PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop

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Lavi-Moshayoff V, Wasserman G, Meir T, Silver J, Naveh-Many T. PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. Am J Physiol Renal Physiol 299: F882–F889, 2010.—Parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) target the kidney to cause a phosphaturia. FGF23 also acts on the parathyroid to decrease PTH expression, but in chronic kidney disease (CKD) there are high-serum PTH and FGF23 levels and resistance of the parathyroid to FGF23. We now report that PTH acts on bone to increase FGF23 expression and characterize the signal transduction pathway whereby PTH increases FGF23 expression. Remarkably, we show that PTH is necessary for the high-FGF23 levels of early kidney failure due to an adenine high-phosphorus diet. Parathyroidectomy before the diet totally prevented the fivefold increase in FGF23 levels in kidney failure rats. Moreover, parathyroidectomy of early kidney failure rats corrected their high-FGF23 levels. Therefore, in early kidney failure, the high-FGF23 levels are dependent on the high-PTH levels. PTH infusion for 3 days to mice with normal renal function increased serum FGF23 and calvaria FGF23 mRNA levels. To demonstrate a direct effect of PTH on FGF23, we added PTH to rat osteoblast-like UMR106 cells. PTH increased FGF23 mRNA levels (4-fold) and this effect was mimicked by a PKA activator, forskolin. PTH also decreased SOST mRNA levels (3-fold). SOST codes for sclerostin, a Wnt pathway inhibitor, which is a PTH receptor (PTH1R) target. The effect of PTH was prevented by added sclerostin. Therefore, PTH increases FGF23 expression which involves the PKA and Wnt pathways. The effect of PTH on FGF23 completes a bone-parathyroid endocrine feedback loop. Importantly, secondary hyperparathyroidism is essential for the high-FGF23 levels in early CKD.

chronic kidney disease; phosphate; osteocyte; secondary hyperparathyroidism

PARATHYROID HORMONE (PTH), fibroblast growth factor 23 (FGF23), and 1,25(OH)2 vitamin D (1,25D) are central to calcium and phosphate homeostasis. PTH binds to its receptor the PTH1R on bone and renal tubular cells to maintain normal serum calcium levels. In bone, the PTH1R is expressed in osteoblasts, osteocytes, and chondrocytes. FGF23 is secreted by osteocytes and osteoblasts and is a major factor involved in phosphate homeostasis (46). It is secreted in response to a high-phosphorus intake as well as 1,25D (19, 34). FGF23 acts on the kidney to increase phosphate excretion and decrease the activity of the 25(OH)-vitamin D 1α-(OH)ase (CYP27B1) and hence intestinal calcium and phosphate absorption (37).

1,25D decreases PTH expression and increases FGF23 secretion. There are therefore well-defined endocrinologic feedback loops involving calcium, phosphate, PTH, 1,25D, and FGF23 (24, 40). FGF23 binds to its receptor, the Klotho-FGF receptor (FGFR)1c complex (25, 43). We recently showed that Klotho and FGFR1 are expressed in the parathyroid and that FGF23 acts to decrease PTH mRNA and serum levels in vivo and in vitro through the MAPK pathway (3). A similar effect was shown in bovine parathyroid primary cell cultures (21). FGF23 also decreases mouse PTH (48) and human PTH in the serum of transgenic mice generated to express the human PTH gene as part of a bacterial artificial chromosome (BAC) (26). Most patients with chronic kidney disease (CKD) develop secondary hyperparathyroidism, which has been related to changes in serum calcium, phosphate, and 1,25D (39, 42). In CKD, serum FGF23 levels are increased together with secondary hyperparathyroidism, indicating resistance of the parathyroid to FGF23 (11). This resistance is associated with a decrease in parathyroid Klotho and FGFR1 levels (8, 20, 23).

The increased serum FGF23 levels in CKD correlate with increased mortality among patients beginning and on maintenance hemodialysis treatment (11, 14). In dialysis patients, plasma FGF23 levels correlate positively with serum phosphate, PTH, calcium, and duration of hemodialysis (12). In patients with primary hyperparathyroidism, there is a decrease in serum FGF23 and in serum calcium after parathyroidectomy (PTX) (18), although not in all studies (47). Patients treated by chronic dialysis with severe secondary hyperparathyroidism who underwent total PTX also showed a decrease in serum FGF23, calcium, phosphate, and PTH (36). On the other hand, patients with hypoparathyroidism have been reported to also have increased serum FGF23 levels attributed to their hyperphosphatemia (10). Kawata et al. (16) showed in an experimental model of primary hyperparathyroidism using transgenic parathyroid-cyclin D1 mice that hyperparathyroidism resulted in increased circulating FGF23 and FGF23 mRNA in calvaria and that PTX normalized FGF23 levels. We now show using a model of secondary hyperparathyroidism that high levels of PTH are necessary for the high-FGF23 levels.

Upon binding to the PTH1R, PTH activates the PKA pathway in exerting both its catabolic and anabolic effects (27). Activation of PKC and intracellular calcium signaling pathways has a more limited role in PTH signaling in bone. PTH-stimulated cAMP production is sufficient for initiation of signaling cascades that increase osteoblast number, but activation of PKC is not (15, 27). Wnt proteins are also important regulators of bone metabolism in both PTH-dependent and -independent responses. Kulkarni et al. (22) showed that PTH added to UMR106 osteoblastic cells changes the expression of several components of the canonical Wnt pathway and stabi-
lized β-catenin in those cells. PTH/PTHrP receptor signaling results in binding of the receptor to Lrp6, phosphorylation of Lrp6, and stabilization of β-catenin in osteoblasts (44). PTH also decreases the expression of Wnt antagonists, Dkk1 and sclerostin (9, 17, 33).

We now show that PTH increases FGF23 expression in vivo and in vitro and that PTX both prevents and corrects the increased serum FGF23 of short-term experimental kidney failure. In rat osteoblastic osteosarcoma UMR106 cells, PTH increases FGF23 mRNA levels as does the PKA activator forskolin. The effect of PTH to increase FGF23 is mediated also by the Wnt pathway. We demonstrate a PTH-FGF23 endocrine feedback axis and propose that secondary hyperparathyroidism is a major factor causing the high-FGF23 levels in CKD.

MATERIALS AND METHODS

Animals. Adult male Sabra rats (150 to 174 g) were fed a control diet or an adenine high-phosphorus diet (0.75% adenine, 1.5% phosphorus; Teklad, Madison, WI) for the indicated time periods of 4 or 7 days to induce renal failure. C57BL/6j male mice 5 to 6 wk of age were used for PTH minipumps experiments. For the different experiments, five to six rats/mice were used in each group. Each experiment was repeated at least twice. All animal experiments were approved by the Hadassah Hebrew University Animal Care and Use Committee.

For PTX, rats were anesthetized by ketamine-xylazine mixture sc brown adipose tissue separated at the median using an electric pencil, and the parathyroids were electrocauterized. The Hadassah Hebrew University Animal Care and Use Committee. Animals.

For continuous sc PTH administration, Alzet osmotic minipumps (model 2002; Alza, Palo Alto, CA) were filled with either rat PTH for an additional 4 days (day 7). Blood was drawn at day 2 after the operation and at the end of the experiment at day 7 (Fig. 1, A–D).

PTX prevents the increased serum FGF23 levels of short-term experimental kidney failure. To study whether PTH affects serum FGF23 levels, we performed PTX or sham surgery before and after feeding the rats an adenine high-Pi diet to induce kidney failure. In the first experiment, rats underwent either PTX or sham surgery and after 3 days were fed the adenine high-Pi diet for a further 4 days (Fig. 1, A–D). In the sham-operated rats, the adenine high-Pi diet at day 7 led to the anticipated increase in serum creatinine (control 0.28 ± 0.05 and 0.65 ± 0.07 mg/dl; n = 4, P < 0.05). PTH and FGF23 levels were increased with no change in serum calcium and phosphate levels (Fig. 1, A–D). There was no effect of the adenine high-Pi diet on 1,25D levels at day 7 (PTX 155 ± 40 pg/ml, sham 205 ± 45 pg/ml; n = 4).

PTX rats had the expected decrease in serum PTH and calcium and an increase in serum phosphate compared with the sham-operated rats (Fig. 1, A–C). Importantly, PTX led to an increase in serum creatinine already at day 7 (0.65 ± 0.07 mg/dl, control 0.28 ± 0.05 mg/dl; n = 4, P < 0.05). Surprisingly, PTX prevented the increase in serum creatinine.
in serum FGF23 induced by the adenine diet observed in the sham rats (Fig. 1D). Therefore, PTX decreases FGF23 levels and prevents the expected increase in FGF23 in early kidney failure.

PTX corrects the increased serum FGF23 levels of short-term experimental kidney failure. To study whether PTX not only prevents but also corrects the increased FGF23 levels induced by the adenine diet, we performed a complementary experiment.
FTX decreases serum FGF23 levels independent of serum calcium. To separate the effects of FTX by PTX from the decrease in serum calcium (Fig. 1), we PTXed rats with normal renal function and corrected serum calcium (Fig. 2A). PTX led to a decrease in serum FGF23 (Fig. 2B) as before (Fig. 1D) but serum FGF23 levels were still decreased despite normalized serum calcium (Fig. 2A). Therefore, the effect of PTX on FGF23 occurs also when serum calcium is normal.

Continuous administration of PTH increases serum FGF23 levels. The effect of PTH administration was studied by continuous subcutaneous minipump infusion in mice with normal renal function, as previously described (1). PTH led to the expected increase in serum calcium and decrease in serum phosphate compared with control mice (Fig. 3, A and B). Serum FGF23 levels were increased by PTH administration as measured both by an intact FGF23 assay (Fig. 3C) and a COOH-terminal FGF23 assay (control 73 ± 7.8 and PTH 202 ± 57 pg/ml; n = 5, P < 0.05). In addition to the increase in serum FGF23 levels, there was also an increase in FGF23 mRNA levels in the mice calvaria (Fig. 3D). Therefore, PTH infusion leads to an increase in FGF23 expression.

Table 1. Primers used for real-time PCR

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat FGF23</td>
<td>5'-TTGGATCCTTGATCACTTGAGAG-3'</td>
<td>5'-TGGCTGGTTCAGAGGATG-3'</td>
</tr>
<tr>
<td>Mouse FGF23</td>
<td>5'-GCCCTCTCAGGCATGAGATCA-3'</td>
<td>5'-GCTTGGATCCTTGATCACTTGAGAG-3'</td>
</tr>
<tr>
<td>SOST</td>
<td>5'-GCCCTCTCAGGCATGAGATCA-3'</td>
<td>5'-GCTTGGATCCTTGATCACTTGAGAG-3'</td>
</tr>
<tr>
<td>HPRT1</td>
<td>5'-TTGGATCCTTGATCACTTGAGAG-3'</td>
<td>5'-GCTTGGATCCTTGATCACTTGAGAG-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-GCCCTCTCAGGCATGAGATCA-3'</td>
<td>5'-GCTTGGATCCTTGATCACTTGAGAG-3'</td>
</tr>
<tr>
<td>RPL13a</td>
<td>5'-GCCCTCTCAGGCATGAGATCA-3'</td>
<td>5'-GCTTGGATCCTTGATCACTTGAGAG-3'</td>
</tr>
<tr>
<td>Ubiquitin C</td>
<td>5'-TTGGATCCTTGATCACTTGAGAG-3'</td>
<td>5'-GCTTGGATCCTTGATCACTTGAGAG-3'</td>
</tr>
<tr>
<td>Actin B</td>
<td>5'-TTGGATCCTTGATCACTTGAGAG-3'</td>
<td>5'-GCTTGGATCCTTGATCACTTGAGAG-3'</td>
</tr>
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FGF23, fibroblast growth factor 23.

PTX increases FGF23 gene expression and decreases SOST gene expression in vitro through the PKA and Wnt pathways. To study the direct effect of PTH on FGF23 expression, we incubated UMR106 cells with rat PTH 1–34. PTH (1 × 10^{-7} M) led to increased FGF23 mRNA levels at 4, 24, and 48 h (Fig. 4A). This effect was also evident at 1 × 10^{-6} M but not 1 × 10^{-8} M (Fig. 4B). PTH acts by activating the PKA and Wnt pathways with a minor role for PKC (15, 44). SOST gene codes for the Wnt inhibitor sclerostin. PTH decreased SOST mRNA levels in UMR106 cells at 4, 24, and 48 h at all doses studied (Fig. 4, C and D) as previously described (2).

The addition of human sclerostin (5 μg/ml) protein to UMR106 cells had no effect on FGF23 mRNA levels (Fig. 5A). However, when sclerostin was added together with PTH, it prevented the increase in FGF23 mRNA levels caused by PTH (Fig. 5A). Similar results were obtained using mouse sclerostin.
Forskolin, the PKA activator, led to an increase in FGF23 mRNA levels (Fig. 5B) together with a decrease in SOST mRNA (Fig. 5C). Therefore, PTH directly regulates FGF23 and sclerostin expression in vitro, in the absence of the secondary changes induced by PTH in vivo.

**DISCUSSION**

Both phosphate and 1,25D act on bone to increase serum FGF23 (10, 13). FGF23, in turn, acts on the kidney to decrease serum phosphate and 1,25D, thereby completing an endocrine feedback loop (30). We recently showed that FGF23 also acts on the parathyroid to activate the MAPK pathway and decrease PTH expression (3). However, in CKD, both PTH and FGF23 are increased due to resistance of the parathyroid to the high-FGF23 levels. This resistance of the parathyroid in CKD is due to downregulation of the FGF23 receptor complex Klotho-FGFR1c in the parathyroids in both experimental CKD and in patients (8, 20, 23). We now show that PTH acts on

![Fig. 3. Continuous PTH administration increases serum FGF23 and FGF23 mRNA levels. Mice were given PTH or vehicle by subcutaneous osmotic minipumps for 3 days when serum calcium (A), phosphate (B), FGF23 (C), and calvaria FGF23 mRNA levels were measured (D). *P < 0.05 (n = 6), PTH treated compared with control. Similar results were obtained in a repeat experiment.](http://ajprenal.physiology.org/)

![Fig. 4. PTH increases FGF23 mRNA and decreases SOST mRNA levels in UMR106 cells. qRT-PCR for FGF23 and SOST mRNA levels after rat PTH 1–34 (10⁻⁸–10⁻⁶ M) for the indicated times. A: time response for FGF23 mRNA levels after PTH (10⁻⁷ M). B: dose response for FGF23 mRNA levels at 24 h. C: time response for SOST mRNA after PTH (10⁻⁷ M). D: dose response for SOST mRNA levels at 24 h. Results are expressed as fold change relative to mRNA in control vehicle treated cells; means ± SE, n = 6. *P < 0.05.](http://ajprenal.physiology.org/)
bone to increase FGF23 expression as part of an additional endocrine feedback loop. We suggest that the secondary hyperparathyroidism of CKD is essential for the high levels of FGF23 in CKD.

We first studied serum FGF23 levels after removing PTH from the system by PTX both before or after the induction of short-term adenine-induced kidney failure. PTX not only prevented the high-FGF23 levels of early kidney failure but also completely corrected the high-serum FGF23 levels in rats with established short-term adenine-induced kidney failure. It is noteworthy that in both sets of experiments of early kidney failure up to 7 days, there was no change in serum 1,25D levels, calcium, and phosphate, which only decrease at later time intervals such as 21 days (28). In the kidney failure rats at 4 days, serum PTH and FGF23 levels were already increased with no change in serum phosphate which may reflect the phosphaturic effect of these two hormones in early kidney failure when there is still responsive renal tissue. It is likely that phosphate retention is the stimulus to the increased secretion of both PTH and FGF23. The increased PTH secretion in early CKD is the tradeoff of hyperparathyroidism to maintain increased phosphaturia (41). The increase of serum FGF23 levels in early CKD would also have a similar phosphaturic effect. In addition, we suggest a direct relationship between PTH and FGF23 in rats with intact parathyroid glands independent of serum calcium, phosphate, or 1,25D. Our results showing that PTX both prevents and corrects the high-FGF23 levels in early kidney failure demonstrate the centrality of an intact parathyroid to the pathogenesis of the high FGF23 of CKD. However, PTX led to changes in serum calcium and phosphate. Correction of serum calcium in PTXed rats with normal renal function did not prevent the decrease in serum FGF23 (Fig. 2). Therefore, in rodents with normal kidney function or early kidney failure, PTH regulates FGF23 expression with and without changes in serum calcium.

Our results in secondary hyperparathyroidism are in agreement with those of Kawata et al. (16) using a mouse model of primary hyperparathyroidism namely PTH-cyclin D1 transgenic mice. In these mice, PTX led to a decrease in PTH, calcium, and FGF23, an increase in phosphate but no change in 1,25D levels. Therefore, PTH and/or calcium correlated with changes in serum FGF23 levels in this model but not 1,25D levels (16). In experimental CKD using 5/6 nephrectomized rats, PTX led to a decrease in the high-serum FGF23 levels and FGF23 expression in bone (35). However, it was difficult to define which factors were responsible for the decrease in FGF23 after PTX in these studies because of changes in serum calcium and 1,25D. Nagano et al. (32) studied adenine-fed rats that developed severe CKD, with high-serum phosphate, PTH, and FGF23 levels, and reduced levels of serum 1,25D. Serum phosphate, PTH, and FGF23 levels were decreased by the oral Pi binder, sevelamer. The changes in serum FGF23 levels began after the onset of changes in serum phosphorus and PTH levels, suggesting that the reduction in serum FGF23 was related to both phosphate and PTH (32).

Fig. 5. Increase in FGF23 mRNA levels by PTH is mediated by PKA and Wnt pathways in UMR106 cells. A: qRT-PCR for FGF23 mRNA levels in UMR106 cells at 24 h after addition of rat PTH 1–34 (10^{-7} M), human sclerostin (5 μg/ml), or PTH together with sclerostin. *P < 0.01 compared with control. #Compared with PTH, n = 4. B: qRT-PCR for FGF23 mRNA levels after rat PTH 1–34 (10^{-7} M) or forskolin (10^{-6} M). C: qRT PCR for SOST mRNA levels after rat PTH 1–34 (10^{-7} M) or forskolin (10^{-6} M). *P < 0.0001 compared with control, n = 14.

Fig. 6. PTH-FGF23 feedback loop. Serum FGF23 is regulated by phosphate, calcium, 1,25D, and PTH. PTH acts on its receptor, the PTH1R, on bone cells to increase FGF23 expression. This effect is mimicked by forskolin, which activates PKA. PTH decreases sclerostin, thus disinhibiting its effect on the Wnt pathway. PKA may also have a direct effect on FGF23. In turn, FGF23 acts on the parathyroid to decrease PTH expression.
There is also a clinical correlate that illustrates the complexity of the relationship between PTH action, calcium, and FGF23. In a patient with an activating mutation of the PTH/PTHrP receptor, namely Jansen’s metaphyseal chondrodysplasia, serum FGF23 concentrations were found to be markedly and persistently elevated despite hypophosphatemia and normal 1,25D levels (5). This observation suggested that serum FGF23 could be governed by activation of the PTH/PTHrP receptor in bone. However, the patient also had a high-serum calcium which itself increases serum FGF23 levels (38). Sato et al. (36) found that in dialysis patients with advanced secondary hyperparathyroidism, subtotal PTX led to the expected decrease in PTH, calcium, and phosphate, which were not correlated with changes in serum 1,25D levels. In contrast, in a large cross-sectional study of CKD patients, Levin et al. (29) showed that the first change in the development of secondary hyperparathyroidism was a decrease in serum 1,25D. The relationships among PTH, calcium, phosphate, 1,25D, and FGF23 are complex involving multiple feedback loops. Finch et al. (7) recently showed in 5/6 nephrectomized rats at 6 wk that the 1,25D analog, paricalcitol, increased serum FGF23 levels while the calcimimetic, cinacalcet, decreased serum PTH at 4 h and had a tendency to decrease serum FGF23 levels at 24 h. Wetmore et al. (45) showed in dialysis patients that cinacalcet decreased not only serum PTH but also serum FGF23 levels. They hypothesized that the decrease in serum FGF23 levels may have been due to changes in serum 1,25D, which were not measured (45). Our present results suggest that the decrease in FGF23 in that clinical study may be due to the decreased serum PTH.

In mice with normal renal function, PTH administration via osmotic minipumps led to the expected increase in serum calcium and decrease in serum phosphate. PTH administration increased calvarial FGF23 mRNA and both intact and COOH-terminal serum FGF23 levels. PTH administration via osmotic minipumps has been shown to decrease calvaria PHEX expression (1), which may be associated with an increase in serum FGF23 levels (4, 49). PTH infusion has been shown to also increase serum 1,25D levels (1), which increases serum FGF23 levels (6). Therefore, in vivo studies do not differentiate among the different factors causing increased FGF23 levels.

To demonstrate a direct effect of PTH on FGF23 mRNA levels, we used the osteoblast-like UMR106 cell line. PTH increased FGF23 mRNA levels in a time- and dose-dependent manner. Thus, PTH acts directly on bone to increase FGF23 expression. PTH1R activates the PKA/cAMP, PKC, and Ca signal transduction pathways, but only PKA/cAMP-mediated signaling has been clearly linked up to now to anabolic effects in bone (15, 27). We show that activation of PKA by forskolin mimics the effect of PTH to increase FGF23 expression. Our studies combine with previous work to suggest that PTH signaling not only activates PKA but also Wnt signaling to influence osteoblast function. PTH decreases the expression of Wnt antagonists, Dkk1 and sclerostin (9, 17). Keller and Kneissel (17) also showed that forskolin inhibits SOST comparable to PTH, while the calcium ionophore ionomycin was ineffective. PTH has been shown to increase the PKA-mediated phosphorylation of Lrp6 at GSK3 and CK1 sites to activate Wnt signaling (44). We now show that PTH and forskolin both decrease SOST gene expression and that added sclerostin interferes with the effect of PTH to increase FGF23 mRNA levels in UMR106 cells. This is the first demonstration that PTH increases FGF23 expression directly and that this effect involves both PKA and Wnt signaling.

In conclusion, the present studies show that PTH increases serum FGF23 and FGF23 mRNA levels. We show that PTH acts directly on bone to increase FGF23 expression which is accompanied by a decrease in SOST expression. In vivo, PTX both prevented and corrected the increased FGF23 of experimental short-term kidney failure demonstrating the importance of PTH in the regulation of FGF23 levels. The bone-parathyroid axis reported previously, namely the effect of FGF23 to suppress PTH expression, represents one arm of an endocrinologic feedback loop (3, 21). The second arm of this loop is the effect of PTH to increase serum FGF23 levels (Fig. 6). In patients with CKD and in rats with experimental CKD, both FGF23 and PTH are increased due to resistance of the parathyroid to FGF23. The finding that PTX completely prevents and corrects the high-FGF23 levels of experimental early kidney failure suggests that the high-FGF23 levels in CKD are due to hyperparathyroidism. It is therefore necessary to control serum PTH levels in CKD patients to attain normal-serum FGF23 levels.

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DISCLOSURES

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