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Parathyroid hormone stimulates ATP-dependent calcium pump activity by a different mode in proximal and distal tubules of the rat

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A new technique was developed to isolate basolateral membrane vesicles individually from proximal and distal tubules of the rat cortex. This new technique enabled us to study differences in their kinetics and mechanisms of hormonal regulation of Ca pump between proximal and distal tubules. The Ca pump in distal tubule has very high affinity (42.6 nM Ca^{2+}) and the one in proximal tubule has relatively low affinity (75.6 nM Ca^{2+}). Parathyroidectomy (PTX) decreased the V_{max} of Ca pump activity in proximal tubule (4.68 ± 0.99 vs. 9.08 ± 2.21 nmol $^{45}Ca^{2+}$/min per mg protein BLMV, $P < 0.05$), while it increased K_m in distal tubule (93.1 ± 11.0 vs. 35.1 ± 16.1 nM Ca^{2+}, $P < 0.05$). Restoration of serum Ca^{2+} concentration by $1,25(OH)_2D_3$ supplement could not reverse these changes by PTX in Ca pump activity in either the proximal or the distal tubule. In conclusion, this study strongly suggested that parathyroid hormone stimulated Ca pump activity by increasing the V_{max} in proximal tubule and by increasing the affinity in distal tubule. $1,25(OH)_2D_3$ does not have a direct effect on the basolateral membrane Ca pump activity.

Introduction

The major portion of renal Ca^{2+} reabsorption is taking place in the proximal tubule and approximately 60% of the filtered Ca^{2+} is reabsorbed in this segment. The paracellular permeability of Ca^{2+} in the proximal tubules is very high and is similar to that for Na^+. Na^+ and water reabsorption through the paracellular pathway generate enough energy as a solvent drug to carry a bulk of Ca^{2+} via the paracellular pathway [1]. Although it is still controversial as to whether PTH stimulates some of the Ca^{2+} transport in the proximal tubule, it is clear that PTH-stimulated active transcellular Ca^{2+} transport is not the main mechanism in this segment [2]. On the contrary, PTH apparently stimulates Ca^{2+} reabsorption in the distal tubule [2]. Ca^{2+} reabsorption in this segment has to be carried out against an electrochemical gradient and the active transcellular transport is the dominant mechanism of Ca^{2+} reabsorption. This segment of the nephron is believed to be the main regulatory part of renal Ca^{2+} transport [3–6].

The proximal and distal tubules must be equipped with different cellular mechanisms for Ca^{2+} transport. An important molecular difference between the proximal and distal tubules is that the latter segment possesses the $1,25(OH)_2D_3$-dependent calcium binding protein (CaBPr) but the former does not [7]. Bronner [8] postulated that Ca^{2+} could diffuse in the cytosolic space in the presence of CaBPr without being trapped by calcium storage organelles, at a speed 30-times faster than in the absence of this protein. For effective transcellular Ca^{2+} transport, the Ca^{2+} pump at the basolateral membrane cannot work efficiently without a continuous supply of its substrate, Ca^{2+}. The proximal tubule lacks CaBPr, which makes transcellular Ca^{2+} movement in this segment much less effective.

Another determinant of transcellular Ca^{2+} transport is the mechanism of Ca^{2+} extrusion at the basolateral membrane. Ca^{2+} has to be pumped out against an electrochemical gradient by an active transport mecha-

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nism. Two active transporters have been identified at this side of the plasma membrane; Na^+/Ca^{2+} exchanger [9,10] and the ATP-dependent calcium pump (Ca pump) [11]. Ramachandran and Brunette [12] found that the Na^+/Ca^{2+} exchanger was located exclusively in the distal tubule of the rabbit. It has been reported in both rat and dog that PTH stimulated Na^+/Ca^{2+} exchange activity in the basolateral membrane vesicles (BLMV) of the renal cortex [9,10]. A number of studies have suggested that Na^{2+}/Ca^{2+} exchanger in renal cortical basolateral membrane operated symmetrically, as was shown in other plasma membrane Na^+/Ca^{2+} exchangers, and that it did not always work as a mechanism for Ca^{2+} exit from the cell [13–16]. The capacity of this exchanger was reported to be 20% of the V_{max} of the Ca pump in renal cortex [17]. These results strongly suggest that the Ca pump is the main mechanism of Ca^{2+} extrusion at the basolateral membrane [13].

The authors have previously reported that thyroparathyroidectomy (TPTX) did not affect the Ca pump activity of the BLMV of rat kidney cortex [18]. However, the BLMV from renal cortex does not originated only from proximal tubules; they also contain basolateral membrane from distal tubules. It is also possible that Ca pump activity and its regulation might differ between proximal and distal tubules in order to carry out their different roles in Ca^{2+} transport. The heterogenous population of basolateral membrane vesicles could thus mask changes in the transport activities in individual segments of the nephron. In order to answer these questions, we developed a new method which enabled us to isolate $(Na^+ + K^+)$-ATPase-rich plasma membrane vesicles separately from proximal and distal tubules. We then characterized Ca pump activity and its regulation by PTH in individual segments of the nephron.

Materials and Methods

Materials

Hank's solution consisted of 137 mM NaCl, 5.5 mM KCl, 0.8 mM $MgSO_4$, 0.33 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1 mM $MgCl_2$, and 10 mM Tris-HCl (pH 7.4). $^{45}CaCl_2$ was obtained from Amersham, while ATP-Mg_2, EGTA, Tris, Hepes and L-cysteine were obtained from Sigma. Percoll was purchased from Pharmacia. The other chemicals were of the highest purity available. All the solutions were filtered through the Millipore filters (0.45 μm) before use.

Animals

Male Sprague-Dawley rats, weighing 150–200 g, fed a normal rat chow were used in this study. Rats were either thyreparathyroidectomized (TPTX rats), parathyroidectomized (PTX rats), or sham-operated and were killed 72 h after surgery. PTX rats were divided into two groups: one group was given daily intraperitoneal injections containing 400 pmol of $1,25(OH)_2D_3$ dissolved in 200 μl of diethyl glycol for 2 days before being killed, in order to normalize serum calcium level (PTX/D rats) and the other group was given only 200 μl of a vehicle (PTX rats).

Dispersion of the renal cortex into the tubular segments

Under ether anesthesia, both kidneys were perfused with ice-cold Hank's solution containing 2.5 mM $CaCl_2$ (solution A) through the inferior vena cava, using a peristaltic pump, until the kidneys became completely pale in color. The kidneys were then further perfused with an additional 30 ml of Hank's solution containing 10 mM $CaCl_2$, 1 mg/ml BSA, and 1 mg/ml collagenase (solution B). Cortices, dissected with scissors from collagenase-treated kidneys, were minced with a razor blade, incubated at 37°C for 30 min in 10 ml of solution B, and gassed with 100% O_2. The minced kidney was washed three times with 20 ml of ice-cold solution A with phenanthroline (solution C) to stop proteolysis. Dispersion of the collagenase-treated cortex into tubular segments was carried out as follows: Minced cortex was placed in a disposable conical tube containing 20 ml of solution A and was vortexed for 15 s. The supernatant was collected into another conical tube on ice. This procedure was repeated several times. The collected supernatant was filtered twice through mesh and was resuspended in 50 ml of solution A. This preparation was the 'pool fraction'. The pool fraction collected from the preparations of three rats was used for further purification.

Discontinuous ficoll gradient

Partial purification of proximal and distal tubules was performed according to the method by Scholer and Edelman [17]. Ficoll was freshly dissolved in solution A at concentrations of 1% (50 ml), 2% (35 ml), 6% (60 ml), 8% (60 ml) and 12% (20 ml) (wt/wt, pH 7.4). A discontinuous gradient consisting of these five layers was generated with a peristaltic pump in a 300 ml beaker. Fifty ml of the pool fraction was carefully loaded on the gradient. After about 15 min when the particle front reached the 8%/12% interphase, two fractions were collected with a 10-ml pipette; one from right above the 8%/12% interphase (fraction A) and the other from right below the 1%/2% interphase (fraction B). All procedures were performed on ice unless otherwise noted.

Hormone-sensitive adenylate cyclase assay

In order to identify the origin of the separated tubular fragments, hormone-sensitive adenylate cyclase activity was assayed by measuring the production of cyclic AMP, using a radioimmunoassay kit (Cyclic AMP

Kit, Yamasa Inc., Tokyo, Japan). 10 μl (protein: 5–10 μg) of fraction A or fraction B was incubated for 15 min at 37°C (total volume, 100 μl) in substrate containing 0.2 mM Tris-ATP, 25 mM phosphocreatinine, 1 mg/ml creatine phosphokinase, 9 mM theophylline, 0.1% (w/v) BSA, 25 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA-Na_2, and 50 mM Tris-HCl (pH 7.4). 10 μl of solution containing bovine [1–34]PTH or vasopressin was also added, where specified in the results. The reaction was stopped by boiling for 3 min, followed by the addition of 100 μl of succinylating reagent. The supernatant after centrifugation (15000 rpm for 15 min.) was used for cyclic AMP assay.

Purification of BLMV from fractions A and B

The purification of BLMV from Fractions A and B was performed by the 'Percoll gradient method' as previously reported for purification from the renal cortex [11,18]. Fractions A and B were centrifuged at 3000 rpm for 15 min. Pellets were resuspended in ice-cold sucrose buffer (0.25 M sucrose, 0.1 mM phenylmethylsulfonyl fluoride, 5 mM Tris-HCl (pH 7.5)) and were homogenized using a Potter homogenizer. Identification of the basolateral membrane-rich fraction in the Percoll gradient was performed by the assay of marker enzyme activities such as those of ouabain-sensitive $(Na^+ + K^+)$-ATPase, maltase, NADH oxidase, and cytochrome-c oxidase as previously described [18].

ATP-dependent Ca pump activity

The activity of the ATP-dependent Ca pump was determined by a method reported previously, which measured $^{45}Ca^{2+}$ uptake by freshly prepared BLMV in the presence of MgATP (1.6 mmol/l) [11,16]. The BLMV samples were preloaded with mannitol (100 mmol/l), KCl (100 mmol/l), and Hepes-Tris (5 mmol/l) solution with a pH adjusted to 7.4. $^{45}Ca^{2+}$ uptake was initiated by the addition of 10 μl of BLMV suspension (6–10 g/l) to 290 μl of assay solution (pH 7.4, 37°C) which contained the following: 100 mmol/l mannitol, 100 mmol/l KCl, 5 mmol/l Hepes-Tris, 0.1 mmol/l EGTA, and $^{45}CaCl_2$ (1 μCi/tube) with either ATPMg (2 mmol/l) or $MgCl_2$ (2 mmol/l). The concentrations of $CaCl_2$ were varied in order to determine both V_{max} and K_m for Ca pump activity at different concentrations of free Ca^{2+} (10^{-8} mol/l, $2 \cdot 10^{-8}$ mol/l, $5 \cdot 10^{-8}$ mol/l and 10^{-7} mol/l). Differences in radioactivity in the absence and the presence of MgATP were expressed as Ca^{2+} pump activity. The uptake of $^{45}Ca^{2+}$ was stopped by dilution with 2 ml ice-cold stop buffer (pH 7.4) that contained the following: 1 mmol/l EGTA, 100 mmol/l mannitol, 100 mmol/l KCl, and 5 mmol/l Hepes-Tris. The samples were then rapidly filtered through 0.65-μm filters (Millipore; Millipore Corp., Bedford, MA), and the filters were rinsed with an additional total of 6 ml of the cold stop solution. Radioactive $^{45}Ca^{2+}$ trapped in the vesicles was determined with a liquid scintillation counter. All experimental points were repeated at least in triplicate.

Analytical technique

Total calcium and phosphorus in serum and urine were measured by Autoanalyzer (Hitachi Industry Co., Tokyo, Japan). Ionized calcium was measured by Autoanalyzer SERA 252 (Horiba Co., Tokyo, Japan). Metabolic cages (Nalge Co., Rochester, NY) were used for the collection of urine. Since three rats were used for one experiment of Ca pump activity assay in both proximal and distal tubules, the mean value of three rats was used for the analysis of laboratory data from one experiment.

Statistical analysis

Data are expressed as the mean ± S.D. Statistical analysis of the data was performed using Anova analysis of variance, with the significance of multiple pairwise comparisons calculated by using a computer system (StatView SE +, Abacus Concept, Inc., CA, USA). A regression line was drawn by the same computer program. A probability level of less than 0.05 was considered significant.

Results

Separation of cortical tubular fragments into fraction-rich in proximal or distal tubules

Fraction A, which migrated into the 8%/12% interphase of the Ficoll gradient, showed not only the morphological characteristics but also the chemical characteristics of the proximal tubule. If more than 80% of tubules possessed brush-borders by the inspection under refractory microscopy, fraction A was designated as the proximal tubule-rich fractions. Fraction B, which migrated into 1%/2% interphase, should not contain any apparent proximal tubules under the microscopy. This fraction usually consisted of individual tubular cells which did not possessed brush-borders. Fig. 1 shows the PAS staining of these two fractions. Table I demonstrates the hormone-sensitive adenylate cyclase activity of these fractions. Fraction A was rich in parathyroid hormone-sensitive adenylate cyclase activity, but showed very small vasopressin-sensitive adenylate cyclase activity. In contrast, fraction B was rich in both hormone-sensitive enzyme activities. Since proximal tubules are vasopressin resistant, proximal tubule-rich fraction (fraction A), which contained 111% of vasopressin stimulation, could have as much as 50% of the distal tubule representation as is present in the fraction B. This result showed less purity of proximal-tubule-rich fraction than the previous report [17].

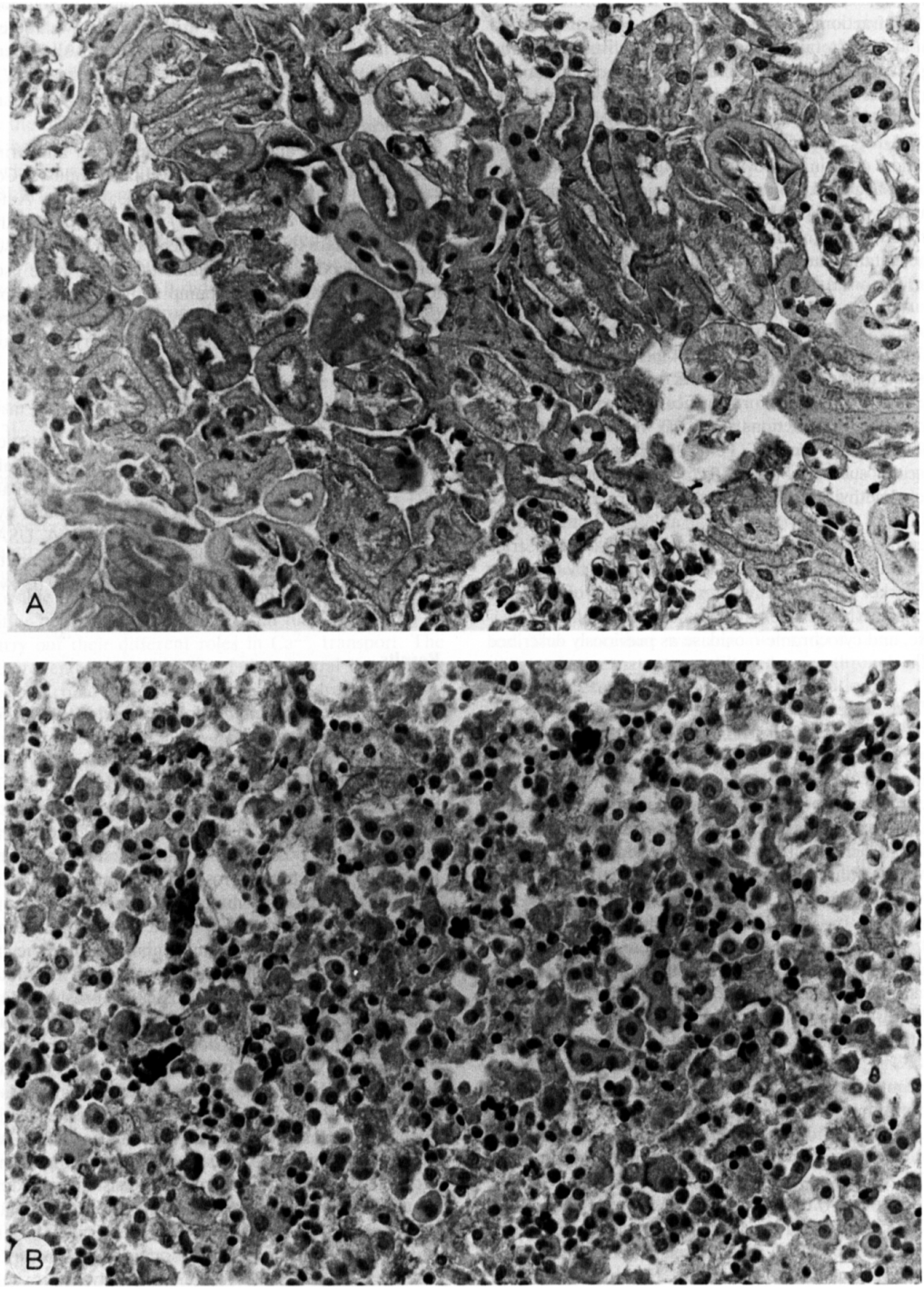

Fig. 1. Morphological characterization of fraction A (A) and B (B) from collagenase treated renal cortex (PAS X500).

TABLE I

Basal and hormone-sensitive adenylate cyclase activity in cortical tubular fractions

Fraction A was collected from the layer right above 8%/12% interphase of the Ficoll gradient and fraction B was collected from the layer right below 1%/2% interphase. Results are expressed as means ± S.D. for three experiments.

	Fraction A	Fraction B
Basal activity (pmol/15 min per mg)	288 ± 26	290 ± 32
Percent change (%)		
Vasopressin 50 mU/ml	111 ± 9	230 ± 67
PTH 10^{-6} M	429 ± 67	225 ± 67

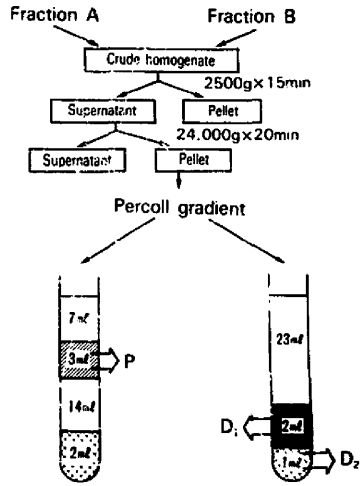

Fig. 2. Isolation of the plasma membrane-rich in $Na^+ + K^+$-ATPase activity in the Fraction A and B.

Purification of the basolateral membrane vesicles (BLMV) from fractions A and B

Following serial centrifugations of the homogenates from fractions A and B, Percoll gradient methods led to the final separation of plasma membrane rich in $(Na^+ + K^+)$-ATPase activity (Fig. 2). After centrifugation, the Percoll gradient showed a pattern of resolution in fraction A which was very similar to that in the cortical homogenate. A fluffy membrane layer (fraction P) was collected. Fraction B gave a different resolution pattern. After centrifugation, two fluffy layers (fractions D1 and D2) were formed in the bottom of the tube. As shown in Table II, the activity of ouabain-sensitive $(Na^+ + K^+)$-ATPase, the specific marker enzyme of the basolateral membrane, was enriched 930% in fraction P and no enrichment was shown for cytochrome-c oxidase, the mitochondrial marker enzyme activity, and NADH oxidase, marker enzyme activity of the endoplasmic reticulum. Maltase, marker enzyme of the brush border membrane, was enriched 204% in fraction P and this enrichment was higher than the basolateral membrane preparation from cortical homogenate. In the distal tubule-rich fraction, D1 had a higher enrichment of $(Na^+ + K^+)$-ATPase activity and a lower enrichment of both cytochrome-c-oxidase and NADH-oxidase activities than D2. No specific marker enzyme was available for luminal membrane of the distal tubule. Ca pump activity was also detected in these fractions and the distribution of activity was identical to that of $(Na^+ + K^+)$ATPase. These results indicated that both Ca pump and $(Na^+ + K^+)$-ATPase activities were located in the same fraction of the plasma membrane. Finally, fractions P and D1 were selected for experimental use as the basolateral membrane from the proximal tubule and the distal tubule, respectively.

TABLE II

Specific activities and enrichment factors for marker enzymes in basolateral membrane preparation from two different cortical tubular fractions

Ca^{2+} pump activity was measured at 10^{-7} M Ca^{2+} (nmol $^{45}Ca^{2+}$/2 min per mg protein) and other enzyme specific activities are reported as nmol/min per mg protein. The values in parenthesis represent the total units of activity in the fraction relative to the total activity for that enzyme in the homogenates of cortical tubular fractions and are reported as the percentage. Results are expressed as means ± S.D. for three experiments. * Significantly lower than the activity of either P or D1 ($P < 0.05$)

	P	D1	D2
Ca^{2+} pump	5.03 ± 0.64	2.81 ± 0.62	1.60 ± 0.28 *
$(Na^+ + K^+)$-ATPase	771 ± 151 (930)	525 ± 41 (680)	333 ± 47 * (430)
Cytochrome-c oxidase	33.6 ± 8.9 (10)	142 ± 24.1 (48)	310 ± 39 (107)
NADH oxidase	432 ± 124 (88)	632 ± 196 (113)	1223 ± 207 (219)
Maltase	86.1 ± 4.4 (204)		

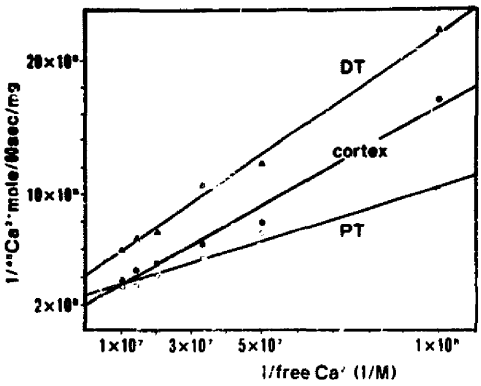

Fig. 3. Difference in kinetics of Ca pump activities in three preparations of basolateral membrane vesicles. Abbreviations are: PT, proximal tubular basolateral membrane vesicles; DT, distal tubular basolateral membrane vesicles; cortex, cortical basolateral membrane vesicles.

Kinetic difference of Ca pump activity between proximal and distal tubule

The separation of basolateral membrane into two different segments of the cortex demonstrated that Ca pump activity possessed a different kinetics between two segments of the nephron (Fig. 3). The Ca pump in the distal tubule had higher affinity for Ca^{2+} than the one in the proximal tubule. K_m of Ca^{2+} was 42.6 nM in the distal tubule and 75.6 nM in the proximal tubule. Kinetic analysis, also performed in the cortical basolateral membrane which had been treated with collagenase, revealed K_m to be 31.5 nM. This value was almost the same as the value obtained from non-collagenase treated cortex (26.2 ± 5.6 nM mean ± S.E., $n = 3$). The V_{max} of Ca pump activity in the collagenase-treated cortex was 3.12 nmol $^{45}Ca^{2+}$/min per mg; this value also was identical to that in non-collagenase treated cortex (2.46 ± 0.12 nmol $^{45}Ca^{2+}$/min per mg, mean ± S.E., $n = 3$). This result indicated that collagenase treatment did not affect Ca pump activity in this study.

Effect of parathyroidectomy

Both PTX and TPTX induced hypocalcemia, hyperphosphatemia and an increase in TRP (Table III). The magnitude of these changes did not differ in these two groups of rats. In a preliminary experiment, varying intraperitoneal doses of $1,25(OH)_2D_3$ were examined and 400 pmol for 2 days was revealed to be a sufficient dose to restore serum calcium level in the PTX rats. Restoration of serum calcium level by $1.25(OH)_2D_3$ supplement increased fractional excretion of Ca^{2+} and normalized total reabsorption of phosphorus in the PTX rats (Table III).

Different effects of PTX on Ca pump activity in proximal and distal tubules were observed. In the proximal tubule, both PTX and TPTX decreased V_{max} of the Ca pump without affecting K_m. In contrast, in the distal tubule, K_m was increased by both PTX and TPTX without a change of V_{max}. Restoration of serum calcium by $1,25(OH)_2D_3$ supplement could not reverse the effect of PTX either in proximal or in distal tubules (Table III).

Discussion

Basolateral membrane vesicle preparations from the renal cortex have been used for many studies which attempted to clarify the mechanisms of membrane transport. In contrast to the brush border membrane, basolateral membrane preparations from the cortex originate from heterogenous segments of the cortical nephron. In fact, Jayakumar et al. demonstrated that a cortical basolateral membrane preparation possessed

TABLE III

Summary of the data in four groups of rats

V_{max} is expressed as nmol $^{45}Ca^{2+}$/min per mg protein and K_m as nM Ca^{2+}. * $P < 0.05$ compared with the value of sham-operated rats. ** $P < 0.05$ compared with the value of PTX rats. Since PTX or TPTX gave much more damage to rats than sham-operation, the condition of operated rats varied at the time of sacrifice. This factor might cause large deviation of the data specially for K_m calculation

	Ca^{2+} ion (mequiv./l)	Serum P (mmol/l)	FECa (%)	TRP (%)
Sham ($n = 8$)	2.72 ± 0.20	2.59 ± 0.28	0.52 ± 0.30	81.8 ± 6.21
PTX ($n = 6$)	1.60 ± 0.57 *	3.84 ± 0.26 *	1.36 ± 1.28	89.5 ± 3.01 *
TPTX ($n = 5$)	1.45 ± 0.54 *	3.69 ± 0.12 *	1.77 ± 1.74	89.3 ± 2.88 *
PTX/D ($n = 6$)	2.52 ± 0.30 **	3.01 ± 0.25 **	4.75 ± 2.85 **	81.6 ± 7.4 **
	V_{max} (PT)	K_m (PT)	V_{max} (DT)	K_m (DT)
Sham ($n = 8$)	9.08 ± 2.21	81.6 ± 2.87	3.18 ± 1.25	35.1 ± 16.1
PTX ($n = 6$)	4.68 ± 0.99 *	85.1 ± 34.9	3.22 ± 1.22	93.1 ± 11.0 *
TPTX ($n = 5$)	4.76 ± 1.37 *	107 ± 71.1	3.01 ± 1.63	103 ± 39.8 *
PTX/D ($n = 6$)	5.59 ± 2.65 *	106 ± 65.8	3.49 ± 0.96	98.3 ± 20.8 *

not only PTH-stimulated, but also vasopressin-stimulated adenylate cyclase activity [9]. The latter activity is located exclusively in the distal tubule of the rat cortex and is not located in the proximal tubule [21]. This heterogeneity in tubular origin has led difficulties in interpreting the data from Ca transport studies which had been used cortical basolateral membrane vesicles, because the mechanism of Ca transport in proximal and distal tubules is known to differ. A technique which could isolate basolateral membranes from individual nephron segments has been desired for a long time. The present study succeeded in finding and describing a technique which could isolate basolateral membrane vesicles separately from proximal and distal tubules. For this purpose, the technique required the collection of a large amount of homogenous tubules in a short time. Such a technique had already been developed by Scholer et al. in 1979 [19]. After the Ficoll gradient procedure, we also succeeded in separating two cortical tubular fractions which showed the characteristic features of proximal and distal tubules, respectively. Although the proximal tubule-rich fraction also contained some vasopressin-stimulated adenylate cyclase activity, further purification of these fractions into basolateral membranes dissolved this problem. Basolateral membrane-rich fractions showed different densities in the Percoll gradient between proximal and distal tubule-rich fractions. Basolateral membrane preparation (fraction P) from proximal tubules was apparently lighter than the one (fraction D1) from distal nephrons. Conclusively, final membrane preparations should have been much more heterogeneous between two segments of the nephron than the crude tubule preparations.

The relative enrichment of both $(Na^+ + K^+)$-ATPase activity and the Ca pump activity was almost equal in fractions P and D1. Since $(Na^+ + K^+)$-ATPase and Ca pump have been located in the basolateral membrane in both proximal and distal tubules [22], the equal enrichment of these two enzyme activities indicated that fractions P and D1 were enriched in basolateral membranes from individual tubules. No enrichment of cytochrome-*c*-oxidase and NADH-oxidase activities indicated only little contamination of mitochondria and endoplasmic reticulum in fractions P and D1. 204% of maltase enrichment in fraction P indicated some contamination of brush border membrane but this enrichment was much less than the one of basolateral membrane. Contamination of luminal membrane in the fraction D1 or D2 could not be assessed because no specific marker enzyme for this purpose was known in the distal cortical nephron.

This new technique made it possible for us to study the difference in Ca pump activity between proximal and distal tubule. We found that Ca pump in the distal tubule had much higher affinity for Ca^{2+} than that in the proximal tubule. The K_m value was 42.6 nM in the distal and 75.6 nM in the proximal tubule. Since cytosolic Ca^{2+} concentration is reported to be higher than 100 nM [15,16], the Ca pump in distal tubules is considered to be a very sensitive system with respect to cytosolic Ca^{2+} level. The high affinity of the Ca pump is suitable for transcellular Ca transport in the distal tubule. In the rat, the distal tubule is the main regulatory segment for renal Ca reabsorption [2]. In this segment, lumen Ca^{2+} is reabsorbed mainly through the transcellular pathway. This segment is equipped with CaBPr which facilitates Ca^{2+} diffusion in the cytosolic space [8]. The basolateral membrane must be equipped with a powerful machine to pump this bulk of Ca^{2+} carried by CaBPr out of the cell. Very high affinity Ca pumps can accomplish this task.

In contrast to the Ca pump in distal tubules, the affinity of the Ca pump in proximal tubules is not very high and this value agrees well with the cytosolic Ca^{2+} level. In the proximal tubule, the major mechanism of Ca^{2+} reabsorption is believed to be that of paracellular diffusion. The absence of CaBPr means that this nephron is less capable of handling transcellular Ca^{2+} reabsorption. The mechanism of Ca exit from the basolateral membrane should be suitable for maintaining cytosolic Ca^{2+} at an adequate level but not for translocating large amounts of Ca^{2+}. This study demonstrated that Ca pump activity in the proximal tubule is, indeed, of such a house-keeping type.

These results for Ca pump activity in individual segments of the nephron do not agree with results for $(Ca^{2+} + Mg^{2+})$-ATPase activity of the mouse kidney. Brunette et al. [23] measured Ca^{2+}-dependent ATP hydrolysis in dissected proximal and distal tubules in the presence of 1 μM or 100 μM Mg^{2+}. They found that K_m was 100 nM Ca^{2+} in proximal and 180 nM Ca^{2+} in distal tubules in the presence of 1 μM Mg^{2+} and there was virtually no activity in the presence of 100 μM Mg^{2+}. The authors have already reported that high-affinity Ca^{2+}-dependent ATPase activity was inhibited competitively by Mg^{2+} in the basolateral membrane of rat renal cortex [20]. This does not necessarily mean that this Ca^{2+}-dependent ATPase activity is a different expression from Ca pump activity; it just means that K_m between ATP hydrolysis and Ca^{2+}-translocating activity cannot be compared simply, because an ATP hydrolysis activity only expresses the phosphorylation step of Ca pumping ATPase. Measuring Ca^{2+}-ATPase activity in a whole tubule also risks contamination by endoplasmic reticulum and mitochondrial Ca^{2+}-ATPase activities.

The question arises whether Ca pump activities are the expression of different Ca^{2+}-ATPases in the proximal and distal tubules. Difference in the affinity could be explained by difference in membrane properties. Borke et al. [7] have provided important information to

answer this question. They reported that monoclonal antibody against the erythrocyte Ca pump bound only to the distal tubules of the rat. This result strongly suggested that the Ca pump in proximal tubules does not have the same epitope as the one in distal tubules. It is known that a wide variety of isoform exists among Ca pumps in various organs [24]. Complete purification and sequence of renal membrane Ca pumps is required to answer this question.

This study clearly demonstrated, for the first time, that PTH stimulated Ca pump activity in a different fashion in proximal and distal tubules. In our previous study, TPTX did not cause any change in Ca pump activity in cortical basolateral membrane vesicles [18]. However, separation of basolateral membranes into those of proximal and distal tubular origin in this study led to results which differed from those of the previous study. Both TPTX and PTX caused a decrease in V_{max} of Ca pump activity in proximal tubules and an increase in K_m in distal tubules. It is apparent in the previous study that the heterogenous cortical basolateral membrane preparation masked the changes in Ca pump activity which were heterogenous in the two parts of the nephron.

Itoh et al. reported that cyclic AMP and PTH directly stimulated '$(Ca^{2+} + Mg^{2+})$-ATPase' activity in the 'proximal basolateral membrane' of the canine kidney [25]. Their study suggested that PTH increased the affinity of ATPase via phosphorylation of a 90 kDa protein, however, two major problems exist in this study. One is that the membrane preparation used was not the proximal but the cortical basolateral membrane. The second is that the assay of ATPase activity did not include any additional Mg^{2+} in the assay mixture and thus expressed high-affinity Ca^{2+}-dependent ATPase activity, which had already been characterized in our previous study [20]. Changing these misconceptions would correct the interpretation of this study as the stimulation of high-affinity Ca^{2+}-dependent ATPase activity by PTH in cortical basolateral membranes. Stimulation of affinity in the cortical basolateral membrane by PTH might reflect stimulation in distal tubules.

Comparing kinetic parameters of cortical and individual tubular basolateral membrane vesicles leads to the estimation of the relative contribution of tubular factors to the cortical membrane preparation. One factor is that the K_m of Ca pump activity in the cortex is much closer to that in distal tubules than to that in proximal tubules. The V_{max} of Ca pump activity was not changed by TPTX either in cortical or in distal basolateral membrane vesicles, but was changed in proximal basolateral membrane vesicles. Two phenomena suggested that the cortical basolateral membrane contains more distal than proximal Ca pump activity. If so, the PTH stimulation of Ca^{2+}-ATPase affinity in Itoh's study can be interpreted as stimulation in distal tubules. Our previous study also demonstrated that secondary hyperparathyroidism because of vitamin D deficiency inhibited the V_{max} of Ca pump activity in cortical basolateral membrane [18]. This result may also be interpreted as the inhibition of Ca pump activity operating in the distal tubule. Thus it is suggested that PTH has dual effects on Ca pump activity depending on its concentration. A physiological concentration of PTH stimulated and a higher concentration inhibited Ca pump activity. Another interesting finding is that restoration of serum Ca in PTX rats by $1,25(OH)_2D_3$ supplement did not influence Ca pump activity in the present study. Our previous study, using vitamin D deficient rats, also failed to show direct effects of $1,25(OH)_2D_3$ [18]. These studies, using different approaches, confirmed that $1,25(OH)_2D_3$ did not have a direct effect on basolateral membrane Ca pump activity either in the proximal or in the distal tubule of the rat.

PTH stimulates Na^+/Ca^{2+} exchange in the cortical basolateral membrane of the rat [9] and in the canine kidney [10]. Since Na^+/Ca^{2+} exchange activity was found exclusively in the distal tubule [12], it is strongly suggested that Na^+/Ca^{2+} exchange is stimulated in the distal tubule. PTH also facilitates the peritubular entry of Ca^{2+} via the cAMP-mediated mechanism in the rabbit cortical connecting tubule [26] and cortical collecting tubule [27]. In these segments, it is most likely that the Ca pump plays a primary role in Ca^{2+} extrusion and that Na^+/Ca^{2+} exchange could be the mechanism of Ca^{2+} entry [16,27].

All of this evidence leads us to propose the following cellular mechanism of renal Ca^{2+} transport as

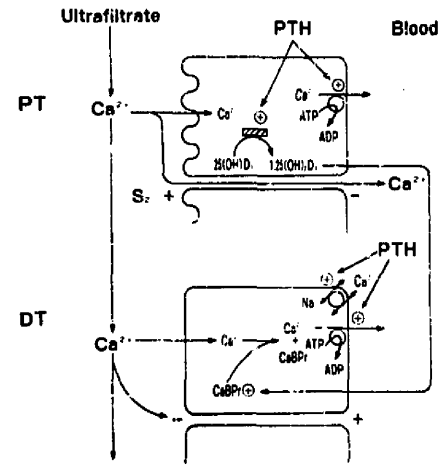

Fig. 4. Schematic presentation of the cellular mechanism of Ca^{2+} transport in the rat kidney. Abbreviations are: PT, proximal tubular cell; DT, distal tubular cell.

illustrated in Fig. 4: PTH increases peritubular Ca^{2+} entry and Ca^{2+} exit through the Ca pump in the distal tubule. In the distal tubule, CaBPr facilitates cytosolic Ca^{2+} diffusion and provides a powerful force which is essential for efficient transcellular Ca^{2+} reabsorption. PTH also stimulates Na^+/Ca^{2+} exchange in this segment. However, this system works symmetrically and performs the fine tuning of cytosolic Ca^{2+} rather than having a primary role in Ca^{2+} exit. The proximal tubule is equipped only with a Ca pump and lacks important systems for transcellular Ca^{2+} transport. PTH also stimulates this Ca pump; however, the Ca pump acts as a house-keeper in this segment. $1,25(OH)_2D_3$ stimulates the production of CaBPr but does not affect the Ca pump directly.

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