NEPHROLITHIASIS IS CHARACTERIZED by the precipitation of salts within the collecting ducts and/or in the renal papillae, leading to stones (2, 16, 18). It is well known that the major defect in kidney stone formation is an imbalance of calcium homeostasis, but there is also some evidence of pH misregulation (16, 18, 38). Recently, several forms of inherited renal stone disease have been described, including Dent’s disease, X-linked recessive nephrolithiasis, and X-linked hypophosphatemic rickets. Hypercalciuria, nephrocalcinosis, and nephrolithiasis are common features of these diseases (29, 33, 34, 40).

The gene responsible for Dent’s disease was identified on the X chromosome within locus Xp 11.22 (19, 22). The gene encodes a new member of the CIC family of voltage-gated-chloride channels, CIC-5 (22, 23). Nine members of the CIC family have been cloned (12). They are likely to play a role in transepithelial transport, cell volume regulation, and membrane excitability (12, 13, 32, 37). The CIC-5 (5) chloride channel has an outwardly rectifying current vs. voltage relationship, is activated by strong depolarizing voltages, and is inhibited by DIDS (35).

Lloyd and co-workers (22, 23) have identified mutations in the CIC-5 gene responsible for Dent’s disease, X-linked recessive nephrolithiasis, and hypophosphatemic rickets. Several authors have also identified mutations in the CIC-5 gene found in patients with other types of nephrolithiasis (14, 22, 23, 29, 33). Several theories regarding the role of CIC-5 in kidney stone formation have been formulated (9, 22, 23). CIC-5 colocalizes with the H^+ -ATPase in intercalated cells of distal tubules (8, 26). These cells are involved in H^+ secretion and acidification of urine, suggesting that CIC-5 may provide the chloride currents that would be necessary to maintain H^+ secretion. It follows that a loss of CIC-5 function would be expected to impair acid secretion and lead to alkalization of urine and precipitation of calcium salts as stones (40).

Parathyroid hormone (PTH) is a peptide hormone produced in the parathyroid glands and released in response to a fall in plasma calcium concentration. It acts to raise plasma calcium concentration by enhancing bone absorption and increasing calcium reabsorption by the kidney (11). PTH also enhances the synthesis of the active metabolite of vitamin D, 1α,25(OH)2 vitamin D3, by kidney cells. PTH regulates several transporters in the kidney, including the Na^+-Pi cotransporter (28), the Na^+/H^+ exchanger (1), and the Na^+/K^+-ATPase (27). PTH also inhibits reabsorption of phosphate by the kidney, augmenting its excretion. The active metabolite of vitamin D, 1α,25(OH)2 vitamin D3 regulates calcium homeostasis by controlling bone resorption, calcium uptake by the intestine, and PTH gene expression (4, 36).

Because the regulation of CIC-5 in kidney is unknown and CIC-5 loss of function mutations leads to the hypercalciuric phenotype, the goal of this study was to determine whether hormones such as PTH and 1α,25(OH)2 vitamin D3, augmenting its excretion. The active metabolite of vitamin D, 1α,25(OH)2 vitamin D3 regulates calcium homeostasis by controlling bone resorption, calcium uptake by the intestine, and PTH gene expression (4, 36).

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homeostasis, also regulate ClC-5 expression.

MATERIALS AND METHODS

Animals and treatment. Ten female Wistar rats, 2 wk pregnant, were housed in a dark room with free access to a vitamin D-deficient diet (0.3% calcium and 0.47% phosphate, Harlan Teklad, Madison, WI) and tap water. Pups from these animals were also raised in the dark room and fed the vitamin D-deficient diet. Animals raised in vitamin D-deficient conditions were used in subsequent experiments. For the control group, pups were raised on a normal diet with a daily 12:12-h light-dark cycle.

Male rats between 6 and 8 wk old and weighing 200–250 g were anesthetized by using ketamine plus acepromazine (0.1 mg plus 0.25 mg/100 g body wt, respectively) by intraperitoneal injection. The rats were thyroparathyroidectomized (TPTX) by using standard surgical techniques. Groups of eight animals each were randomly chosen and submitted to the following treatments (n = 8 each group): 1) control group: sham operated and treated by an osmotic pump with Ringer’s solution; 2) vitamin D-deficient: sham operated and treated by an osmotic pump with Ringer solution; 3) vitamin D-deficient, TPTX: sham treated with Ringer solution by an osmotic pump with Ringer solution; and 4) vitamin D-deficient, TPTX: PTH replete with rat 1-34 PTH at 10^−7 M diluted in Ringer solution per day by an osmotic pump.

The animals were kept in a dark room for 3 days after surgery with daily care and free access to tap water and a vitamin D-deficient diet. On the second day the rats were placed in individual metabolic cages to collect 24-h urine samples. On the third day the animals were given an overdose of pentobarbital sodium. Urine and blood were collected and properly stored until use. The kidneys were perfused with cooled Ringer solution and then dissected into cortex and medulla. All the procedures are in agreement with The Johns Hopkins Animal Use Committee.

RNA and protein isolation. Total RNA was isolated by using TRIzol (GIBCO-BRL, Grand Island, NY) according to the manufacturer’s protocol without any modification. Total RNA was treated with RNase-free DNase at 10 U/μl (Boehringer-Mannheim, Indianapolis, IN) for 1 h at 37°C. Then the samples were digested with proteinase K (Boehringer-Mannheim) at 10 μg/ml for 30 min at 50°C. RNA was extracted from the mix by using phenol:chloroform-isomylalcohol (GIBCO-BRL) extraction and precipitated in 100% ethanol plus 0.5 M ammonium acetate (RNase free) overnight at −80°C. RNA concentration was measured by spectrophotometer (Beckman Instruments) at optical densities of 260 and 280 nm.

Urine and serum analysis for calcium. Twenty-four-hour urine was collected and analyzed for calcium and creatinine by using kits (Sigma Diagnostics, St. Louis, MO) according to the manufacturer’s protocol.

Serum analysis for 1α,25(OH)2 vitamin D3, PTH, and calcitonin (CT). The concentration of 1α,25(OH)2 vitamin D3 was measured by HPLC/competitive binding assay; ARIA was used to determine the PTH and calcitonin concentrations (Peninsula Laboratories, Belmont, CA) following the manufacturer’s protocols. A gamma counter (Beckman Instruments) was used to measure radioactive counts.

Quantification of the CIC-5 transcripts by RNase protection assay (RPA). To quantify CIC-5 mRNA, we performed a RPA. A CIC-5 PCR product was generated from rat cortex cDNA. cDNA was generated by using the Retroscript kit (Ambion, Austin, TX) following the manufacturer’s protocol. The primers used for the generation were forward primer 5’-CAGTGT-GCGTTCTCTTTGCG3’ (nucleotides 992–1012) and reverse primer 5’-GCTGTCACATAGGACC3’ (nucleotides 1238–1256) encompassing 264 bp. The thermal cycles were 94°C, 5 min (“hot start”; 1 cycle), followed by 94°C, 1 min; 53°C, 1 min; and 72°C, 1 min (40 cycles). The reaction mix consisted of (in mM) 200 Tris·HCl, pH: 8.0, 0.1 EDTA, 1.4-dithiothreitol, 50% (vol/vol) glycerol, 0.2 dNTPs, 50 MgCl2 and 2.5 U of Taq polymerase. This PCR product was subcloned into pCR Script SK+ vector and used to transform in Epicurian XL-Blue Escherichia coli (Stratagene, La Jolla, CA). Cloned CIC-5 cDNA were sequenced by using Sequenase 2.0 (Amersham Life Science, Cleveland, OH) following the manufacture’s protocol and confirmed by The Johns Hopkins Core Sequence Facility.

Plasmids were digested with a HindIII restriction enzyme, and the linearized template was used to generate antisense RNA. The antisense RNA was made following the protocol provided by the Maxi Script kit (Ambion, Austin, TX) using α-32P]UTP (800 μCi/mmol, NEN-DuPont, Boston, MA). The antisense RNA probe was hybridized for 24 h at 45°C with 50 μg of total RNA from the rat groups. For control, 5 μg of yeast tRNA were used to check RNase digestion and specificity of the probes. The CIC-5 probe was 75 bases longer, and the β-actin was 56 bases longer than the expected RNase-protected fragments. The samples were loaded onto 4.5% acrylamide/8 M urea denaturing gels, and the protected fragments were visualized by exposing the dried gel to an autoradiogram at −80°C. The bands obtained after 1-day exposure were analyzed by laser densitometry for β-actin. The same films were exposed for 3–5 days, and the bands of CIC-5 were also analyzed by laser densitometry. The data were then normalized and expressed as a ratio of CIC-5 over β-actin.

Generation of anti-serum against CIC-5 and Western blotting. A fragment encompassing 225 bp was amplified by RT-PCR from cDNA of rat kidney cortex. The region chosen was the extracellular loop between the transmembrane domains D8 and D9. This region was chosen because it has lower homology among CIC-3, CIC-4, and CIC-5. This region was chosen by another group to generate antibodies against human CIC-5 (5). Primers used for PCR were forward primer 5’-GGTGTCCTTTGCG3’ (nucleotides 1237–1256) and reverse primer 5’-AAAGCCAGCTGCCACATG3’ (nucleotides 1445–1462). The PCR fragment was amplified by using the following thermal cycle: 94°C for 5 min for 1 cycle followed by 94°C, 1 min 53°C, 1 min, and 72°C, 1 min (40 cycles). Reagents used for this reaction are the same as described above. The PCR fragment was subcloned into PCR-Script SK+ vector and used to transform Epicurian XL-Blue-competent E. coli (Stratagene). The PCR product CIC-5 was sequenced by using Sequenase 2.0 (Amersham Life Science) following the manufacture’s protocol. The vector was digested with BamHI I and Sst I, generating two fragments. The longer fragment of 225 bp, encompassing an open-reading frame of CIC-5, was subcloned into the same restriction sites of pTrC His-2 C myc-tagged vector (Invitrogen, Carlsbad, CA), used to transform DH5α-competent cells (Stratagene), and sequenced by using Sequenase 2.0 (Amer- shan, Life Science). The fusion protein was induced by 1 mM IPTG for 9 h and affinity-purified by using Nickel columns (Invitrogen, Carlsbad, CA). Purity of the fusion protein was examined by SDS-PAGE and Western blotting with anti-myc antibody (Invitrogen), and both have shown a protein with a mobility of 13 kDa. This fusion protein was used to immunize chickens.

For Western blotting 50 μg of protein were loaded per lane in 4–20% Ready gel (Bio-Rad, Hercules, CA), transferred to
polystyrene, polyvinylidene difluoride membranes (Sequigel, Bio-Rad), or nitrocellulose (Bio-Rad), and blocked with 5% dry milk in 0.5% Tween 20/PBS for 1 h at room temperature. The antisera was diluted 1:4,000 in 5% dry milk in 0.05% Tween 20/PBS overnight at 4°C. The detection system consisted of biotinylated anti-chicken light chain IgG and streptavidin-conjugated peroxidase (Sigma Diagnostics). Both were incubated at room temperature diluted 1:2,000. The membranes were developed by enhanced chemiluminescence (ECL) using the hyperfilm ECL (Amersham Life Science). For each set of experiments as a control for the specificity of the antibody, the same dilution of primary antibody was absorbed with 100 μg/ml of the purified fusion protein. A band of 80 kDa was identified and disappeared with incubation with the fusion protein.

Chemicals and Materials. Rat parathyroid hormone (1-34), calcium, and creatinine kits were purchased from Sigma Chemical. Osmotic pumps were purchased from Alzet MiniPumps model 1003D (Alza, Palo Alto, CA). The metabolic cages were purchased from Nalgene (Nalgene, Rochester, NY).

Statistics. All results are expressed as means ± SE. The samples were analyzed by ANOVA and non-paired Bonferroni post hoc tests. Results were significant at P < 0.05.

RESULTS

Serum levels of PTH, CT, and 1α, 25(OH)2 vitamin D3. Effects of vitamin D deficiency and replacement with PTH were verified by measuring the serum concentrations of these hormones. These data are shown in Fig. 1. Control rats have serum PTH concentrations of 100 ± 15 pg/ml, serum CT of 34 ± 4 pg/ml, and 1α,25(OH)2 vitamin D3 of 71 ± 18 pg/ml (n = 5, P < 0.05). In vitamin D-deficient rats in all experimental conditions (sham operated, TPTX rats and those repleted with PTH) there is a fourfold decrease in serum 1α,25(OH)2 vitamin D3 concentration to 18 ± 4 pg/ml (n = 24, P < 0.05, combined data for all vitamin D-deficient animals) compared with control rats. PTH and CT increase in vitamin D-deficient rats compared with the control animals (145 ± 15 pg/ml, n = 5, P < 0.05) and CT (59 ± 15 pg/ml, n = 5, P < 0.05). As expected TPTX, vitamin D-deficient animals caused a decrease in both PTH (51 ± 20 pg/ml, n = 5, P < 0.05) and CT (24 ± 6 pg/ml, n = 5, P < 0.05) 3 days after the surgery. Replacement with PTH in vitamin D-deficient, TPTX animals increases PTH to 150 ± 5 pg/ml (n = 5) but did not change the levels of CT (20 ± 4 pg/ml, n = 5, P < 0.05).

Calcium homeostasis. Serum calcium concentrations and Ca2+ excretion rates were analyzed to quantify the impact of our experimental maneuvers on calcium metabolism (Fig. 2A).

The control group had a serum calcium concentration of 9.51 ± 1.35 mg/dl (n = 5) and a urinary calcium/creatinine ratio of 0.14 ± 0.04 (n = 5). Serum calcium decreased in vitamin D-deficient rats by twofold (4.37 ± 0.87 mg/dl, n = 20, P < 0.05). Neither TPTX nor PTH replacement had any effect on serum calcium.

To determine the effect of PTH on the kidney we analyzed urinary excretion of calcium. Vitamin D-deficient animals had a calcium/creatinine ratio of 0.13 ± 0.03 (n = 5, P < 0.05) similar to control animals, 0.14 ± 0.04 (n = 5, P < 0.05). Consistent with the role of PTH in renal calcium transport, calcium excretion increased fivefold in vitamin D-deficient, TPTX rats to 0.46 ± 0.13 (n = 5, P < 0.05). Whereas, replacement of PTH in vitamin D-deficient TPTX rats led to a fivefold increase in calcium excretion to 0.12 ± 0.04 (n = 5, P < 0.05) (Fig. 2B). Thus as confirmed by our results, PTH acts on the kidneys promoting calcium reabsorption (11).

It is well known that PTH mobilizes calcium and phosphate from bone (11). Despite a reduction in calcium excretion in PTH-replete animals (Fig. 2B) serum calcium did not rise (Fig. 2A). This suggests that despite the increase in PTH levels in the PTH-replete
animals and the resulting reduction in calcium excretion, calcium continued to be excreted. In addition, these animals still had reduced levels of 1α,25(OH)2 vitamin D3, which impaired intestinal calcium absorption. This impaired intestinal absorption, and continued excretion of calcium did not allow restoration of serum calcium back to control levels.

Quantification of ClC-5 by RPA. RPA was performed to quantify the changes in ClC-5 mRNA levels in each experimental group. In the kidney cortex ClC-5 mRNA was equal in both control and vitamin D-deficient-sham operated rats (n = 8, P < 0.05) (Fig. 3, A and B). However, ClC-5 mRNA decreased 45% in vitamin D-deficient, TPTX animals (n = 8, P < 0.05). These data demonstrate that CIC-5 mRNA levels are not sensitive

![A: serum calcium analysis.](imageA)

*Fig. 2. A: serum calcium analysis. Vitamin D deficiency leads to a 2-fold decrease in serum calcium compared with control. Replacement of PTH does not increase serum calcium. Data are expressed as means ± SE (n = 5, P < 0.05). B: urinary calcium analysis. Urinary calcium is expressed by ratio between calcium over creatinine clearances. Urinary calcium is 4-fold higher in vitamin D-deficient, TPTX rats. Replacement with PTH brings urinary calcium back to control values (n = 5; P < 0.05). A and B: *compared with control and + compared with vitamin D-deficient, TPTX.*

![B: urinary calcium analysis.](imageB)

![A: representative RNAse protection assay of mRNA isolated from renal cortex of rats.](imageC)

**Fig. 3.** A: representative RNAse protection assay of mRNA isolated from renal cortex of rats. Control for RPA: lane 1, ladder; lane 2, yeast tRNA + β-actin digested; lane 3, yeast tRNA + β-actin undigested; lane 4, yeast tRNA + ClC-5 digested; lane 5, yeast tRNA + ClC-5 undigested. Data from rats subjected to the following treatments: lane 6, control; lane 7, vitamin D-deficient sham-operated; lane 8, vitamin D-deficient TPTX; lane 9, vitamin D-deficient TPTX PTH replete. Samples were loaded onto 4.5% acrylamide/8 M urea denaturing gels, and protected fragments were visualized by exposing dried gel to an X-ray film at −80°C. It should be noted that differences in band intensity are caused by changes in mRNA expression and variation in the loading of mRNA. Simultaneous measurement of β-actin was used to normalize for variations in loading of mRNA into different lanes. To accomplish this, bands obtained after 1-day exposure were analyzed by laser densitometry for β-actin (see inset at bottom right). The same films were exposed for 3–5 days, and bands of ClC-5 were also analyzed by laser densitometry. Data were then normalized and expressed as a ratio of ClC-5 over β-actin. B: laser densitometry of RPA data obtained from samples of renal cortex. Data (n = 8) are normalized to β-actin as described in Fig. 3A. Vitamin D-deficient TPTX rats show a 45% decrease in ClC-5 message. Replacement with PTH restores ClC-5 mRNA levels to control values (P < 0.05; *compared with control; + compared with vitamin D-deficient TPTX).*
to changes in circulating levels of 1α,25(OH)₂ vitamin D₃ but do respond dramatically to a reduction in PTH levels. This is shown more clearly when PTH was infused into vitamin D-deficient TPTX animals. Replacement of PTH resulted in a restoration in CIC-5 mRNA levels to values equivalent to that seen in control animals (n = 8, P < 0.05). These results suggest that PTH regulates CIC-5 mRNA in the kidney cortex. In contrast, despite the changes in 1α,25(OH)₂ vitamin D₃ or PTH levels induced in our experimental protocols, there were no changes in CIC-5 mRNA expression in the kidney medulla (n = 8) (Fig. 4, A and B), suggesting that 1α,25(OH)₂ vitamin D₃ and PTH do not regulate CIC-5 expression in the medulla.

Detection of CIC-5 protein by Western blots. Because mRNA and protein expression do not always correlate, we generated an antibody against CIC-5 to assess CIC-5 protein expression. The antibody detected a major protein of 80 kDa, a mobility predicted for CIC-5. The antibody also detects two minor proteins of 50 and 35 kDa. The major 80-kDa band was easily detected in kidney and colon but faintly in brain, lung, and bone (Fig. 5). The smaller 50- and 35-kDa bands were detected only in kidney. The identity of the minor 50- and 35-kDa bands on the gel is not known. It is possible that they represent either different isoforms or degradation products of CIC-5.

The pattern of detection by the antibody that showed a major 80-kDa CIC-5 protein band in different tissues as shown in Fig. 5, matches with the distribution of RNA expression published previously (Refs. 5, 21–23, 35). On the other hand, the pattern of detection of CIC-5 protein did not overlap with the tissue distribution of CIC-3 and CIC-4 mRNA (Refs. 13, 26). Thus cross-reactivity of our antibody with CIC-3 and CIC-4 is unlikely. No bands were detected when the antibody was preincubated with the purified fusion protein used to generate the anti-CIC-5 antibody, confirming that the antibody recognizes specifically the fusion protein (see Fig. 6A, B).

In the renal cortex a 80-kDa CIC-5 protein was detected in both control and vitamin D-deficient animals. However, in vitamin D-deficient TPTX rats the 80-kDa protein disappeared. Consistent with our data on mRNA expression, these results show that PTH regulates CIC-5 protein expression in cortex. The observation that protein expression was restored when the animals were injected with PTH (n = 5) is further evidence that PTH does indeed regulate CIC-5 protein expression (Fig. 6A).

In medulla the major 80-kDa CIC-5 protein band was detected in all experimental groups (Fig. 6B). These data are identical to those obtained by mRNA expression, again suggesting that, although CIC-5 is expressed in the medulla, the expression is not regulated either by 1α,25(OH)₂ vitamin D₃ or PTH.

DISCUSSION

Two major hormones regulate mineral metabolism, PTH and 1α,25(OH)₂ vitamin D₃. PTH acts mainly in cortical segments of the nephron (11) whereas, 1α,25(OH)₂ vitamin D₃ receptors are predominantly expressed in the kidney medulla (11). In this work, we investigated whether PTH and/or 1α,25(OH)₂ vitamin D₃ could modulate the expression pattern of CIC-5 mRNA and protein in rat kidney cortex and medulla.

1α,25(OH)₂ vitamin D₃ depletion alone does not affect CIC-5 expression in renal cortex. Our data suggest that PTH does play a role in modulating expression of CIC-5 mRNA and protein levels in kidney cortex. The modulation of CIC-5 expression may be modulated through second-messenger systems directly related to PTH. Alternatively, it is also possible that CIC-5 mes-
sage is regulated by changes in Ca\textsuperscript{2+} excretion subsequent to changes in PTH.

Although, ClC-5 is expressed in the medulla, it is not regulated by hormones involved in calcium homeostasis. This differential modulation of ClC-5 in cortex but not in medulla is probably indicative of regional differences in the role ClC-5 in calcium reabsorption in cortical vs. medullary nephron segments.

Other investigators have also identified chloride channels activated by PTH in the proximal tubule. For example, Suzuki and co-workers (39) studied a chloride channel in the apical cell membrane of the proximal convoluted tubule by using patch-clamp analysis. They found a rectifying chloride-selective channel with conductances of 33 pS at positive potentials and 22.5 pS at negative potentials, which were blocked by DIDS. PTH activates this channel via the action of protein kinase A or by protein kinase C, but not by calcium.

How do chloride channels play a role in calcium absorption? Gesek and Friedman (7) have described a chloride conductance that promotes the driving force for calcium reabsorption in mouse distal convoluted tubule (DCT) cells. Blocking the chloride channel also blocks calcium entry across the apical membrane. They suggest that activating a chloride channel would hyperpolarize the plasma membrane, allowing calcium to flow through the plasma membrane via calcium channels (24), thereby enhancing calcium reabsorption.

They found that PTH increases the chloride conductance of the DCT, but the effect is not immediate. It occurs only after a long latency (~8 min). This may reflect differences in the signaling mechanisms between CT and PTH, with PTH acting via a modulation of protein expression (7). In our work we showed that PTH modulates ClC-5 in the renal cortex via an increase in mRNA and protein expression. Clearly, one possibility is that the channel identified by Gesek and Friedman (7) is ClC-5. The channel described by Gesek and Friedman has outwardly rectifying current vs. voltage relationship. This feature resembles the characteristics of ClC-5 chloride channel. Although the properties of their channel in the DCT are similar to ClC-5, ClC-5 mRNA and protein have been detected primarily in the glomerulus, proximal tubules, and connecting and collecting ducts (26, 35). This raises the possibility that another kind of chloride channel similar to ClC-5 and also regulated by PTH is responsible for calcium reabsorption in the DCT.

To determine whether changes in ClC-5 expression correlates with calcium excretion, we analyzed urinary calcium. The urinary excretion of calcium in vitamin D-deficient rats did not change compared with controls. These animals were also able to maintain ClC-5 mRNA and protein in kidney cortex similar to control values. When vitamin D-deficient animals are TPTX, there is an increase in calcium excretion that is inversely proportional to the amount of ClC-5 mRNA and protein.
in kidney cortex, suggesting that this chloride channel may play a role in calcium reabsorption.

Several diseases involved with imbalances in calcium and phosphate homeostasis are referred to as idiopathic hypercalciuria. Some patients with this disorder have lower levels of PTH in the absence of changes in 1α,25(OH)2 vitamin D3 (4), whereas, other forms of normocalcemic hypercalciuria in humans may involve disordered regulation of 1α,25(OH)2 vitamin D3 (3). In some cases, hypercalciuria may lead to kidney stone formation (16, 18, 38). Our results predict that patients with idiopathic hypercalciuria associated with lower levels of PTH may have reduced expression of CIC-5, which in turn would contribute to enhanced renal calcium excretion.

Mutations that disrupt the function of the CIC-5 chloride channel have been detected in several types of inherited forms of nephrolithiasis. These patients show low-molecular-weight proteinuria, hypercalciuria, and nephrolithiasis (10, 23, 25, 32). We have shown that lower levels of CIC-5 expression correlate with higher urinary calcium excretion. Therefore, the loss of function of CIC-5 could lead to the hypercalciuric phenotype.

Interestingly, cases of hyperparathyroidism have been reported in some patients with both idiopathic nephrolithiasis and X-linked hypophosphatemia (15). Some mutations in the CIC-5 gene found in X-linked hypophosphatemic patients generate a lower conductance channel compared with wild type (14, 22, 23). We showed that replacement of PTH in vitamin D-deficient parathyroidectomized rats restores CIC-5 protein levels. Although not tested here, higher doses of PTH may induce even higher expression of CIC-5. It is also possible that hyperparathyroidism in these patients would increase the CIC-5 protein, partially offsetting the reduction in conductance.

A feature that is often associated with mutations in CIC-5 is low-molecular-weight proteinuria (5, 23, 25). β2-Microglobulin is the major protein found in the urine from people who lose CIC-5 function. This protein is normally reabsorbed by endocytosis in proximal tubules of nephron. In the proximal tubule, CIC-5 protein is located in early endosomes colocalizing with the vacuolar H+ ATPase (8) that pumps H+ into the endosomes acidifying them. We speculate that CIC-5 provides the chloride conductance necessary to neutralize the positive charge generated by H+ within the endosome. Thus a mutation in the CLC5 gene that interrupts channel function would be expected to disrupt normal endosome function, leading to increases in β2-microglobulin excretion.

Protein endocytosis and subsequent degradation is an important mechanism for the regulation of membrane proteins. For example, PTH enhances the catabolism of type II Na+-P2 co-transporter in opossum kidney cells by targeting it for lysosomal degradation (28). Likewise, Devuyst et al. (5) have demonstrated a colocalization of endocytosed albumin and transfected CIC5 suggestive of a role of CIC5 in albumin endocytosis. These data taken together, we speculate that PTH could regulate CIC-5 expression to maintain the number of vesicles able to recycle both membrane and endocytosed proteins in proximal tubules. Furthermore, a disruption of CIC-5 function could impair not only protein endocytosis but also the internalization of calcium crystals in renal epithelia (20).

In conclusion, we have shown that alterations in mineral homeostasis can affect the expression of CIC-5 in the kidney. PTH plays a role in regulating both mRNA and protein expression in kidney cortex. CIC-5 protein regulation in kidney cortex correlates with the level of urinary calcium excretion. It is well known that PTH is a major mediator of calcium reabsorption in kidney and its site of action is mainly in the kidney cortex. This paper suggests that PTH may exert part of its action on calcium reabsorption by regulating CIC-5 mRNA and protein expression.

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