The aging suppressor klotho: a potential regulator of growth hormone secretion

Shahmoon S, Rubinfeld H, Wolf I, Cohen ZR, Hadani M, Shimon I, Rubinek T. The aging suppressor klotho: a potential regulator of growth hormone secretion. Am J Physiol Endocrinol Metab 307: E326–E334, 2014. First published June 17, 2014; doi:10.1152/ajpendo.00090.2014.—Klotho is a transmembranal protein expressed predominantly in the distal tubules of the kidney, choroid plexus, and anterior pituitary. Klotho can also be cleaved and shed as a circulating hormone. Klotho-deficient mice (kl/kl) mice develop a phenotype resembling early aging. Several lines of evidence suggest a role for klotho in the regulation of growth hormone (GH) secretion. The kl/kl mice are smaller compared with their wild-type counterparts, and their somatotropes show reduced numbers of secretory granules. Moreover, klotho is a potent inhibitor of the IGF-I pathway, a negative regulator of GH secretion. Therefore, we hypothesized that klotho may enhance GH secretion. The effect of klotho on GH secretion was examined in GH3 rat somatotrophs, cultured rat pituitaries, and cultured human GH-secreting adenomas. In all three models, klotho treatment increased GH secretion. Prolonged treatment of mice with intraperitoneal klotho injections increased mRNA levels of IGF-I and IGF-I-binding protein-3 mRNA in the liver, reflecting increased serum GH levels. In accord with its ability to inhibit the IGF-I pathway, klotho partially restored the inhibitory effect of IGF-I on GH secretion. Klotho is known to be a positive regulator of basic bFGF signaling. We studied rat pituitaries and human adenoma cultures and noted that bFGF increased GH secretion and stimulated ERK1/2 phosphorylation. Both effects were augmented following treatment with klotho. Taken together, our data indicate for the first time that klotho is a positive regulator of GH secretion and suggest the IGF-I and bFGF pathways as potential mediators of this effect.

Aging suppressor klotho: a potential regulator of growth hormone secretion

Klotho-deficient mice (kl/kl mice) mature normally until 3 wk of age, at which time they develop a severe aging phenotype that includes osteoporosis, hypoglycemia, hypogonadism, and growth retardation (18). Klotho is a type 1 transmembrane protein expressed predominantly in the distal tubules of the kidney, choroid plexus, and pituitary glands. The extracellular region of klotho contains two weakly homologous domains, KL1 and KL2, which can be shed from the cell surface (10). Ample data indicate klotho as an active circulating hormone. Klotho can be detected in body fluids, including the blood and cerebrospinal fluid (CSF), expression of klotho in a single organ can rescue the kl/kl phenotype (18), intraperitoneal (ip)

administration of klotho rescues some features of kl/kl phenotype (12), and its injection inhibits tumor formation in athymic mice (2). Several activities have been attributed to klotho, including regulation of phosphate homeostasis (19), regulation of calcium shunting (6, 9), and inhibition of WNT signaling. Importantly, klotho is also a modulator of two major signaling pathways, the IGF-I and the fibroblast growth factor (FGF) pathways. Klotho is a potent inhibitor of IGF-I activity in hepatocytes and myocytes (21), and we have also noted the inhibition of the IGF-I pathway and direct interaction between klotho and the IGF-I receptor (IGF-IR) in cancer cells (2, 30, 37, 38). The activity of klotho toward the FGF pathway is ligand and cell type dependent. Thus, klotho is an essential cofactor for the activation of the FGF receptors (FGFRs) by FGF23 but can either enhance or inhibit activation of these receptors by bFGF (2, 20, 35, 38).

Growth hormone (GH) secretion from the pituitary is a tightly regulated process. Positive regulators include GH-releasing hormone and ghrelin, whereas negative regulators include somatostatin and IGF-I (8). Recent data also suggest a role for bFGF in the process (22). Indirect evidence suggests a role for klotho in the regulation of GH secretion. Studies of kl/kl mice revealed smaller GH-secreting cells (somatotropes) and a reduced number of secretory granules compared with control mice (18). Moreover, klotho inhibits activation of a major negative regulator of GH secretion, namely the IGF-I pathway.

To our knowledge, the role of klotho in regulating GH secretion from somatotropes has not yet been elucidated. Therefore, we aimed to study the role of klotho in regulating GH secretion. Our data indicate klotho, for the first time, as a positive regulator of GH secretion from normal somatotropes and pituitary adenomas and suggest the IGF-I and bFGF pathways as possible mediators involved in this process.

MATERIALS AND METHODS

Antibodies and reagents. The reagents used were soluble human klotho (R & D Systems, Minneapolis, MN); soluble mouse klotho, which was produced as described previously (2, 21); basic FGF (bFGF; Biological Industries, Kibbutz Be’er Haemek, Israel); IGF-I and FGF23 (PeproTech, Rocky Hill, NJ); and U-10126 (Calbiochem, Gibbstown, NJ). Antibodies used were anti-diphosphorylated and total ERK1/2 (Sigma, St. Louis, MO) and anti-HA (Covance, Princeton, NJ). Mouse klotho with an HA tag at the COOH terminus expression vector was a generous gift from Y. Nabeshima (Kyoto University).

Human samples. Pituitary adenoma specimens for primary cultures and for mRNA expression studies were collected following informed consent during transsphenoidal procedures. The study was approved.
Table 1. Clinical characteristics of patients with pituitary adenomas

<table>
<thead>
<tr>
<th>Tissue No.</th>
<th>Size, mm</th>
<th>Sex/Age, yr</th>
<th>Immunostaining</th>
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<tbody>
<tr>
<td>A1</td>
<td>11</td>
<td>F/31</td>
<td>GH, FSH, LH, and TSH</td>
</tr>
<tr>
<td>A2</td>
<td>8</td>
<td>M/35</td>
<td>GH, PRL, and FSH</td>
</tr>
<tr>
<td>A3</td>
<td>10</td>
<td>F/61</td>
<td>GH and TSH</td>
</tr>
<tr>
<td>A4</td>
<td>19</td>
<td>M/49</td>
<td>GH, PRL, scattered FSH, and TSH</td>
</tr>
<tr>
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<td>NA</td>
<td>F/43</td>
<td>GH</td>
</tr>
<tr>
<td>A6</td>
<td>12</td>
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</tr>
<tr>
<td>A7</td>
<td>9</td>
<td>M/39</td>
<td>GH</td>
</tr>
</tbody>
</table>

M, male; F, female; NA, not available; GH, growth hormone; PRL, prolactin.

Table 2. Primer sequences

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
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<td>CGCGGAGCAGTACCCGCTGA CTGATGTTTCCTGGAGCAGGTTGGG</td>
<td>IGFBP-3</td>
</tr>
<tr>
<td>IGF-1</td>
<td>TGCGCGCTCTGGCTGCTGAACC</td>
<td>CGCGGAGCAGTACCCGCTGACCATCGGGAAGCAAGGACAGGTTGGG</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>CGCGGAGCAGTACCCGCTGACCATCGGGAAGCAAGGACAGGTTGGG</td>
<td>CGCGGAGCAGTACCCGCTGACCATCGGGAAGCAAGGACAGGTTGGG</td>
</tr>
<tr>
<td>β-Actin</td>
<td>CGCGGAGCAGTACCCGCTGACCATCGGGAAGCAAGGACAGGTTGGG</td>
<td>CGCGGAGCAGTACCCGCTGACCATCGGGAAGCAAGGACAGGTTGGG</td>
</tr>
<tr>
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<td>CGCGGAGCAGTACCCGCTGACCATCGGGAAGCAAGGACAGGTTGGG</td>
</tr>
<tr>
<td>Mouse</td>
<td>GGGCTGGGAGAGGGAATGATGCTTT</td>
<td>CGCGGAGCAGTACCCGCTGACCATCGGGAAGCAAGGACAGGTTGGG</td>
</tr>
</tbody>
</table>

IGFBP-3, IGF-binding protein-3.

Mice studies. Athymic mice were used to evaluate the effects of klotho on hepatic IGF-I and IGF-I-binding protein-3 (IGFBP-3). For the experiment, 6-wk-old female athymic mice were treated for 3 wk with daily ip injections of mouse klotho [produced as described earlier (2, 21)]: 10 μg/kg, 25 μg/kg, or saline control (n = 5/group). At the end of the experiment, mice were euthanized, livers were homogenized, and RNA was extracted from the tissue for IGF-1 and IGFBP-3 mRNA analyses.

Western blot. Cells were harvested and lysed for total protein extraction in radio immunoprecipitation assay buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.25% Na-deoxycholate, 1 mM EDTA, and 1 mM NaF) together with a protease inhibitor cocktail (Sigma). A total of 50 μg of protein extracts were loaded on 10% polyacrylamide gels, separated electrophoretically, and blotted onto nitrocellulose membranes (Pall, Port Washington, NY). The membranes were then immunoblotted with the indicated antibodies. Band intensities were quantified using ImageJ software.

Statistical analysis. Results are presented as means ± SD. Categorized variables were compared between the study groups using Fisher’s exact test, and continuous variables were compared using a t-test. All significance tests were two-tailed, and P < 0.05 was considered to be statistically significant.

RESULTS

Klotho induces GH secretion from pituitary cells. We first aimed to study the effects of klotho on the secretion of GH. This was examined in three different experimental systems: rat GH-secreting GH3 cell line, cultures of rat normal pituitary, and cultures of human pituitary adenomas. GH3 cells are used extensively for the study of GH secretion. Klotho is highly expressed in the pituitary (18); thus, to mimic the effects of endogenous klotho, we chose to overexpress klotho in these cells. The cells were transfected with either a control vector or klotho expression vector, and GH secretion to the media was determined. Overexpression of klotho increased GH secretion by 47% (P < 0.05; Fig. 1A). We next examined the effects of klotho on rat pituitary cultures. For each experiment, pituitaries of six 8-mo-old male rats were harvested, and cells were...
maximal effect of 50% induction was noted at 0.1 ng/ml ($P < 0.005$; Fig. 1C). Five of seven tumor-derived cultures were responsive to klotho, and a significant increase in GH secretion was observed following klotho treatment with a mean elevation of 28%, ranging from 15 to 54% ($P < 0.05$; Fig. 1D). No correlation was found between the clinical characteristics and the response to klotho.

In vivo administration of klotho increases mRNA levels of hepatic IGF-I. In mice, GH secretion is pulsatile, and its levels fluctuate rapidly and depend on multiple factors, including stress at the time of blood collection. Thus, direct analysis of GH levels following klotho administration may not accurately reflect GH secretion and activity (32). Hepatocytes are the primary site for GH action and respond to GH stimuli by synthesis of IGF-I and IGFBP-3 (25, 36, 39). Thus, their levels serve as an accurate, albeit indirect, measurement for GH levels. We determined IGF-I and IGFBP-3 mRNA levels in livers of mice treated daily for four wk with ip injections of klotho (10 or 25 µg/kg klotho) or a vehicle control ($n = 5$/group). mRNA levels of IGF-I and IGFBP-3 were elevated in the klotho-treated groups by ≤30 and 47%, respectively, compared with control group ($P < 0.05$ for both comparisons; Fig. 2). A slight decrease in IGF-I expression is observed at 25 µg/kg compared with 10 µg/kg; however, this decrease was not significant ($P = 0.11$).

Klotho abrogates IGF-I inhibitory effects on GH secretion. IGF-I is a physiological inhibitor of GH secretion, and klotho is a potent inhibitor of IGF-I signaling (2, 21, 38). We aimed to examine the ability of klotho to counteract the inhibitory effect of IGF-I on GH secretion in GH-secreting adenomas. These adenomas express IGF-IR, albeit at lower levels compared with normal somatotrophs (17). We studied GH secretion in response to IGF-I alone and in combination with klotho in three cultured GH-secreting adenomas. IGF-I treatment moderately decreased GH secretion (12–31% decrease, $P < 0.05$; Fig. 3). Cotreatment of IGF-I and klotho partially released the IGF-I inhibitory effect ($P < 0.05$; Fig. 3).

bFGF elevates GH secretion, and klotho further increases its secretion. Klotho is an important regulator of the FGF pathway. $kll/kll$ and FGF23-knockout mice share a similar phenotype, and klotho is a mandatory cofactor for the interaction pooled. To represent physiological conditions, the activity of klotho was tested at concentrations that are within the normal range of serum klotho levels (0.05–0.5 ng/ml) (40). GH levels in the media were determined after 24 h of treatment. A dose-dependent increase in GH secretion was observed, with the highest effect of 70% increase achieved at 0.5 ng/ml ($P < 0.05$ for 0.5 ng/ml compared with vehicle-treated controls; Fig. 1B). We next tested the effect of klotho on GH secretion from human pituitary GH-secreting adenomas. For these studies, fresh tissue samples were obtained during surgery from seven patients with acromegaly (numbered A1–A7). Each tumor sample was cultured separately and treated for 24 h with klotho or a vehicle control. Klotho’s dose dependence effect on GH secretion was studied in adenoma A2, and the results show that
of FGF23 with FGFR1, -3, or -4 (15, 20, 35). Klotho also regulates the activation of FGFRs in breast and pancreatic cancer cells (2, 38). FGFRs expression in pituitary adenomas is deregulated compared with the normal pituitary (1), and we verified FGFR expression in GH3 cells and noted the expression of FGFR1, -2, and -3 (not shown). The role of bFGF in GH secretion has not yet been fully established (3, 23). Therefore, we first tested the ability of bFGF to regulate GH secretion in normal rat pituitary cells. Treatment with bFGF increased GH secretion by 26% at 24 h (P < 0.05; Fig. 4A). Cotreatment with klotho further increased GH secretion by 83% (P < 0.05; Fig. 4A). Next, we studied the effect of bFGF and klotho treatment on GH secretion in five cultured human GH-secreting adenomas. bFGF induced a significant increase in GH secretion in three out of five adenomas (adenomas A4, A5, and A6; Fig. 4B). Cotreatment with klotho further increased GH secretion by ≤80% in three adenomas (P < 0.05 compared with single treatment, adenomas A1, A4, and A6; Fig. 4B). Because of cell number limitations, we were unable to study the effect of cotreatment in adenoma A7. Importantly, even in adenoma A1 culture that did not respond to klotho or bFGF, cotreatment elicited a 42% increase in GH secretion (P < 0.0001), manifesting the prominent role of the interaction of bFGF and klotho (Fig. 4B).

**ERK1/2 mediate klotho-induced GH secretion.** ERK1/2 are major downstream effectors of the bFGF pathway. We analyzed the effects of klotho and bFGF on the activation of ERK1/2 in GH3 cells. Klotho increased ERK1/2 phosphorylation in a time-dependent manner, peaking at 15 min. (Fig. 5A). As expected, bFGF also increased ERK phosphorylation (Fig. 5B). The interaction of bFGF and klotho on ERK1/2 phosphorylation was evaluated; treatment with bFGF increased ERK1/2 phosphorylation fivefold, whereas cotreatment with klotho (using klotho transfection) further increased this effect 8.5-fold (Fig. 5C). In contrast to soluble klotho treatment, klotho transfection did not result in a robust increase in ERK phosphorylation. This is due probably to the extended period of time that cells are exposed to klotho upon transfection (48 h) compared with the short duration of soluble klotho treatment (≤1 h). To study the role of the ERK pathway in mediating klotho and bFGF effects on GH secretion, we treated GH3 cells with U-0126, a specific inhibitor of MEK1, the upstream kinase of ERK1/2. U-0126 inhibited ERK1/2 phosphorylation following treatment with bFGF or klotho (P < 0.005; Fig. 5D). Notably, U-0126 significantly inhibited bFGF- and klotho-induced GH secretion from cultured GH adenoma (A7) cells (P < 0.05; Fig. 5E). Treatment of normal rat pituitary cells with U-0126 alone had no effect on GH secretion, but U-0126 abolished bFGF-mediated GH secretion (Fig. 5F).

Endogenous klotho can operate as a cofactor for FGF23 activity in the pituitary (20, 35). We aimed to examine the ability of soluble klotho to modulate bFGF and FGF23 activity. For these studies, GH3 cells were treated with klotho, bFGF, and FGF23 alone and in combination, and phosphorylation of ERK1/2 was evaluated; treatment with bFGF increased ERK1/2 phosphorylation fivefold, whereas cotreatment with klotho and bFGF effects on GH secretion, we treated GH3 cells with U-0126, a specific inhibitor of MEK1, the upstream kinase of ERK1/2. U-0126 inhibited ERK1/2 phosphorylation following treatment with bFGF or klotho (P < 0.005; Fig. 5D). Notably, U-0126 significantly inhibited bFGF- and klotho-induced GH secretion from cultured GH adenoma (A7) cells (P < 0.05; Fig. 5E). Treatment of normal rat pituitary cells with U-0126 alone had no effect on GH secretion, but U-0126 abolished bFGF-mediated GH secretion (Fig. 5F).

**Klotho expression is reduced in acromegaly.** Although klotho may act as a circulating hormone, it is also expressed in various endocrine tissues, including the pituitary (18), and may also have paracrine activities. We aimed to examine klotho expression in normal pituitary and pituitary adenomas. Klotho mRNA levels were measured in GH-secreting adenomas (n = 19), nonfunctioning pituitary adenomas (n = 21), and normal pituitary (n = 3). Low klotho expression (defined as <80% of the value of normal samples) was noted in only one of 21 (4.7%) of the nonfunctioning adenomas (Fig. 6A) but in 12 of 19 (63%) of the GH-secreting adenomas (P < 0.0001; Fig. 6B).

**DISCUSSION**

Growth retardation is a prominent phenotype in klkl mice, and studies of their pituitary gland denoted atrophic somatotropes with reduced number of secretory granules (18). Our data indicate for the first time that klotho is a potential new...
player in the complex regulatory machinery governing GH secretion. We were able to demonstrate using three independent systems, the GH3 somatotrope cell line, cultures of normal rat pituitary, and human GH-secreting adenomas, that klotho can induce a modest yet significant increase in GH secretion. Our data also suggest an interaction between klotho and the IGF-I- and FGF-signaling pathways in the pituitary.

Direct GH measurement does not reflect GH/IGF-I activity in humans and mice (4). Serum levels of IGF-I, the direct GH downstream effector, are affected by binding to various binding proteins and are also considered to be unreliable indicators of GH activity (27). Therefore, hepatic mRNA levels of IGF-I and IGFBP-3 can be used to assess GH activity (13, 28). We noted increased hepatic IGF-I and IGFBP-3 mRNA levels following treatment with klotho. This observation provides indirect support for the role of klotho in regulating GH secretion and is supported by a recent publication by Chen et al. (12), who noted an increase in hepatic ALS expression in kl/kl mice treated with klotho injections. Mice were treated with klotho for 4 wk, and the last injection was administered on the same day of blood and tissue collection. Thus, the results may reflect either short-term or long-term effects of klotho on GH secretion.

The mechanism underlying klotho activity has not yet been elucidated. Evidence for a klotho receptor has not been provided. Yet, klotho has been shown to modulate several receptors, among them being IGF-IR (2, 21, 38) and FGFRs (2, 20, 35, 38). IGF-I is produced in the liver in response to GH. Whereas IGF-I is the main peripheral mediator of GH activity, in the pituitary it inhibits GH secretion. GH-secreting adenomas, although they express lower IGF-IR levels (17), retain some responsiveness to IGF-I. We noted that klotho can partially override the IGF-I inhibitory effect in IGF-I-responsive adenoma cultures. These data suggest that some of klotho effects on pituitary GH secretion may be mediated by inhibition of IGF-I actions.

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**A**

Rat normal pituitary

**B**

Adenoma A1

Adenoma A4

Adenoma A5

Adenoma A6

Adenoma A7

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Fig. 4. Basic fibroblast growth factor (bFGF) elevates GH secretion, and klotho further increases its secretion. A: rat normal pituitaries were harvested, and cells were cultured in 48-well plates at 5 × 10^4 cells/well. Forty eight hours later, cultures were treated with klotho (0.1 ng/ml), bFGF (50 ng/ml), their combination, or C in SFD. Aliquots were removed after 24 h for GH measurements. B: 5 human GH adenoma tissues were harvested, and cells were treated as in A. *P < 0.05; **P < 0.005; ***P < 0.0001.
Fig. 5. Klotho augments bFGF-induced ERK1/2 phosphorylation. A: GH3 cells were treated with soluble klotho (0.1 ng/ml) or a control vehicle at different times, as indicated. Cells were then lysed and immunoblotted with phosphorylated (p) and total (t)ERK1/2. B: cells were processed as in A and serum starved for 48 h prior to treatment with bFGF (50 ng/ml) or a vehicle. C: GH3 cells were transfected with klotho-HA (KL) or with empty vector (pcDNA3); 24 h later, cells were serum-starved for 48 h, stimulated with bFGF (10 ng/ml) for 15 min, lysed, and immunoblotted with indicated antibodies. Experiments were repeated at least 3 times. D: GH3 cells were pretreated for 5 min with U-0126 (10 μM) either alone or in combination with klotho (0.1 ng/ml) or bFGF (10 ng/ml) both for an additional 15 min. Cells were then lysed and immunoblotted with indicated antibodies. Longer (long) and shorter (short) exposure times are presented. E: human acromegaly culture was prepared as described in Fig. 4B, and after 48 h, cells were treated with klotho (0.1 ng/ml), bFGF (50 ng/ml), or in combination with U-0126 in SFD medium. Controls were treated with vehicle solution. Aliquots were removed after 24 h for GH measurements. F: rat pituitary culture was prepared as described in Fig. 4A. After 48 h, cells were treated with bFGF (50 ng/ml), U-0126, or their combination in SFD medium. Controls were treated with vehicle solution. Aliquots were removed after 24 h for GH measurements. G: GH3 cells were treated as indicated or with a control vehicle. Cells were then lysed and immunoblotted with phosphorylated and total ERK1/2. *P < 0.05; **P < 0.005.
expression level (determined as 1) of normal pituitaries (N). Fig. 6. Klotho expression in pituitary adenomas. Klotho mRNA levels were determined by quantitative RT-PCR in GH-secreting (A) or nonfunctioning pituitary adenoma specimens (B) and are shown relative to the average expression level (determined as 1) of normal pituitaries (N).

To our knowledge, the effects of bFGF on GH secretion from normal pituitary have not yet been studied. We found that FGFR1, -2, and -3 are expressed in GH3 cells and observed that bFGF can induce a modest increase in GH secretion from GH-secreting adenomas and normal rat pituitary (Fig. 4). These data indicate bFGF as a regulator of GH secretion. In support of these findings, Atkin et al. (3) showed that bFGF promotes GH secretion in a unique subset of adenomas cosecreting GH and prolactin, and Liu et al. (22) noted that bFGF induces GH mRNA synthesis in GH4 cells. On the other hand, bFGF did not affect GH expression or secretion in GH3 cells (5, 23). This discrepancy, as well as the mixed response detected by us, may reflect the heterogeneity of GH-secreting adenomas. This heterogeneity was demonstrated recently by RNA and micro-RNA expression arrays that revealed a high degree of variation between GH-secreting adenomas (14, 24, 31).

The results presented here suggest that klotho mediates GH release through activation of the ERK1/2 pathway. Activation of the ERK1/2 pathway by klotho was also noted in oligodendrocyte cells (11), breast cancer (38), and pancreatic cancer cells (2). It has been shown recently that somatostatin or the AMP-activating kinase inhibitor AICAR can inhibit GH secretion from GH-secreting adenomas, and this inhibition was associated with ERK1/2 phosphorylation (34). Association between GH secretion and activation of ERK1/2 has been noted in other conditions as well (reviewed in Ref. 7). In accordance with these observations, we noted activation of ERK1/2 by klotho in GH3 cells, and cotreatment of klotho and bFGF further increased ERK phosphorylation in these cells. Moreover, inhibition of ERK1/2 activation, using the MEK1 inhibitor U-0126, abolished klotho-induced GH release from GH-secreting adenomas and normal pituitary. Taken together, these data suggest that klotho enhances GH secretion by activation of the ERK pathway.

Klotho is an essential cofactor for FGF23 activity in the kidney and in the pituitary (19, 35). However, this effect is mediated mainly by membrane-bound klotho and not by soluble klotho (20). We examined mostly the activity of soluble klotho and, as expected, did not observe enhancement of FGF23 activity by klotho. Because klotho is highly expressed in the pituitary, including in GH-secreting cells (26, 33), it is possible that FGF23 participates in the regulation of GH secretion through interaction with endogenous membrane-bound klotho.

Recently, Neidert et al. (26) and Sze et al. (33) have reported on the increased levels of circulating klotho in patients with acromegaly, which reverted to normal shortly after tumor resection. Conversely, we observed reduced klotho blood levels in children with GH deficiency (Wolf I, Sahmoon S, Ben Ami M, Levy-Shraga Y, Mazor-Aronovitch K, Pinsas-Hamiel O, Yeshayahu Y, Hemi R, Kanety H, Rubinek T, and Modan-Moses D, unpublished data). Possibly, the increased IGF-I levels observed in acromegaly may enhance the shedding of klotho (10), thus forming a positive feedback loop where increased GH secretion, followed by increased IGF-I production, leads to elevated circulating klotho levels, which in turn further increase GH secretion. Thus, inhibiting klotho activity in the pituitary may stop this positive loop and serve as a potential strategy for the treatment of acromegaly.

We noted a decrease in klotho mRNA levels in GH-secreting adenomas compared with normal pituitary and nonfunctioning adenomas. In a recent study, immunohistochemistry analyses did not reveal a significant difference in klotho staining between acromegaly and nonfunctioning adenomas (26). However, we measured mRNA levels, whereas in the described study klotho protein was evaluated. Therefore, it is possible that the discrepancy is the result of enhanced stability of the klotho protein compared with klotho mRNA. Studies in other tissues have not supported this notion, as a correlation between klotho mRNA levels and protein was observed in the pancreas (2) and the ovary (Lojkin I, Rubinek T, Orsulik S, Schwarmann O, Karlan BY, Bose S, and Wolf I, unpublished observations). However, perhaps klotho stability is differentially regulated in the pituitary.

GH treatment was insufficient to correct the growth-retarded phenotype of the kl/kl mice (16). However, the systemic phenotype observed in these mice is attributed to multiple metabolic aberrations, among them impaired levels of calcium, phosphate, vitamin D, and FGF23, which are not corrected by GH supplementation.

In conclusion, our data suggest klotho as a positive regulator of GH secretion and as a modulator of IGF-I and bFGF activity in the pituitary. More studies are needed to establish the precise physiological role klotho plays in the complex regulatory machinery of GH secretion.

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