2+} was followed by a transient increase in $[\text{Ca}^{2+}]_i$ reflecting depletion of intracellular stores. The subsequent addition of extracellular Ca^{2+} was followed by a rapid increase in $[\text{Ca}^{2+}]_i$ reflecting store operated Ca^{2+} entry (SOCE). The pretreatment with lithium was followed by a significant augmentation of the thapsigargin-induced increase in $[\text{Ca}^{2+}]_i$ and of the increase in $[\text{Ca}^{2+}]_i$ following re-addition of extracellular Ca^{2+} (Fig. 1).

The additional treatment with Orai1 inhibitor 2-APB ($50 \mu\text{M}$) significantly blunted the thapsigargin-induced elevation of $[\text{Ca}^{2+}]_i$ and virtually abrogated the increase in $[\text{Ca}^{2+}]_i$ following re-addition of extracellular Ca^{2+} (Fig. 2).

In order to test whether the effect of lithium on SOCE was mediated by NF- κB , experiments were repeated in the absence and presence of NF- κB inhibitor of wogonin ($100 \mu\text{M}$).

Fig. 1. Thapsigargin-induced intracellular Ca^{2+} release and subsequent SOCE in UMR106 cells without and with pretreatment with lithium. A. Representative original tracings showing the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in Fura-2/AM loaded UMR106 cells prior to and following removal of extracellular Ca^{2+} , addition of the sarco-endoplasmic Ca^{2+} ATPase (SERCA) inhibitor thapsigargin ($1 \mu\text{M}$) and re-addition of extracellular Ca^{2+} without (white circles) and with (black circles) prior lithium treatment (2 mM , 24 h). B,C. Arithmetic means $\pm$ SEM ($n = 20-60$) of the peak (left) and slope (right) values of $[\text{Ca}^{2+}]_i$ increase following addition of thapsigargin reflecting Ca^{2+} release from intracellular stores (B) and of $[\text{Ca}^{2+}]_i$ increase following re-addition of extracellular Ca^{2+} reflecting store-operated Ca^{2+} entry (C) in UMR cells incubated in the presence (black bars) and absence (white bars) of lithium (2 mM , 24 h) **($p < 0.01$), ***($p < 0.001$), unpaired Student's t -test.

Fig. 2. Effect of Orai1 inhibitor 2-APB on thapsigargin-induced intracellular Ca^{2+} release and subsequent SOCE in lithium treated UMR106 cells. A. Representative original tracings showing the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in Fura-2/AM loaded UMR106 cells prior to and following removal of extracellular Ca^{2+} , addition of the sarco-endoplasmic Ca^{2+} ATPase (SERCA) inhibitor thapsigargin (1 μ M) and re-addition of extracellular Ca^{2+} in control cells (white circles), and in lithium-pretreated cells without (black circles) or with (grey circles) simultaneous treatment with Orai inhibitor 2-APB (10 μ M). B,C. Arithmetic means $\pm$ SEM (n = 22-60) of the peak (left) and slope (right) values of $[Ca^{2+}]_i$ increase following addition of thapsigargin reflecting Ca^{2+} release from intracellular stores (B) and of $[Ca^{2+}]_i$ increase following re-addition of extracellular Ca^{2+} reflecting store-operated Ca^{2+} entry (C) in untreated control cells (white bars) or in lithium pretreated (2 mM, 24 h) UMR106 cells in the absence (black bars) or presence (grey bars) of Orai inhibitor 2-APB (50 μ M). **($p < 0.001$), ***($p < 0.001$), ANOVA.

Again, SOCE was triggered in UMR106 cells by removal of extracellular Ca^{2+} and addition of the sarco-endoplasmic Ca^{2+} ATPase (SERCA) inhibitor thapsigargin (1 μ M) followed by readdition of extracellular Ca^{2+} . As illustrated in Fig. 3, SOCE was increased by treatment with lithium (2 mM, 24 h), an effect abrogated by wogonin (100 μ M).

In order to determine whether the lithium-induced increase in the cytosolic Ca^{2+} concentration could participate in the stimulation of FGF23 release, FGF23 transcript levels were determined by qRT-PCR. As illustrated in Fig. 4, lithium (2 mM) significantly increased FGF23 transcript levels in IPO cells (Fig. 4A) and UMR106 cells (Fig. 4B-C), an effect significantly blunted by Orai inhibitors 2-APB (50 μ M) or YM58483 (100 nM).

A further series of experiments explored whether lithium is effective through upregulation of the transcription factor NF- κ B. To this end, the effect of lithium on the nuclear factor NF- κ B subunit p65 transcript levels was tested. As illustrated in Fig. 5, lithium treatment indeed up-regulated the NF- κ B p65 transcript levels in UMR106 cells (Fig. 5A). As illustrated in Fig. 5B, lithium treatment enhanced the nuclear localization of NF- κ B p65.

In order to test whether the effect of lithium on Orai1 transcript levels in UMR106 cells requires NF- κ B, UMR106 cells were treated with lithium in the absence or presence of NF- κ B inhibitors withaferin A or wogonin. As illustrated in Fig. 6, both inhibitors virtually abrogated the stimulating effect of lithium on Orai1 transcript levels in UMR106 cells.

A final series of experiments tested whether the effect of lithium on FGF23 transcript levels in UMR106 cells was similarly dependent on NF- κ B. To this end, FGF23 transcript levels were determined in UMR106 cells with or without NF- κ B inhibitors withaferin or wogonin. As illustrated in Fig. 7, the stimulating effect of lithium on the FGF23 transcript levels in UMR106 cells was virtually abrogated by both, wogonin (100 μ M) and withaferin A (500 nM).

Fig. 3. Thapsigargin-induced intracellular Ca^{2+} release and subsequent SOCE in UMR106 cells without and with pretreatment with lithium in the absence or presence of wogonin. A. Representative original tracings showing the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in Fura-2/AM loaded UMR106 cells prior to and following removal of extracellular Ca^{2+} , addition of the sarco-endoplasmic Ca^{2+} ATPase (SERCA) inhibitor thapsigargin (1 μ M) and re-addition of extracellular Ca^{2+} in untreated control cells (white circles) or in cells pretreated with lithium (2 mM, 24 h) without (black circles) and with (grey circles) presence of wogonin (100 μ M). B,C. Arithmetic means $\pm$ SEM ($n = 23-46$) of the peak (left) and slope (right) values of $[Ca^{2+}]_i$ increase following addition of thapsigargin reflecting Ca^{2+} release from intracellular stores (B) and of $[Ca^{2+}]_i$ increase following re-addition of extracellular Ca^{2+} reflecting store-operated Ca^{2+} entry (C) in UMR cells incubated in the absence of lithium (white bars) and in the presence of lithium (2 mM, 24 hours) without (black bars) and with (grey bars) additional treatment with wogonin (100 μ M). * ($p < 0.05$), ** ($p < 0.001$), *** ($p < 0.001$), ANOVA.

Fig. 4. Effect of lithium on FGF23 transcript levels in IPO and UMR106 cells. A. Arithmetic means $\pm$ SEM ($n = 12-17$) of the FGF23 transcript levels in IPO cells without (white bar) and with (black bar) prior lithium treatment (2 mM). B. Arithmetic means $\pm$ SEM ($n = 6$) of the FGF23 transcript levels in UMR106 cells without (white bars) and with (black bars) prior lithium treatment (2 mM, 24 h) in the absence (left bars) and presence (right bars) of Orai inhibitor 2-APB (50 μ M). C. Arithmetic means $\pm$ SEM ($n = 16-18$) of the FGF23 transcript levels in UMR106 cells without (white bars) and with (black bars) prior lithium treatment (2 mM, 24 h) in the absence (left bars) and presence (right bars) of Orai inhibitor YM58483 (100 nM). *, ** ($p < 0.01$, $p < 0.001$) indicates difference from control. ### ($p < 0.001$) indicates difference from lithium alone.

Discussion

The present observations demonstrate that lithium up-regulates the Ca^{2+} channel Orai1 and enhances store operated Ca^{2+} entry (SOCE). Both effects are virtually abrogated by pharmacological inhibition of the transcription factor NF- κ B. The present observations further reveal that lithium enhances NF- κ B transcript levels. Lithium up-regulates FGF23

Fig. 5. Effect of lithium on NF-κB p65 in UMR106 cells. A. Arithmetic means $\pm$ SEM ($n = 6$) of the NF-κB p65 transcript levels in UMR106 cells without (white bar) and with (black bar) prior lithium treatment (2 mM, 24 h). B. Confocal microscopy of NF-κB p65 abundance in UMR106 cells without (upper panels) and with (lower panels) prior lithium treatment (2 mM) (Nuclear Draq5, Red; NF-κB, Green), scale bar 20 μ m. * ($p < 0.05$), unpaired Student's t-test.

Fig. 6. Effect of lithium on Orai1 transcript levels in UMR106 cells in the absence or presence of NF-κB inhibitors withaferin A or wogonin. A. Arithmetic means $\pm$ SEM ($n = 6$) of the Orai1 transcript levels in UMR106 cells without (white bars) and with (black bars) prior lithium treatment (2 mM, 24 h) in the absence (left bars) and presence (right bars) of NF-κB inhibitor withaferin A (500 nM). B. Arithmetic means $\pm$ SEM ($n = 12-15$) of the Orai1 transcript levels in UMR106 cells without (white bars) and with (black bars) prior lithium treatment (2 mM) in the absence (left bars) and presence (right bars) of NF-κB inhibitor wogonin (100 μ M). **, *** ($p < 0.01$, $p < 0.001$) indicates difference from control. ### ($p < 0.001$) indicates difference from lithium alone.

transcript levels, an effect virtually abrogated by pharmacological inhibition of Orai1 and NF-κB. Previous observations demonstrated the stimulation of Orai1 transcription and protein by NF-κB [61-63] and the stimulating effect of SOCE on FGF23 transcription and protein expression [64]. Effects of lithium on FGF23 release may in addition be modified by GSK3 phosphorylation [65], a known effect of lithium [66-71].

It is noteworthy, that the serum FGF23 level is elevated in gene targeted mice expressing WNK-resistant SPAK [72] or OSR1 [73]. Both SPAK and OSR1 stimulate renal tubular Na-Cl co-transporter, and lack of those kinases is expected to result in cell shrinkage, which fosters Ca^{2+} oscillations [74]. Orai1 is upregulated by the serum & glucocorticoid inducible kinase SGK1 [61-63, 75], which is in turn markedly upregulated by cell shrinkage [76]. Orai1 is further regulated by the AMPK activated kinase [77]. Future studies will be required to explore whether this kinase participates in the regulation of FGF23 release.

Fig. 7. Effect of lithium on FGF23 transcript levels in UMR106 cells in the absence or presence of NF- κ B inhibitors withaferin A or wogonin. A. Arithmetic means $\pm$ SEM ($n = 12$) of the FGF23 transcript levels in UMR106 cells without (white bars) and with (black bars) prior lithium treatment (2 mM, 24 h) in the absence (left bars) and presence (right bars) of NF- κ B inhibitor withaferin A (500 nM). B. Arithmetic means $\pm$ SEM ($n = 12$) of the FGF23 transcript levels in UMR106 cells without (white bars) and with (black bars) prior lithium treatment (2 mM, 24 h) in the absence (left bars) and presence (right bars) of NF- κ B inhibitor wogonin (100 μ M). *** ($p < 0.001$) indicates difference from control. ### ($p < 0.001$) indicates difference from lithium alone.

FGF23 plasma concentrations are further enhanced in patients suffering from chronic kidney disease or injury [45, 78-89]. Notably, plasma FGF23 levels increase prior to plasma phosphate concentrations and prior to the development of hyperparathyroidism [22, 80-82, 87]. FGF23 plasma levels are also elevated in diabetic nephropathy [90] and polycystic kidney disease [91-93]. Notably, in those diseases, FGF23 originates at least in part from formation in kidney. The signaling underlying the triggering of renal FGF23 formation in those diseases remains elusive [91, 93]. It is tempting to speculate that deregulated intracellular Ca^{2+} signaling could contribute to the renal formation of FGF23. The hyperphosphatemia of renal insufficiency results in vascular calcification [94], which is counteracted by Klotho and FGF23 [95, 96].

FGF23 downregulates 1α hydroxylase and thus the formation of $1,25(\text{OH})_2\text{D}_3$ [3, 5], an effect requiring Klotho as a co-receptor [23, 97]. Conversely, $1,25(\text{OH})_2\text{D}_3$ stimulates the release of FGF23 [98]. Lithium treatment increases FGF23 serum levels despite a decrease of $1,25(\text{OH})_2\text{D}_3$ serum concentrations [47], which is in turn expected to lower FGF23 release [99-102].

$1,25(\text{OH})_2\text{D}_3$ stimulates both, renal and intestinal phosphate transport [13]. FGF23 reduces renal tubular phosphate reabsorption by decreasing $1,25(\text{OH})_2\text{D}_3$ formation and by more directly inhibiting Na^+ -coupled phosphate transport in proximal renal tubules [2, 5]. FGF23 release is thus expected to trigger phosphaturia and decrease the serum phosphate concentration. As high serum phosphate concentrations foster vascular calcification and are associated with accelerated aging and decreased life span [103], an increased FGF23 release could counteract vascular calcification, aging and early death. Lithium has indeed been shown to attenuate tissue calcification [104]. Klotho [24] and FGF23 [25] are both powerful regulators of aging and lack of either, Klotho [24] or FGF23 [5] accelerates the development of several age-related disorders and leads to early death.

FGF23 is primarily produced in osteoblasts and its major biological and biomedical function is the regulation of $1,25(\text{OH})_2\text{D}_3$ formation [3, 5]. The effect of lithium on FGF23 expression may thus be considered a side effect unrelated to the antidepressant activity of the drug [105]. The observed effect of lithium may, for instance, contribute to the known impact of lithium on inflammation [106]. However, $1,25(\text{OH})_2\text{D}_3$ has been shown to be a powerful regulator of mood [107-109] and the influence on FGF23 may indirectly modify the neurobiological

effects of lithium. Moreover, NF- κ B [110, 111] and Orai1 [112-114] are expressed in the brain and it is tempting to speculate that the effect of lithium on NF- κ B and Orai1 may contribute to the neuronal effects of this widely used drug. Moreover, SOCE contributes to the regulation of function and to the pathophysiology of neurons [115] and glial cells [114-116]. Known neurobiological and neuro-psychiatric effects of lithium include not only antidepressant activity but as well neuroprotection against several neurotoxic paradigms including Alzheimer disease [117]. Notably, Orai1 has been shown to confer survival of several cell types [118-122]. To the best of our knowledge, an effect of lithium on Orai1 expression and function has never been shown. In view of the present observations, extensive additional experimentation is warranted clarifying the neurobiological significance of lithium sensitive up-regulation of NF- κ B, Orai1, FGF23 and/or related growth factors.

In conclusion, the present observations reveal that an increase in the cytosolic Ca^{2+} concentration stimulates the expression of FGF23 in UMR106 osteoblast cells. Lithium upregulates cytosolic Ca^{2+} release and subsequent store-operated Ca^{2+} entry, which are mainly accomplished by Orai1 and its regulator STIM1.

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Disclosure Statement

All authors disclose that they have not any potential conflict of interest (e.g., consultancies, stock ownership, equity interests, patent-licensing arrangements, lack of access to data, or lack of control of the decision to publish).

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