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Selective decrease of mRNAs encoding plasma membrane calcium pump isoforms 2 and 3 in rat kidney

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Background. Although the existence of multiple isoforms of plasma membrane calcium ATPase (PMCA) is now well documented, their biological functions are not yet known. In this study, we set out to investigate the potential role of PMCA isoforms, previously identified in renal cortical tissue, in tubular reabsorption of calcium (Ca^{2+}).

Methods. With use of reverse transcription-polymerase chain reaction analysis, we determined levels of mRNAs encoding isoforms of PMCA1 through PMCA4 in renal cortex, liver, and brain of rats with hypercalciuria induced by feeding with a low-phosphate diet (LPD) as compared with Ca^{2+}-retaining rats that were fed a high-phosphate diet (HPD).

Results. We observed that in hypercalciuric LPD-fed rats, the mRNAs encoding isoforms PMCA2b and PMCA3(a + c) are significantly lower (Δ approximately −50%) than in HPD-fed hypocalciuric rats, whereas no changes in mRNAs encoding isoforms PMCA1b and PMCA4 were observed, and mRNA encoding calbindin 28 kDa was increased. On the other hand, the content of mRNAs encoding PMCA2b and PMCA3(a + c) in liver and brain, respectively, was not changed.

Conclusion. These findings are evidence that expression of PMCA isoforms in the kidney can be selectively modulated in response to pathophysiologic stimuli. The association of a decrease in mRNA encoding PMCA2b and PMCA3(a + c) with hypercalciuria suggests that the two PMCA isoforms may be operant in tubular reabsorption of Ca^{2+} and its regulation.

The family of plasma membrane Ca^{2+}-ATPase (PMCA) isozymes [1, 2] is a key component of active transport of Ca^{2+} from the cytosol to the extracellular space [3, 4]. The active extrusion of Ca^{2+} across the basolateral plasma membrane and its regulation is likely related to several diverse functions of functionally and structurally polarized epithelial cells. First, PMCAs are operant in maintaining cellular homeostasis of Ca^{2+}, that is, the maintenance of a steep concentration gradient of Ca^{2+} [Ca^{2+}_o] >> [Ca_i^{2+}] across plasma membranes [3–5]. Likewise, changes of cytosolic Ca^{2+} caused by an influx from extracellular fluid, extrusion across plasma membrane, or efflux from intracellular stores or Ca^{2+} and Ca^{2+}-reuptake in response to regulatory stimuli are key components of the signaling systems that employ Ca^{2+} as a second messenger [6]. Thus, in functionally and ultrastructurally polarized cells, such as those that transport Ca^{2+} across renal epithelial layers, PMCAs, along with the Na^+/Ca^{2+} exchanger, are major active transporters that generate, maintain, and regulate transepithelial Ca^{2+} movement [7]. The Ca^{2+}-binding protein calbindin 28 kDa, which is present in cytoplasm of distal tubular epithelia and the expression of which is dependent on $1,25(OH)_2$-vitamin D_3 (calcitriol), promotes Ca^{2+} reabsorption by sequestering/buffering Ca^{2+} that influxes from tubular lumen, and by accelerating Ca^{2+} diffusion across the cell [3, 8], it also can stimulate PMCA [9].

Investigations of recent years revealed that PMCAs exist in a number of isoforms that are splice variant products of four distinct isogenes, PMCA1 through PMCA4 [10]. It stands to reason to assume that various isoforms of PMCA subserve diverse cellular functions. However, the nexus between individual PMCA isoforms and specific cell functions has not yet been established. Isoforms such as PMCA1 and PMCA4 are believed to be widely expressed and to serve primarily to maintain Ca^{2+} homeostasis in a wide variety of tissues and cell types [10, 11]. On the other hand, PMCA2 and PMCA3 isoforms are apparently tissue specific [10, 11]. Physiological regulation of gene expression of these and other PMCA isoforms is not yet known.

Our preceding studies revealed that rat kidney parenchyma contains mRNAs encoding PMCA1, PMCA2, PMCA3, and PMCA4 [12, 13]. However, these mRNAs encoding PMCA isoforms are unevenly distributed in major renal tissue zones, as well as in different nephron segments [12, 13]. Isoforms PMCA1 and PMCA4 are

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expressed in all zones of renal parenchyma and also in all nephron segments studies to date [12–14]. On the other hand, PMCA isoforms 2 and 3 are expressed only in renal cortex and/or in the outer medulla, but not in inner medulla [12, 13].

According to our working hypothesis, certain PMCA isoforms have specific roles in vectorial transport of Ca^{2+} across renal epithelial tubular wall, and we postulate that the degree of expression of such PMCA isoforms will change in functional states that are characterized by different rates of renal tubular reabsorption of Ca_i^{2+}. As a first step to test this hypothesis, we set out to determine whether the expression of some PMCA isoforms is different in functional states of intense renal tubular Ca^{2+} reaborption-conversion, as compared with states with low tubular reclamation of Ca^{2+} and consequently high urinary output of Ca^{2+}, that is, hypercalciuria.

It is now well established that feeding rats with a low-phosphate diet (LPD) causes a dramatic increase in urinary excretion of Ca^{2+} because of a decrease of tubular Ca^{2+} reabsorption [15, 16]. In response to feeding with a LPD, urinary excretion of Ca^{2+} increases more than 20-fold, with little or no changes in plasma Ca^{2+} [15, 16]. Therefore, it follows that this hypercalciuria in response to phosphate deprivation caused by feeding with a LPD is due, solely or predominantly, to decreased tubular reabsorption of filtered Ca^{2+} from tubular fluid. To discern whether this change in the rate of tubular Ca^{2+} reabsorption may relate to the expression of some specific PMCA isoforms, we investigated steady-state content of cognate mRNAs encoding isoforms PMCA1 through PMCA4 in kidney cortex of rats that were fed a high-phosphate diet (HPD) or LPD.

Our results show that in response to feeding a LPD, the suppressed tubular reabsorption of Ca^{2+} is associated with marked (approximately 50%) decrease in the level of mRNAs encoding PMCA2 and PMCA3, whereas mRNA encoding PMCA1 and PMCA4 remained unchanged.

METHODS

Male Sprague-Dawley rats (6 to 8 weeks old) were fed with regular diet for at least one week before starting the treatment. After this period, rats were placed into metabolic cages, were divided into two groups, and were fed for seven days with a LPD (0.2% Pi) and a HPD (1.2%); after seven days, rats were completely and stably adapted to LPD, both in terms of hypophosphaturia as well as hypercalciuria [15]. Urine was collected on day 1 and day 7 of the experiment to determine urinary excretion of phosphate and Ca^{2+}. On day 7, rats were anesthetized with pentobarbital, and the kidneys were perfused with sterile Hank's solution containing 1% bovine serum albumin (BSA) [12, 13]. Then the kidneys were excised, and the animals were sacrificed by an overdose of pentobarbital. The kidneys were macrodissected into cortex, outer medulla, and inner medulla and were stored frozen in liquid nitrogen until the mRNA isolation. mRNA isolation was performed using the FastTrack kit (Invitrogen, Carlsbad, CA, USA).

Reverse transcription (RT) was done as described in detail in our previous study [12]. Polymerase chain reaction (PCR) was performed in 100 μl by using the Geneamp RNA-PCR kit (Perkin Elmer, Norwalk, NJ, USA). To 20 μl from the RT mix, in each case the primers, Taq polymerase and buffers were added, and the PCR was started. To prevent misamplification, TaqStart antibody (Clontech, Palo Alto, CA, USA) was added to the mixture to avoid mispriming, and the tubes were kept on ice until the start of the PCR process. After two minutes of initial melt at 94°C, PCR was carried out for 35 cycles of one minute at 94°C, one minute at 52°C, and one minute at 72°C, with a final extension step of seven minutes at 72°C in a thermal cycler Perkin Elmer 9600 system [12, 13].

For amplification of rPMCA1, we employed primers 5′-CCCTGAGGAGGAATTGGCG-3′ and 5′-GATTG AACTTGATTCT-3′ [17]; for rPMCA2, the primers were 5′-CACCATCCCTACCAGCAGGC-3′ and 5′-CAGG TCGGTGTCATCGATG-3′. For rPMCA3, the primers were 5′-GTCATTGCCACTATCCC-3′ and 5′-AGCTA CGGAATGCTTTCACC-3′ [18]; and for rPMCA4, the primers were 5′-ATGCCGAGATGGAGCTTCGC-3′ and 5′-CAGCATCCGACAGGCGCTTG-3′ [16]. For amplification of calbindin 28 kDa, we employed primers 5′-AGAACTGCTCATCATCGGCT-3′ and 5′-ACTTT CTCTTCTGCCACCGA-3′ [19].

Analysis of the PCR products

Thirty-five microliters of each of the PCR mixtures were size fractionated in 1.5% agarose gels and stained with ethidium bromide [12–14, 20, 21], and the PCR products were sequenced. Negative controls were performed in the absence of RT and gave no noticeable products in all cases.

The steps in analysis by Southern blot, that is, transfer to supported nitrocellulose, fixation of the DNA, prehybridization, hybridization washing, and exposition, were performed as described in our preceding studies [12–14]. For detection of PMCA1b, we used a probe for 5′ > ATC CGA GTG GTG AAT GCA TTT CGT AGC TCT TTA TAC GAA GGG TTA GAG AA < 3′ [17]. For rPMCA2b, we used a probe for 5′ > ATC CGC GTC GTG AAG GCG TTC CGT AGC TCT CTC TAT GAA GGG TTA GAA AA < 3′ [22]. For rPMCA(a + c), we used a probe for 5′ > CAC CAG CCA GCT CAA GTG CCT GAA GAA AGC AGG GCA TGG GCC TGG GAA GG < 3′ [22], and for rPMCA4b, we used a probe for 5′ > ATG CCG AGA TGG AGC TTC GCC GAG GCC AGA TCC TCT GGG TCC GTG

GCC TGA ACC < 3′ [22]. Labeling of the probes with [^{32}P]-γ-ATP (6000 Ci/mol) was done by the T_4 polynucleotide kinase method by using a 5′ Terminus labeling system (GIBCO-BRL, Gaithersburg, MD, USA). Labeled oligonucleotides were separated from γ-[^{32}P]ATP by using Nuc-Trap Push columns (Stratagene, La Jolla, CA, USA).

To make a relative quantitation of the amount of mRNA for each of the PMCAs present in the samples, the PCR was carried out for 23, 28, and 35 cycles for each sample. After electrophoresis and Southern blotting with specific probes, the radioactivity associated with the appropriate band was estimated with the aid of a Phosphoimager (Molecular Dynamics, Sunnyvale, CA, USA). Then the radioactivity of the band corresponding to DNA amplified from a LPD rat was compared with a band corresponding to DNA amplified from a HPD rat under the same conditions. Special care was taken to compare samples processed in parallel and blotted side by side in the same sheet of reinforced nitrocellulose. The ratio of LPD/HPD was obtained for all pairs of samples and averaged.

To confirm that the results observed did not depend on the degree of amplification, that is, the number of cycles during the PCR, the amount of radioactivity in the bands corresponding to LPD rats was plotted against the radioactivity present in the equivalent band corresponding to HPD rats. In all cases, the data fitted to a straight line with an intercept equal to 0 (data not shown), thus indicating that the results were independent of the magnitude of the amplification. The slope of the line reflects the ratio of mRNA encoding a particular PMCA isoform present in LPD versus mRNA encoding the same isoform present in HPD, provided that the RT and PCR were carried out in the same conditions and started from the same amount of mRNA. Results with both methods of analysis were consistent and gave concordant results.

Calcium in the urine was measured by atomic absorption spectrometry [15, 16], and urine phosphate content was determined by the method of Chen, Toribara, and Warmer [23]. The excretion of Ca^{2+} ($U_{Ca}V$) and Pi ($U_{Pi}V$) was expressed as mg/24 hr [15].

The results were evaluated with the use of two-tailed *t*-test for group or paired comparisons, as appropriate. *P* values < 0.05 were considered significant.

RESULTS

The results summarized in Table 1 affirm that rats responded adequately [13] and reached steady state of Pi and Ca^{2+} excretion in response to feeding with LPD or HPD. Feeding with LPD caused profound hypercalciuria. Urinary excretion of Ca^{2+} ($U_{Ca}V$) was initially not different between the two groups, but then increased nearly 40-fold after seven days of feeding with LPD (Table 1). After seven days of feeding with LPD and HPD, respectively, urinary excretion of Ca^{2+}, and by extension, tubular reabsorption of Ca^{2+}, and urinary excretion of Pi were fully stabilized [13]. In rats fed a HPD, even low $U_{Ca2+}V$ was still significantly reduced, as compared with the initial control period (Table 1). Also, excretion of phosphate ($U_{Pi}V$) was appropriately reduced in LPD rats (Table 1). However, the plasma Ca^{2+} levels on day 7 in LPD rats (2.0 ± 0.12 mM, mean ± SEM, $N = 12$) were not different from HPD rats (2.09 ± 0.01 mM; mean ± SEM, $N = 12$).

Table 1. Effect of feeding with low phosphate diet (LPD) and high phosphate diet (HPD) upon urinary excretion of calcium ($U_{Ca}V$) and phosphorus ($U_{Pi}V$)

	HPD	LPD	*P* value[a]
Urinary calcium *mg/day/100 g*			
Day 1	0.6 ± 0.15	0.3 ± 0.06	NS
Day 7	0.2 ± 0.04[b]	7.9 ± 1.2[c]	<0.01
Urinary Pi *mg/day/100 g*			
Day 1	20.7 ± 4.7	13.4 ± 2.6	NS
Day 7	227 ± 3.7[c]	0.687 ± 0.14[b]	<0.01

[a] Denotes significance of differences between HPD group and LPD
[b] Value significantly lower ($P < 0.05$, *t*-test) than on day 1
[c] Value significantly higher ($P < 0.01$, *t*-test) than on day 1

We have analyzed mRNA extracted from renal cortex, liver, and brain of LPD rats as compared with HPD rats. For a better comparison, analysis was performed with the use of paired design; that is, mRNA from LPD rats and from HPD rat was analyzed simultaneously under identical experimental conditions. The size of the products and Southern blot analysis (Fig. 1) show that PMCA1b, PMCA2b, PMCA3(a + c), and PMCA4b were all detected in renal cortical extract in preparation from renal cortex as we reported previously [11]. However, the amount of mRNA encoding PMCA2b and PMCA3(a + c) was clearly lower in LPD rats, as compared with HPD rats. On the other hand, mRNA encoding isoforms PMCA1b and PMCA4b and β-actin were not different between the two groups (Fig. 1). The summary of analysis of all the mRNA samples from 10 pairs of rats (Fig. 2) shows that the LPD/HPD was significantly ($P < 0.01$, *t*-test) lower for PMCA2b and PMCA3(a + c), thus documenting lower abundance of mRNAs encoding these two PMCA isoforms. On the other hand, the LPD/HPD ratios for PMCA1b and PMCA4b were not decreased; in contrast, there was a tendency to a slight increase (Fig. 2). The relative (Δ-%) extent of decrease in LPD/HPD ratio was not different between PMCA2b and PMCA3(a + c). In comparison with mRNA PMCA2 and mRNA PMCA3 that were decreased or mRNA PMCA1B and mRNA PMCA4b that were not changed (Figs. 1 and 2), the expression of mRNA encoding calbindin

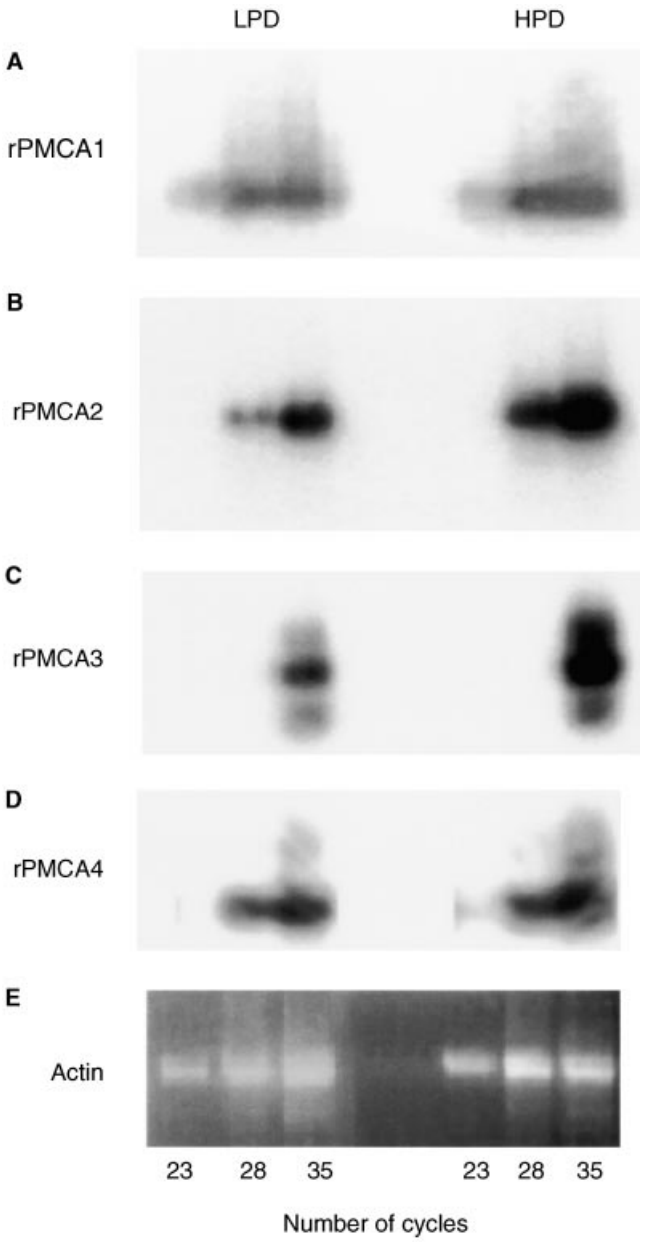

Fig. 1. Southern blot analysis of PCR products for plasma membrane Ca^{2+}-ATPase (PMCA)-1 (*A*), PMCA2 (*B*), PMCA3 (*C*), and PMCA4 (*D*) in rats fed low (LPD)- or high (LPD)-phosphate diet. (*E*) This shows an agarose gel electrophoresis of the PCR products for β-actin in the same rats. RT-PCR was performed as described in the **Methods** section. The number of amplification cycles in each case is indicated at the bottom of the figure.

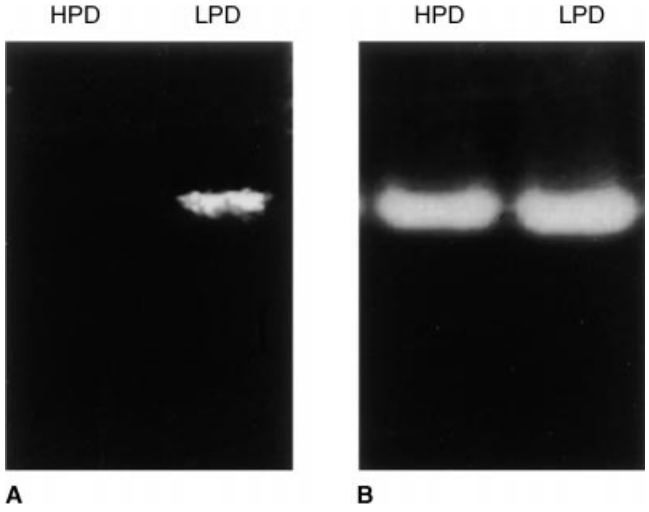

Fig. 2. Electrophoresis of RT-PCR products for (*A*) calbindin 28 kDa (band 552 bp) and (*B*) β-actin in mRNA from kidney cortex of rats fed low-phosphate diet (LPD) or high-phosphate diet (HPD). The procedure is described in the **Methods** section. The mRNA were pooled from three kidneys for each preparation.

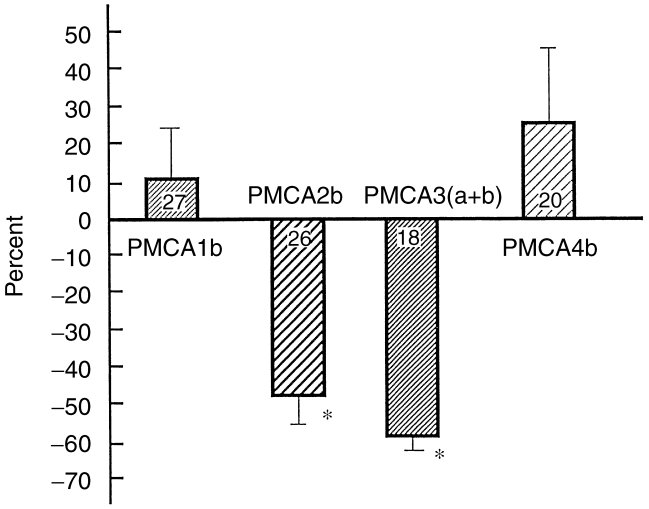

Fig. 3. Changes in mRNAs encoding the four PMCA isoforms in renal cortex of rats in response to low-phosphate diet (LPD), as compared with high-phosphate diet (HPD). Each bar denotes mean values of LPD/HPD ratios (mean ± SEM). *N* is the number of observations shown within the bars. The asterisk denotes the values significantly different from zero (*$P < 0.01$, *t*-test). Details are discussed in the **Methods** section.

28 kDa, a Ca^{2+}-binding protein that is postulated to promote reabsorption of Ca^{2+} from tubular lumen into interstitial fluid [8], was markedly increased in cortex of LPD rats (Fig. 3).

To explore whether a decrease in contents of mRNAs encoding PMCA2b and PMCA3(a + c) in cortex of LPD rats is a generalized phenomenon or rather kidney specific, the mRNA extracted from renal cortex was compared with mRNA extracted from liver and brain of LPD and HPD rats. Although PMCA2b was decreased in kidney cortex, no such decrease was found in mRNA in brain and liver (Table 2). Likewise, whereas mRNA encoding PMCA3(a + c) was decreased in renal cortex, PMCA3 in brain remained unchanged. No difference of PMCA1b between LPD and HPD was found either in kidney cortex or liver (Table 2).

Table 2. Expression of mRNAs encoding isoforms PMCA1-3 in liver, brain and kidney cortex

Isoform	Brain ($N = 4$)	Kidney cortex ($N = 10$)	Liver ($N = 4$)
PMCA1b	ND	1.1 ± 0.14[a]	1.25 ± 0.1
PMCA2b	1.41 ± 0.15	0.53 ± 0.07[b]	1.33 ± 0.18
PMCA3(a + c)	1.02 ± 0.1	0.41 ± 0.04[b]	ND

The values denote the ratio of mRNA values of LPD rat to paired HPD rat in the same organ. Details are in the **Methods** section.

[a] Denotes mean + SEM, ND is not determined; [b] Values significantly different ($P < 0.01$, *t*-test) from those in brain or liver, also significantly different from PMCA1b in kidney cortex

DISCUSSION

Members of the PMCA superfamily undoubtedly play a key role in cellular homeostasis of Ca^{2+}. Thanks to the availability of recombinant DNA technology, a number of PMCA isoforms were identified [1, 2]; however, the relationship and role of specific PMCA isoforms to specific cell functions involving Ca^{2+} fluxes remains virtually unknown.

The results of this study provide, to our knowledge, the first evidence that expression of specific PMCA isoforms do selectively change in response to a functional state that is characterized by large differences in renal epithelial transport of Ca^{2+}. Our results show that in a state of decreased tubular Ca^{2+} reabsorption; that is, lower Ca^{2+} transport, the abundance of mRNAs encoding two PMCA isoforms, PMCA2 and PMCA3, were markedly decreased, whereas expression of PMCA1b and PMCA4b was not changed (Fig. 2). In contrast, mRNA encoding calbindin 28 kDa was increased (Fig. 3). In extrarenal tissues from phosphate-depleted rats (LPD), namely liver and brain, mRNAs encoding PMCA2 and PMCA3 were not decreased (Table 2). This finding indicates that a decrease of PMCA2 and PMCA3 is not a generalized phenomenon and that it is specific for the kidney. Our results thus suggest that the increase in urinary Ca^{2+} excretion and decreased tubular Ca^{2+} reabsorption may be due to or at least are associated with suppressed expression of PMCA2 and PMCA3.

The expression of mRNA encoding calbindin 28 kDa in renal cortex was enhanced in LPD rats (Fig. 3), a finding consistent with reported enhancement of mRNA calbindin 28 kDa in kidneys of rats after 17 days of phosphate deprivation [24]. Studies of renal epithelial cells in culture indicate that calbindin 28 kDa is essential for increase of Na+–Pi cotransport in response to LPD [25]. The increase in expression of calbindin 28 kDa is stimulated by calcitriol [26, 27], and enhanced mRNA calbindin 28 kDa in our experiments (Fig. 3) is most likely due to action of calcitriol, a hormone that is elevated in response to LPD [28], consequent to marked increase in 25-hydroxycholecalciferol-1-α-hydroxylase [29, 30]. On the other hand, secretion of parathyroid hormone (PTH), which may enhance calbindin 28 kDa in the kidney [31], is suppressed in response to feeding with LPD [31, 32], and calcitonin has no apparent effect on renal calbindin 28 kDa [33]. Calbindin 28 kDa accelerates reabsorption in tubules probably by binding and sequestering Ca^{2+} that enters the cell from lumen across apical membrane and accelerates Ca^{2+} diffusion [34]. Calbindin 28 kDa can also directly enhance Ca^{2+} entry across luminal membranes of distal tubular cells [35]. Moreover, calbindin 28 kDa may also directly activate PMCA [9]. Thus, our findings—which are that in LPD, both Ca^{2+} reabsorption (Table 1) and expressions of PMCA2 and PMCA3 (Fig. 2) are decreased, whereas expression of calbindin 28 kDa is increased (Fig. 3)—support the notion that PMCA2 and/or PMCA3 may represent a rate-limiting step in transepithelial reabsorption of Ca^{2+} in renal cortical tubules.

Although the increase of mRNA abundance is not in all instances paralleled by increased expression of encoded protein [36], recent reports suggest that this is not the case in a relationship between PMCA isoforms and Ca^{2+} transport [7, 37–40]. For example, transfection of aortic endothelial cells with PMCA1a [38], or myogenic L6 cells with PMCA4 [37], or expression of PMCA1 and PMCA4 in the course of the cell cycle in vascular smooth muscle cells [39] resulted in a severalfold increase of Ca^{2+}-ATPase activity, as well as to an increased amount of PMCA protein. A study on a myogenic cell line has shown that when PMCA is overexpressed in the stably transfected cells, the resting cytosolic Ca^{2+} level is significantly reduced as compared with controls [37]. Thus, it is reasonable to expect that changes in abundance of mRNA encoding PMCA isoforms has an impact on both the amounts of PMCA protein and the Ca^{2+} pumping activity.

Our results indicate that decreased transport of Ca^{2+} in tubular cells may be caused by, or at least associated with, a decreased expression of PMCA2 and PMCA3 isoforms (Fig. 2), and several implications of our findings should be critically considered. Evident association of selectively decreased expressions of PMCA2 and PMCA3 with lowered lumen-to-interstitium transepithelial Ca^{2+} flux may suggest that the two PMCA isoforms may have a direct function in transcellular Ca^{2+} transport and, in particular, at the step of active Ca^{2+} extrusion from cell interior across the basolateral membrane to the interstitial fluid [1–3]. However, it is also conceivable that one or two of PMCA isoforms that were decreased in LPD-induced hypercalciuria may be part of intracellular Ca^{2+} signaling pathways that regulate the tubular transport of Ca^{2+} [6, 40, 41]. The notion that PMCA2 and PMCA3 are involved in specific transport or regulatory functions of renal tubules is consistent with a current view that these two PMCA isoforms serve as components of tissue-specific functions in various cell types [10, 11], whereas

PMCA1 and PMCA4, which were not changed in our experiments, may have a "housekeeping" function in the maintenance of basic Ca^{2+} homeostasis of the cell [8, 9]. It is therefore noteworthy that in spite of an increase of calbindin 28 kDa (Fig. 2), expressions of both PMCA2 and PMCA3 were reduced (Fig. 2), and Ca^{2+} reabsorption was decreased (Table 1). These findings argue in favor of the notion that PMCA2 and PMCA3 are determining factors in transepithelial Ca^{2+} flux in renal cortical tubules.

The question arises as to whether observed selective decreases in PMCA2 and PMCA3 in kidneys of LPD rats may be accounted for by changes in calciotropic hormones that are known to occur in response to Pi deprivation. Synthesis of calcitriol in the kidney is the state of Pi deprivation is enhanced, and the plasma levels of calcitriol are elevated [28, 29, 42]. However, calcitriol was reported to cause an increase PMCA [43, 44], an effect opposite to suppression of PMCA that was observed in our study (Figs. 1 and 2). Furthermore, mRNA encoding calcitriol-dependent protein calbindin 28 kDa [8, 26] was induced in LPD rats (Fig. 3), and this indicates that renal tubular cells responded appropriately to elevated calcitriol; however, PMCA2 and PMCA3 were suppressed (Figs. 1 and 2). Parathyroid hormone (PTH) was reported to increase PMCA activity in the kidney [45], but plasma levels of PTH are decreased in response to LPD [32, 46]. Calcitonin decreased PMCA activity in liver [44], had no effect on PMCA in bone cells [47], and increased PMCA in erythrocytes [48]. Thus, it seems unlikely that any of the three hormones caused, at least directly, decrease of PMCA2 and PMCA3 observed in LPD rats.

It would be premature even to speculate how the decrease in mRNAs encoding for PMCA2 and PMCA3 relates to Ca^{2+} reabsorption in specific tubular segments; however, in this context, it is of interest to note that *in vivo* inhibition of DNA transcription by administration of actinomycin D and inhibition of protein synthesis by cycloheximide in rats blocked the hypercalciuric response to feeding with LPD [16]. Moreover, it should be noted that both isoforms were detected both in renal cortex and in isolated cortical tubules [12, 13]. Therefore, it stands to reason that PMCA2 and PMCA3 are likely involved in functions of tubular segments that are located in the cortical zone of the kidney parenchyma. Distal tubular segments are a major site of active and regulated Ca^{2+} reabsorption [3, 4, 49]; only PMCA1 and PMCA4 were expressed in proximal tubular cells [50]. In view of this, it may be speculated that observed changes in PMCA2b and PMCA3(a + c) occur in cells of distal nephron.

As in previous studies, feeding with LPD caused a large increase in urinary Ca^{2+} excretion (Table 1). It has been well established in previous studies [15, 16] that in dietary Pi depletion, caused by feeding by LPD, urinary Ca^{2+} excretion greatly increases, without changes in plasma Ca^{2+} levels. Consequently, an increase in urinary Ca^{2+} output is bound to be due to suppression of transport of Ca^{2+} across tubular epithelium into peritubular space. However, the relationship between the extent of decrease in expression of PMCA isoforms to the extent of decrease of Ca^{2+} transport in cortical nephron segments may be only speculated on and remains to be determined.

Results presented herein are, to our knowledge, the first observations showing that a selective change in expression of PMCA isoforms occurs in response to physiological stimuli. Decreased PMCA2 protein was found in renal cortex of rats with genetic hypertension, but mRNAs encoding isoform PMCA2 and PMCA4 were not changed [51]. No difference was found in expression of mRNA encoding PMCA1 and PMCA4 in mesangial cells of control and genetically hypertensive rats [52]. The administration of calcitriol to vitamin D-deficient chickens increased the abundance of mRNA encoding for PMCA in duodenal enterocytes; however, PMCA isoforms were not identified [43]. At the same time, it is noteworthy that expression of PMCA1 in rabbit intestinal mucosa along the intestinal tract coincides with the intensity of Ca^{2+} reabsorption [53].

In conclusion, we present evidence that PMCA2 and PMCA3 isoforms are selectively decreased, compared with PMCA1 and PMCA4 isoforms, in a state of decreased Ca^{2+} transport across renal epithelia. Regardless of whether this association is causal or coincidental, the results provide the first evidence that PMCA isoforms are selectively modulated in response to physiologic/pathophysiologic stimuli. These results invite future focused investigations to determine the location and local extent of differences in expression of PMCA2 and PMCA3, to explore the question whether PMCA2 and PMCA3 proteins are also changed, and what the impact is on Ca^{2+} transport across plasma membranes.

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