Sodium Gradient-dependent Calcium Uptake in Renal Basolateral Membrane Vesicles

EFFECT OF PARATHYROID HORMONE*

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The Na⁺/Ca²⁺ exchange system in rat renal cortex basolateral membrane vesicles was studied. Uptake and efflux of Ca²⁺ in the membrane vesicles were stimulated by trans-Na⁺. The enhancement of Ca²⁺ uptake by the intravesicular > extravesicular Na⁺ gradient was inhibited by ionophores that dissipated the gradient, and was increased by an outside negative membrane potential. Na⁺-dependent Ca²⁺ uptake was saturable with respect to both Ca²⁺ and Na⁺. A [Ca²⁺]o of 8 μM was calculated. The relationship between Na⁺ concentration and rate of Ca²⁺ efflux was sigmoidal; a [Na⁺]o of 15 mM and a Hill coefficient of 2.5 were estimated. Removal of parathyroid glands from the rats resulted in a 40% decrease in the Na⁺-dependent Ca²⁺ uptake. Infusion of parathyroid hormone (the synthetic tetratriacontapeptide) into these animals fully restored the activity. The isolated basolateral membrane possessed parathyroid hormone-sensitive adenylate cyclase. These findings may suggest a mechanism by which parathyroid hormone regulates the reabsorption of Ca²⁺ in the kidney.

In many tissues, a Na⁺/Ca²⁺ exchange system plays a key role in the extrusion of cellular Ca²⁺ and the regulation of the cytosolic Ca²⁺ concentration (1). The carrier mechanism has been studied most extensively in excitable tissues, such as nerve, heart, and skeletal muscle (2). Little is known about the exchange system in nonexcitable tissues. In the kidney, support for the presence of a Na⁺/Ca²⁺ exchange comes from findings that in microperfused tubules removal of Na⁺ from, or additions of ouabain to, the peritubular fluid inhibits active Ca²⁺ transport (3), and alterations in the magnitude of the electrochemical gradient for Na⁺ across the peritubular cell membrane affect the intracellular level of Ca²⁺ (4).

Normally, mammalian kidneys reabsorb about 98% of the filtered Ca²⁺. Classical physiological studies indicate that more than half of the Ca²⁺ is reabsorbed in the proximal convoluted tubule, but important fractions of the total filtered load are taken up in the pars recta, thick ascending limb of Henle, distal convoluted tubule, and collecting duct segments of the nephron (5–7). The mechanisms by which Ca²⁺ is reabsorbed in the nephron has not been fully elucidated, but evidence suggests that reabsorption in the proximal tubule is mainly passive and secondary to Na⁺ reabsorption, although there is an active component, which is also Na⁺-dependent (5). On the other hand, modulation of the Ca²⁺ excreted in the urine appears to occur primarily in the final nephron segments (5).

Ca²⁺ reabsorption in the kidney, a process that contributes importantly to Ca²⁺ homeostasis in the whole animal, is regulated by various hormones, including parathyroid hormone, calcitonin, 1,25-dihydroxycholecalciferol, and adrenal corticosteroids. How these hormones affect the renal handling of Ca²⁺ is poorly understood, in part attributable to the paucity of information about the systems by which Ca²⁺ is removed from the glomerular filtrate and transferred transepithelially across the renal tubule. It is generally assumed, because renal cytosolic free Ca²⁺ concentrations are in the submicromolar range (8) whereas plasma Ca²⁺ is about 2.5 mM and the membrane potential is cell interior negative, that Ca²⁺ enters the tubular cell across the luminal (microvillar) segment of the plasma membrane by a diffusional mechanism, but that the cation has to be transported actively out of the cell across the basolateral segment of the plasma membrane. There is evidence for at least two different transport systems for Ca²⁺ in the basolateral membrane. One, is suggested by the presence in the membrane of a high affinity Ca²⁺-ATPase, which by analogy with the function of this enzyme in other tissues, serves as a Ca²⁺ pump (9–12). The other is the secondary active Na⁺/Ca²⁺ exchange. Direct studies on the exchange system in basolateral membranes have not been studied, with the exception that it has been noted that Na⁺ inhibits ATP-driven Ca²⁺ accumulation by plasma membrane vesicles (9, 13) and Na⁺ enhances the loss of Ca²⁺ from basolateral membrane vesicles preloaded with the divalent cation (13).

In the present paper, characteristics of the Na⁺ gradient-dependent uptake and efflux of Ca²⁺ by kidney cortex basolateral membrane vesicles are reported. Evidence is presented suggesting that renal Na⁺/Ca²⁺ exchange activity may be regulated by parathyroid hormone. A portion of this work has appeared in abstract form (14).

EXPERIMENTAL PROCEDURES

Preparation of Membrane Vesicles—The renal cortices from two male Sprague-Dawley rats (250–350 g) were used for each preparation. Basolateral membrane vesicles were isolated by the Percoll gradient procedure, described previously in detail (15). In experiments in which the intravesicular medium was varied, the membrane vesicles were prelabeled by dialuting Fraction II (the fraction enriched in basolateral membranes) and carrying out the entire washing procedure in the described medium. The efficacy of the preloading technique to vary the intravesicular medium, thereby to establish gradients of Na⁺, K⁺, choline⁺, and other ions across the membrane, was ascertained previously (15). The quality of the membrane preparations, evaluated by specific activities and enrichments of enzyme markers, was essentially the same as reported earlier (15). The specific activity of (Na⁺ + K⁺)⋯

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Renal Na⁺/Ca²⁺ Exchange and Effects of Parathyroid Hormone

ATPase ranged from 0.52 to 0.57 μmol/min·mg⁻¹ protein, representing 10- to 12-fold enrichments relative to the cortex homogenate. The enrichments of malate and cytochrome c oxidase, enzyme markers for the brush-border membrane and mitochondrion, respectively, were 0.9 and 0.2, respectively. NADPH-cytochrome c reductase, a marker enzyme for the endoplasmic reticulum, was not determined with different membrane preparations; however, other studies in which the basolateral membranes were prepared by an identical method as used here reported that the enzyme was significantly de-enriched (16, 17). The specific activities and enrichment factors of (Na⁺ + K⁺)-ATPase, malate, and cytochrome c oxidase from sham-operated and TPTX rats did not differ. Adenylate cyclase activity was measured as described previously (18, 19).

Transport Measurements—Freshly prepared basolateral membrane vesicles were used. All incubations were carried out at least in triplicate. Each experiment was repeated a minimum of three times with different membrane preparations.

Uptake and efflux of [Ca²⁺] were measured by a Millipore filtration technique (20, 21), using 0.65-μm filters. The filters were presoaked in the buffered mannitol medium for several hours prior to use in order to minimize background (zero time) radioactivity. To determine Ca²⁺ uptake into membrane vesicles preloaded to 60 μg of protein with 100 mM mannitol and 100 mM K⁺, Na⁺, or Tris⁺, as Cl⁻ or SCN⁻ salts, adjusted to pH 7.5 with 5 mM Hepes/Tris, was preincubated for 1 min at 20 °C. Incubation at 20 °C was initiated by the addition of 95 μl of an isotonic (300 mosm) medium containing 100 mM mannitol and 100 mM K⁺, Na⁺, or Tris⁺, as Cl⁻ or SCN⁻ salts, adjusted to pH 7.5 with 5 mM Hepes/Tris buffer, and 0.1 mM free Ca²⁺ (labeled with 1 μCi of [Ca⁴⁺]), buffered with 1 mM nitritotriacetic acid. The free Ca²⁺ concentration was determined by a Ca⁺⁺ electrode. Incubations, 5 s unless noted otherwise, were terminated by the addition of 3 ml of an ice-cold stopping solution containing 1 mM EGTA, 100 mM mannitol, and 100 mM KCl, adjusted to pH 7.5. The membrane vesicles on the Millipore filter were rinsed with an additional total of 12 ml of the cold stopping solution. The quenching procedure was completed in about 2 s. The radioactive Ca²⁺ remaining in the vesicles was determined with a liquid scintillation counter. Na⁺-dependent Ca²⁺ uptake was calculated by subtracting the uptake with membrane vesicles containing intravesicular K⁺ from the uptake obtained with vesicles preloaded with a Na⁺-containing medium. With these conditions of incubation, Ca²⁺ uptake was linear with respect to membrane protein concentration.

To determine Ca²⁺ efflux, 2 μl of a suspension of membrane vesicles (40 to 60 μg of protein), preloaded with 150 mM NaCl in 5 mM Hepes/Tris buffer, pH 7.5, was preincubated with 38 μl of a Ca²⁺ uptake medium containing 150 mM KCl, 5 mM Hepes/Tris buffer, pH 7.5, and 100 μM [Ca²⁺]. After 10 min of uptake, the membrane suspension was diluted 4-fold with an efflux medium containing, as final concentration, 100 mM NaCl and 1 mM EGTA or 50 mM CaCl₂, 100 mM NaCl, and 1 mM EGTA. Efflux was terminated by the addition of ice-cold stopping solution and the Ca²⁺ remaining in the vesicles measured by Millipore filtration.

Thyroparathyroidectomy and Infusion of Animals—Rats were anesthetized with ether and surgically TPTX or sham-operated. Completeness of thyroparathyroidectomy was verified by the decrease in serum calcium concentration, from 2.05 ± 0.04 mM before to 1.55 ± 0.05 mM 48 to 72 h after surgery. No significant differences were found in specific activity and the enrichment factor for (Na⁺ + K⁺)-ATPase in membrane vesicles from sham and TPTX animals. Animals infused with TPTX rats were infused with medium ± parathyroid hormone. In these experiments, the animals 48-72 h after surgery were anesthetized with Inactin (200 mg/kg body weight) and placed on an electrically heated pad to maintain body temperature at 36.5-38.0 °C. Temperature was monitored rectally with a thermistor probe. Tracheotomy was performed with the insertion of a polyethylene 200 tube and the rat was allowed to breathe spontaneously. The right jugular vein was cannulated with a polyethylene 50 tube for infusion. A second tube was inserted into the right carotid artery for collection of blood. The infusion medium contained 50 mM NaCl, 2 mM KCl, 1.6 mM CaCl₂, 0.7 mM MgCl₂, and 1 mM Hepes/Tris, pH 7.4, with or without 48 units/ml of parathyroid hormone. The animal was given a primary dose (0.13 ml/min) for 1 min and then infused at a constant rate (4.4 μl/min) for 3 h. At the end of the infusion period, the animals were killed and the kidneys were rapidly removed.

Materials—[⁴⁰Ca] (38 Ci/g) was obtained from New England Nuclear. Valinomycin was purchased from Sigma Chemical Co. The ionophores A23187, nigericin, and monensin were donated by Eli Lilly Laboratories. Parathyroid hormone (the synthetic tetracontapeptide, 901 units/mg) was from Beckman. Other chemicals were of highest purity available from commercial sources. All solutions were filtered through 0.45-μm Millipore filters prior to use (22).

**RESULTS**

Na⁺-dependent Uptake of Ca²⁺—Fig. 1 shows the time course of uptake of 100 μM Ca²⁺ by renal cortex basolateral membrane vesicles, preloaded with either K⁺ or Na⁺. When the intravesicular medium contained 100 mM K⁺, 0.64 ± 0.05 nmol of Ca²⁺/mg of protein was taken up in 5 s. Uptake at 10 s was less than twice the uptake at 5 s. Thus, Ca²⁺ accumulation was not linear -th time, at least during these time periods. When Na⁺ replaced K⁺ in the intravesicular medium, thereby imposing a Na⁺ gradient (intravesicular [Na⁺] > extravesicular [Na⁺]) on the vesicle system, uptake of Ca²⁺ was enhanced. The uptake of 5 s was 1.03 ± 0.03 nmol/mg of protein, approximately 60% greater than that found with K⁺ in the intravesicular medium. Accumulation of Ca²⁺ was higher with intravesicular Na⁺ than with K⁺ throughout most of the time course of the incubation. However, the relative enhancement decreased with time, and at 1 h, when steady state uptakes were approached, the difference between the Ca²⁺ accumulated in vesicles containing Na⁺ and those containing K⁺, was not significantly different. Fig. 1 also illustrates the Na⁺-dependent Ca²⁺ uptake, i.e., uptake in the presence of intravesicular Na⁺ minus uptake in the presence of intravesicular K⁺. A value of about 0.4 nmol/mg of protein was found at 5 s with 100 μM free Ca²⁺ in the medium. The specificity for Na⁺ was evident from other experiments. Uptakes of Ca²⁺ in the presence of intravesicular Rb⁺, choline⁺, and Li⁺ were the same as that with K⁺ in the intravesicular medium, and always less than that with Na⁺ (data not shown). Also, Ca²⁺ uptakes were essentially identical when K⁺ or choline⁺ was in the extravesicular medium and the intravesicular medium contained Na⁺ (data not shown), demonstrating...
ing that Na+-dependent Ca2+ uptake was independent of an extravesicular > intravesicular K+ gradient (Fig. 1).

To test whether the initial (5 s) uptake of Ca2+ by the basolateral membranes represented transport into vesicles rather than membrane binding, the effect of intravesicular volume on the uptake of Ca2+ was examined. In the experiments illustrated in Fig. 2, intravesicular space was decreased by increasing the medium osmolarity with sucrose. Na+ gradient-dependent uptake of Ca2+ was found to be inversely proportional to medium osmolarity from 0.3 to 0.8 osmolar and, thus, directly related to intravesicular space. Approximately 10% of the uptake of 0.3 osmolar, the final osmolality in all subsequent uptake measurements, was estimated by extrapolation to infinite medium osmolarity. This relatively small calculated uptake might suggest the slight binding of Ca2+ to the membrane during the incubation time period, or perhaps, the slow leak of sucrose into the vesicle, thus negating zero intravesicular volume. The finding that almost all the Ca2+ was taken up into the intravesicular space would argue against the possibility that intravesicular Na+ enhanced Ca2+ uptake, relative to intravesicular K+, merely by exposing additional Ca2+-binding sites on the basolateral membrane.

The Na+ gradient-dependent stimulation of Ca2+ uptake was inhibited by ionophores that dissipated the Na+ gradient. Monensin (25 μg/mg membrane protein) and nigericin (25 μg/mg membrane protein) which mediated the electroneutral exchange of Na+ for H+ in renal membe

![Fig. 2. Effect of osmolarity on the Na+ gradient-dependent uptake of Ca2+ in basolateral membrane vesicles.](image)
Renal \( \text{Na}^+ / \text{Ca}^{2+} \) Exchange and Effects of Parathyroid Hormone

Fig. 3. The relationship between \( \text{Ca}^{2+} \) concentration of \( \text{Ca}^{2+} \) uptake. Membrane vesicles were preloaded with \( \text{Na}^+ \) (●) or \( \text{K}^+ \) (○) and the uptakes were measured after 5-s incubations as described in Fig. 1. The free \( \text{Ca}^{2+} \) concentrations were estimated keeping nitrilotriacetic acid concentration constant at 1 mM. Each datum represents the mean ± S.E. of five experiments, each carried out in triplicate. The dashed line represents the \( \text{Na}^+ \) minus \( \text{K}^+ \) uptakes, at each concentration of \( \text{Ca}^{2+} \). The plot on the left shows an Eadie-Hofstee transformation of the \( \text{Na}^+ \)-dependent uptake.

Fig. 4. The relationship between intravesicular \( \text{Na}^+ \) concentration and \( \text{Ca}^{2+} \) uptake. Membrane vesicles were preloaded with 150 mM KSCN in 5 mM Hepes/Tris, pH 7.5, or with the different concentrations of NaSCN replacing KSCN, keeping osmolarity constant. Uptakes of 25 \( \mu \text{M} \) free \( \text{Ca}^{2+} \) were measured at 5 s. The \( \text{Na}^+ \)-dependent \( \text{Ca}^{2+} \) uptake was calculated by subtracting the uptake in the absence of \( \text{Na}^+ \) (150 mM KSCN) from the uptake with the different concentrations of NaSCN. Each datum represents the mean ± S.E. of four experiments, each carried out in triplicate.

efflux is shown in Fig. 6. When 100 mM \( \text{Na}^+ \) was in the extravesicular medium, the earliest measured rate of \( \text{Ca}^{2+} \) efflux increased from 0.46 to 0.75 nmol/15 s · mg\(^{-1}\) of protein. Throughout the efflux period, more \( \text{Ca}^{2+} \) was removed from the vesicles in the presence of extravesicular \( \text{Na}^+ \) than with the \( \text{K}^+ \) medium. After 5 min, about 70% of the accumulated \( \text{Ca}^{2+} \) exited from the vesicle. In other experiments, when the membrane vesicle suspension was diluted 4-fold in a \( \text{Na}^+ \) or \( \text{K}^+ \) medium containing 1 mM EGTA and 1.2 \( \mu \text{M} \) ionophore A23187, approximately 90% of the intravesicular \( \text{Ca}^{2+} \) effluxed from the vesicles in 5 min (not illustrated). After 30 min, efflux was nearly complete in the presence of EGTA and the ionophore, in the presence of either extravesicular \( \text{Na}^+ \) or \( \text{K}^+ \). The finding of \( \text{Ca}^{2+} \) efflux stimulated by extravesicular \( \text{Na}^+ \) coupled with the demonstration of \( \text{Ca}^{2+} \) uptake increased by intravesicular \( \text{Na}^+ \) provided further evidence for the presence of a \( \text{Na}^+ / \text{Ca}^{2+} \) exchange system in these renal basolateral membrane vesicles.

Fig. 5. Inhibition of \( \text{Na}^+ \)-dependent \( \text{Ca}^{2+} \) uptake by \( \text{La}^{3+} \) and divalent cations. Uptakes and measurements of \( \text{Na}^+ \)-dependent uptakes were as reported in Fig. 1. Inhibitory cations were present in the uptake media, added at zero time. Each datum represents the mean ± S.E. of three to five experiments, each carried out in triplicate.

Fig. 6. \( \text{Na}^+ \)-dependent efflux of \( \text{Ca}^{2+} \) from basolateral membrane vesicles. Membrane vesicles were loaded with \( \text{Ca}^{2+} \) by incubating for 10 min a 2-\( \mu \)l suspension of vesicles (about 50 \( \mu \text{g} \) of protein), containing intravesicularly 150 mM NaCl in 5 mM Hepes/Tris buffer, pH 7.5, in 38 \( \mu \)l of medium containing 150 mM KCl in the Hepes/Tris buffer and 100 \( \mu \text{M} \) 45\text{Ca}^{2+} (total). Efflux was initiated by diluting the vesicle suspension 4-fold with a solution of 1 mM EGTA, 5 mM Hepes/Tris buffer, and either 150 mM KCl (●) or 50 mM KCl and 100 mM NaCl (○). Each datum represents the mean ± S.E. for four experiments, each replicated three to five times.
Membrane vesicles were obtained from animals treated as described in Fig. 1, with the exception that the 100 μM free Ca\(^{2+}\) was obtained with 174 μM CaCl\(_2\) and 250 μM nitrilotriacetic acid. Membrane vesicles were obtained from animals treated as described in the text. The comparison between sham-operated and TPTX rats represents the mean ± S.E. of six animals, each preparation replicated six to seven times. The comparison between TPTX and parathyroid hormone (PTH)-infused animals had an n value of 7, each replicated six to seven times. The comparison between sham-operated and TPTX rats, respectively. In another series of experiments (Fig. 8), rats, TPTX 48 to 72 h previously, were infused for 3 h with medium without or with parathyroid hormone (44 units of the synthetic tetraetriaccontapeptide). Infusion of the hormone caused a 45% increase in Na\(^{+}\)-dependent Ca\(^{2+}\) uptake, 0.155 ± 0.016 compared to 0.107 ± 0.010 nmol/5 s·mg\(^{-1}\) of protein in vesicles from sham-operated and PTH rats, respectively. In this series of experiments 0.199 ± 0.012 and 0.220 ± 0.015 nmol/5 s·mg\(^{-1}\) of protein in vesicles from TPTX rats and animals infused with parathyroid hormone, respectively. It was found in these experiments that serum Ca\(^{2+}\) levels fell from 2.05 ± 0.04 mM before surgery to 1.55 ± 0.05 mM 48 to 72 h after surgery. However, with infusion of parathyroid hormone, the serum level of Ca\(^{2+}\) rose to 1.85 ± 0.02 mM. To preclude the possibility that the increased Na\(^{+}\)-dependent Ca\(^{2+}\) uptake in the TPTX rat infused with parathyroid hormone was a secondary response to the elevated serum Ca\(^{2+}\) rather than from a more direct action of the hormone, the concentration of Ca\(^{2+}\) in the serum of TPTX rats was raised to 1.78 ± 0.12 mM by adding 200 mM CaCl\(_2\) to an infusion medium from which hormone was deleted. As shown in Fig. 8, Na\(^{+}\)-dependent Ca\(^{2+}\) uptake in K\(^{+}\)-loaded vesicles did not differ significantly, being in this series of experiments 0.199 ± 0.012 and 0.220 ± 0.015 nmol/5 s·mg\(^{-1}\) of protein in vesicles from TPTX rats and animals infused with parathyroid hormone, respectively.
cytolytic activity. That this was indeed the case is shown in Table I. In the presence of a concentration of GTP which did not significantly enhance basal adenylate cyclase activity, parathyroid hormone increased activity 6-fold. The augmentation of hormone-stimulated adenylate cyclase by the guanine nucleotide was noted earlier (19). Arginine vasopressin caused only a moderate activation, whereas calcitonin and glucagon were without significant effects. Adenylate cyclase activity in the basolateral membrane preparation was markedly stimulated by forskolin and NaF.

**DISCUSSION**

The present results demonstrated that uptake and efflux of Ca\(^{2+}\) in renal cortex basolateral membrane vesicles were enhanced by trans-Na\(^+\). Other monovalent cations were ineffective. The stimulation of Ca\(^{2+}\) uptake by the Na\(^+\) gradient was inhibited by ionophores that dissipated the gradient. Na\(^+\)-dependent Ca\(^{2+}\) uptake was saturable with respect to both Ca\(^{2+}\) and Na\(^+\). These findings were consistent with the presence of a Na\(^+\)/Ca\(^{2+}\) exchanger in the basolateral membranes of the kidney cortex.

Accumulation of Ca\(^{2+}\) by these vesicle preparations probably represented a combination of the Ca\(^{2+}\) taken up into the intravesicular medium and that bound to the membrane. This was evident from the findings that the initial rate of Ca\(^{2+}\) uptake was directly related to the intravesicular space and the total Ca\(^{2+}\) accumulated at the steady state (60 min) in the vesicles greatly exceeded the uptake predicted from intravesicular space determinations, estimated from the uptakes in these vesicles of D-glucose and L-glutamate, at equilibrium (15). The excess Ca\(^{2+}\) taken up, however, was likely bound to the inside of the membrane vesicle. First, Ca\(^{2+}\) uptake was terminated by the addition of EGTA to the incubation medium, and afterwards, the membrane vesicles were washed rapidly but thoroughly, with the chelator. This stopping procedure should have removed readily accessible externally bound Ca\(^{2+}\). Second, the membrane vesicles could be depleted completely of their accumulated Ca\(^{2+}\) by treating the vesicles with the Ca\(^{2+}\) ionophore A23187 in the presence of EGTA. These results would favor the view that an increased uptake of Ca\(^{2+}\) found in the presence of trans-Na\(^+\) reflected the stimulation of the transmembrane movement of Ca\(^{2+}\) into the intravesicular medium, relative to the entry found in the absence of K\(^+\), and the subsequent binding of the divalent cation to the vesicle interior. The possible enhancement of Ca\(^{2+}\) binding to the membrane vesicle interior by intravesicular Na\(^+\) was not unambiguously excluded, although the decreased Ca\(^{2+}\) efflux by extravesicular Na\(^+\), which presumably could enter the vesicle passively, would argue against this possibility.

The Na\(^+\)-dependent Ca\(^{2+}\) uptake system in the renal membrane exhibited Michaelis-Menten kinetics with respect to Ca\(^{2+}\). A [Ca\(^{2+}\)]\(_{50}\) of 8 µM was calculated. This value might be considered high relative to the reported concentration of tubular cell cytosolic free Ca\(^{2+}\) in the submicromolar range (8). However, the [Ca\(^{2+}\)]\(_{50}\) for the renal exchanger was in close agreement with other estimated values for the carrier in cardiac membrane preparations (27, 28). The present findings that Na\(^+\)-dependent Ca\(^{2+}\) uptake was stimulated by a valinomycin-induced K\(^+\) potential (outside negative) and by anions of high conductance were consistent with the view that the Na\(^+\)/Ca\(^{2+}\) exchange system in the basolateral membrane was electrogenic. This conclusion was supported by the finding of a Hill coefficient with respect to Na\(^+\) of 2.5. A similar conclusion was deduced previously from experiments in the squid axon (29) and heart (30).

The renal basolateral membrane vesicle preparation used in the present studies probably represented a mixed population of inside-out and right-side-out orientated vesicles. Although this question was not addressed experimentally in this paper, examinations by others indicated that about 75% of the vesicles with assignable orientation were right-side out (16, 31). Assuming that a similar orientation was the case with the vesicles employed in this study, Na\(^+\)-dependent Ca\(^{2+}\) efflux in the vesicle system would mimic the transport of Ca\(^{2+}\) across the basolateral membranes in situ from cell to interstitial fluid. However, both Na\(^+\)-dependent Ca\(^{2+}\) efflux and uptake were found in the renal vesicle system, like that reported for Na\(^+\)/Ca\(^{2+}\) exchange in cardiac sarcosomelemal vesicles (27). Indeed, with the heart membranes, it was suggested that the Ca\(^{2+}\)-binding sites on the two sides of the exchange transport mechanism were symmetrical. By analogy, Ca\(^{2+}\) could move out of or into the renal cell in situ, the direction of movement of Ca\(^{2+}\) across the basolateral membrane would depend on the relative electrochemical gradients of Ca\(^{2+}\) and Na\(^+\) as well as on the potential across the membrane.

Previous in vivo studies demonstrated that parathyroid hormone increased renal tubular Ca\(^{2+}\) absorption and decreased urinary Ca\(^{2+}\) excretion (5). Subsequently, it was found that the hormone stimulated Ca\(^{2+}\) transport in the isolated perfused tubule, indicating a direct action on the kidney (32-35). The physiological mechanism by which parathyroid hormone augmented Ca\(^{2+}\) transport remained unknown. The present results showed that Na\(^+\)/Ca\(^{2+}\) exchange activity in basolateral membrane vesicles from TPTX rats decreased by about 40% and this activity was restored when the synthetic tetracontapeptide of parathyroid hormone was infused in the TPTX animal. The parathyroid hormone status of the animal had no effect on Ca\(^{2+}\) uptake in the presence of an intravesicular > extravesicular K\(^+\) gradient, in the absence of Na\(^+\). These findings provided evidence supporting a hypothesis that the enhancement of renal Ca\(^{2+}\) transport by parathyroid hormone was concomitant with an increase in the activity of the Na\(^+\)/Ca\(^{2+}\) carrier in the basolateral membrane. In the microperfused tubule from TPTX rats, Ca\(^{2+}\) transport was restored to control levels by infusion of synthetic parathyroid hormone (1-34) and by cyclic AMP analogs (35). Thus, it is tempting to speculate that cyclic AMP might act as a messenger to increase Na\(^+\)/Ca\(^{2+}\) exchange activity. Interestingly, it was reported that the release of Ca\(^{2+}\) from neonatal mouse calvaria in organ culture stimulated by parathyroid hormone was inhibited by ouabain and ionophores which presumably increased intracellular Na\(^+\) concentration; thus, it was proposed that the hormone stimulated bone resorption via a Na\(^+\)/Ca\(^{2+}\) exchange mechanism (36). It should be recognized that in the experiments described in the present study levels of thyroid hormone and calcitonin would also be depleted in the TPTX rat, and that these hormones might directly or indirectly affect renal calcium transport (37, 38). The possible effects of thyroid hormone and calcitonin on the carrier were not excluded and remain to be tested. However, the long half-life of thyroid hormone and the fact that the animals were used 2 to 3 days after surgery makes a potential effect of thyroid hormone less likely.

Previous studies demonstrated the functional heterogeneity of the nephron with respect to Ca\(^{2+}\) transport (5-7). Although roughly half of the Ca\(^{2+}\) reabsorbed in the nephron was found to occur in the proximal tubule and the presence of Na\(^+\)/Ca\(^{2+}\) exchange system in this segment of the nephron was suggested (39), significant fractions of the filtered Ca\(^{2+}\) and, importantly, the regulation of the Ca\(^{2+}\) content in the final urine appeared to take place distally. Additionally, it was reported
that parathyroid hormone clearly enhanced Ca\(^{2+}\) reabsorption in the cortical thick ascending limb of Henle (33, 34) and the cortical granular epithelium of the distal convoluted and collecting tubule (35, 40), whereas the hormone's action on Ca\(^{2+}\) transport in the proximal tubule was mainly equivocal (5). In the distal regions of the nephron, the presence of a parathyroid hormone-sensitive Ca\(^{2+}\) transport was concordant with a parathyroid hormone-stimulated adenylate cyclase (41, 42). In the present study, it was shown that the basolateral membrane vesicle preparation which contained Na\(^+/Ca\(^{2+}\) exchange activity responsive to the parathyroid hormone status that parathyroid hormone clearly enhanced Ca\(^{2+}\) reabsorption demonstrated substantial parathyroid hormone-enhanced cyclase.

Because the basolateral membranes were derived from the renal cortex, it was not unreasonable to expect elements of hormone-stimulated adenylate cyclase in the membrane preparation, i.e. responsive to parathyroid hormone but not to calcitonin and glucagon (Table I), might suggest that the membranes were derived predominantly from the proximal tubule. This finding would be consistent with the fact that the bulk of the nephrogenous elements in the cortex would be proximal tubules and with other reports of hormone-stimulated adenylate cyclase in basolateral preparations from the renal cortex (17, 18). In addition, the proximal tubule would be the locus of parathyroid hormone-sensitive phosphate transport (5) and 25-hydroxyvitamin D\(_3\) hydroxylation (43). On the other hand, the cortical thick ascending limb of Henle in the rat should contain calcitonin-, glucagon-, and parathyroid hormone-stimulated adenylate cyclase as well as some vasopressin-stimulated adenylate cyclase. Thus, the failure to find significant activations of the cyclase by calcitonin and glucagon in the isolated membranes would not be coincident with their derivations from the thick ascending limb of Henle. The possibility that during the preparation of the basolateral membranes calcitonin- and glucagon-responsive adenylate cyclase was lost was not precluded, however. Despite this enigma, the present study did demonstrate that the activity of the Na\(^+/Ca\(^{2+}\) exchange, which has a role in the transepithelial movement of Ca\(^{2+}\) in the kidney, was modulated by parathyroid hormone. The precise loci of the carrier in the heterogeneous nephron and its sensitivity to the hormone remain to be determined.

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