Stimulation of Calcium Uptake by Parathyroid Hormone in Renal Brush-Border Membrane Vesicles

RELATIONSHIP TO MEMBRANE PHOSPHORYLATION

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The effect of parathyroid hormone (PTH) on Ca²⁺ uptake was studied in brush-border membrane vesicles (BBMV) prepared from the kidneys of dogs administered 4–5 μg/kg of bovine PTH 1-84 in vivo. PTH stimulated Ca²⁺ uptake at 20 s of incubation from control values of 231 ± 21 to 306 ± 30 pmol/mg of protein, p < 0.001. The stimulation of Ca²⁺ uptake by PTH was not reversed by incubation of the BBMV with the Ca²⁺ ionophore, despite the fact that Ca²⁺ uptake was several times greater than the expected uptake at equilibrium, indicating that most of the uptake represented Ca²⁺ binding to the BBMV. In BBMV from kidneys exposed to PTH, hypotonic lysis or increasing protein, BBMV. Ca²⁺ binding was initially to the exterior of the membrane. In BBMV from kidneys exposed to PTH, Ca²⁺ ionophore, despite the fact that Ca²⁺ uptake was several times greater than the expected uptake at equilibrium, indicating that most of the uptake represented binding of Ca²⁺ to the exterior of the vesicle membrane.

Incubation of BBMV from kidneys exposed to PTH with gentamicin, which competes with Ca²⁺ for anionic phospholipid-binding sites, reversed the stimulatory effects of PTH on Ca²⁺ uptake. These data also indicated that the largest fraction of Ca²⁺ uptake in the presence of a chemical potential represented binding of Ca²⁺ to BBMV. Ca²⁺ binding was initially to the exterior of the membrane, then translocated within the membrane and to the interior vesicular face as assessed by chelation of Ca²⁺ bound to the BBMV by ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid. Incubation of BBMV from kidneys exposed to PTH with gentamicin, which competes with Ca²⁺ for anionic phospholipid-binding sites, reversed the stimulatory effects of PTH on Ca²⁺ uptake. Phosphorylation of BBMV and PTH treatment in vivo had similar effects on BBMV phospholipid composition increasing the levels of anionic phospholipids. Phosphorylation of the BBMV also produced gentamicin-inhibitable increases in membrane Ca²⁺ binding. Phosphorylation of BBMV from kidneys exposed to PTH was inhibited suggesting a higher state of phosphorylation in vivo. The data demonstrate that PTH administered in vivo stimulated Ca²⁺ binding in BBMV that was gentamicin inhibitable and associated with an increase in the membrane content of anionic phospholipids.

The initial step in Ca²⁺ reabsorption by the renal tubule is Ca²⁺ translocation from the tubular fluid phase of the BBM to the cytosolic side of the membrane. Ca²⁺ movement across the BBM proceeds along favorable concentration and electrical gradients. This portion of the cell membrane has been reported to be lacking the mechanisms of Ca²⁺ transport which are associated with Ca²⁺ flux from the renal tubular cells. These mechanisms, active transport utilizing energy derived from ATP ([Ca²⁺ + Mg²⁺]·ATPase) and the Na⁺/Ca²⁺ exchange mechanism, are functional in the basolateral membranes of renal tubular cells (1, 2).

In recent years, preparations of BBMV of renal tubular cells have been utilized to study the solute transport processes of this membrane. Using BBMV with largely a right-side-out orientation, Ca²⁺ uptake and release have been analyzed (1, 3). These studies demonstrate that, in the presence of inwardly directed chemical potentials for Ca²⁺, uptake represents largely binding of Ca²⁺ to the vesicular membranes. The bound Ca²⁺ rapidly distributes within the membrane and to the interior face of the vesicle membrane.

Brush border membrane vesicles have also been utilized to analyze phosphate (P₄) transport. A Na⁺-dependent co-transport mechanism capable of uphill Pi movement has been characterized in BBMV (4–6). The effects of hormonal stimuli on this Na⁺-dependent P₄ co-transport, including those of PTH, have been investigated (4, 7–9). These studies demonstrated that PTH inhibited Na⁺-dependent P₄ co-transport in BBMV by stimulation of cyclic AMP-dependent protein phosphorylation of the BBM. In the course of these studies, cyclic AMP-independent phosphorylation of BBM phospholipids was demonstrated (9). We have subsequently related phosphorylation of anionic BBM phospholipids to an increase in Ca²⁺ binding in BBMV (3). Since PTH stimulates the metabolism of these phospholipids in isolated renal cortical tubular segments (10) and since PTH is a major regulator of the cellular physiology of calcium (11–13), including Ca²⁺ transport (14–16), the present studies were undertaken to examine the effects of PTH on Ca²⁺ uptake in BBMV.

**EXPERIMENTAL PROCEDURES**

Preparation of BBMV—Mongrel dogs weighing 15–20 kg were fed standard Purina dog chow (Ralston Purina, St. Louis, MO) and were studied according to protocols previously described (4, 17). Briefly, dogs were anesthetized with pentobarbital, and jugular vein and urinary bladder catheters were placed for obtaining blood samples and urine collections. Baseline endogenous creatinine clearances and P₄ clearances were obtained, after which control kidneys were removed. Fifteen to 30 min after the first nephrectomy, a second set of urinary bladder catheters were placed for obtaining blood samples and urine collections. Baseline endogenous creatinine clearances and P₄ clearances were obtained, after which control kidneys were removed. Fifteen to 30 min after the first nephrectomy, a second set of

The abbreviations used are: BBM, brush-border membrane; BBMV, brush-border membrane vesicles; PTH, parathyroid hormone; Mes, 2-(N-morpholino)ethanesulfonic acid; TLC, thin layer chromatography; PA, phosphatidic acid; PC, phosphatidylethanolamine; DPI, phosphatidylinositol 4'-monophosphate; TPI, phosphatidylinositol 4',5'-diphosphate; MPI, phosphatidylinositol; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.
control clearances was obtained. Then, bovine PTH (1-84, 3000 units/mg) was given intravenously, 4.5 μg/kg, body weight. Twenty min later urine was collected for 10 min and the experimental kidney was removed. The renal artery of each kidney was immediately catheterized and perfused with 75–100 ml of ice-cold saline and the kidneys were placed in ice.

BBMV were isolated from homogenates of both kidneys by the MgCl2 precipitation technique described previously in detail (4, 9, 17). Isolated BBMV were washed, recentrifuged, and resuspended in 10 mM MgCl2, 10 mM KF, 240 mM mannitol, 5 mM Mes/Tris, pH 6.5 (suspension solution). Membrane preparations were evaluated by electron microscopy and by measuring specific activities of marker enzymes. The results of these studies have been reported previously. PTH did not affect the microscopic appearance of the membranes or the activities of the enzyme markers (4, 9, 17).

Phosphorylation of BBMV—In those experiments utilizing phosphorylation of BBMV (Figs. 4 and 5), phosphorylation was carried out in hypotonic solutions to cause opening of the vesicles, allowing access of ATP to the vesicular interior as previously reported (3, 9). The solutions used for this purpose were the suspension solutions without mannitol containing 10 μM ATP. Unless indicated otherwise, incubation of BBMV with 10 μM ATP was allowed to proceed for 30 s at 30 °C. At the end of solution incubation, ATP was added to initiate Ca2+ uptake. Opening and rescaling of BBMV by exposure to hypotonic media was demonstrable by release of tritiated glucose accumulated in the presence of sodium chloride gradients and by uptake of sucrose, which is normally excluded, into the intravesicular space (3).

Measurement of Calcium Uptake in BBMV—Ca2+ uptake was measured by a modification of the Millipore filtration technique of Aronson and Sacktor (18) as previously reported (4). Samples of BBMV containing 100–300 μg of membrane protein (10 μl) were incubated in a shaking water bath for 1 min at 30 °C. Then the hypotonic solutions used to induce phosphorylation were added, and incubation was continued for 30 s at 30 °C, before 200 μl of solutions used to initiate Ca2+ uptake were added. In the majority of experiments where phosphorylation of BBMV was not utilized, ATP was deleted from the hypotonic solution, but the hypotonic addition was made so that all BBMV in the present report were handled similarly. The standard solution used to initiate Ca2+ uptake consisted of 48 mM mannitol, 10 mM MgCl2, 10 mM KF, 5 mM Mes/Tris, pH 6.5, and 25 μM CaCl2, with tracer 45CaCl2 (2–5 × 106 cpm/ml). In some instances, Ca2+ concentrations were buffered by addition of EGTA to the solutions. Ca2+ concentration of all solutions was determined by a Ca2+-specific electrode (Orion Research Inc., Cambridge, MA). The uptake of Ca2+ at selected times was terminated by dilution and rapid filtration of the BBMV incubation medium. Solutions used for dilution consisted of ice-cold 100 mM mannitol, 5 mM Mes/Tris, pH 6.5, or 1 mM Mes/Tris, pH 6.5. In some instances, 5 mM EGTA was added to the solutions used to terminate Ca2+ uptake. Filters were dissolved in 1 N NaOH (1 ml) and suction-filtered into Aquasol (New England Nuclear), and radioactivity was counted in a liquid scintillation spectrometer (model 460 CD, Hewlett-Packard, Downers Grove, IL). Values for nonspecific retention of 45Ca on Millipore filters were subtracted from the values of all membrane samples. All incubations were carried out in triplicate with freshly prepared membrane vesicles.

The results are expressed as mean ± S.E.

In experiments in which the uptake of 3H-glucose was analyzed, 50 μM D-glucose was added to solutions containing 120 mM NaCl, 10 mM MgCl2, 10 mM KF, and 5 mM Mes/Tris, pH 6.5. Glucose uptake was stopped by dilution of the incubation medium with 5 ml of ice-cold 150 mM NaCl and rapid filtration.

Analysis of BBM Phospholipids—Analysis of BBM phospholipids was performed by determination of P, content of phospholipid extracts separated by TLC. Samples of BBMV were added to 3 ml of ice-cold chloroform:methanol (1:1) with tetrabutylammonium sulfate (final concentration 10 mM). The volume of each sample was reduced to 1.5 ml under a stream of N2 at 22 °C. Then 1 ml of 0.1 N HCl and 1 ml of chloroform were added, and the samples were centrifuged at 1000 × g for 10 min. The aqueous phases were drawn off and discarded. Then, the lipid phases were washed with theoretical upper phase volumes and were resuspended 3 times. The interface and upper phases were washed and the chloroform dissolved lipid phase was combined with the wash. Chloroform was evaporated to dryness, and the remaining lipids were reconstituted in chloroform:methanol (1:1), and spotted on TLC plates pre-coated with Silica Gel 60 (E. Merck, Darmstadt, Germany). TLC plates were developed unidimensionally in several solvent systems designed for separation of specific phospholipids as previously described (3). In some instances a solvent system not previously reported by us, chloroform, methanol, 10% methylamine (60:36:10), was used for separation of the phosphoinositides, phosphatidylcholine and phosphatidylethanolamine from other phospholipids. The respective phospholipids were removed from the TLC plates and analyzed for phosphate content using the method of Bartlett (19).

The results of the clearance studies performed prior to the removal of the experimental kidney were similar to those reported previously (4, 17). Specifically, the administration of bovine PTH 1-84 did not affect plasma calcium, phosphorus, or creatinine during the time of the study. The creatinine clearance was stable during the urine collections following the first nephrectomy, and the excretion of phosphorus (both absolute and fractional) was increased in each case following PTH administration (data not shown).

Effect of PTH on Ca2+ Uptake—Calcium uptake in BBMV isolated from kidneys exposed to PTH was higher compared to BBMV from control kidneys at both 20 s (estimate of initial rates) and 90 min (steady state) of incubation (Fig. 1). Under the experimental conditions utilized in these experiments, the driving force for Ca2+ uptake was an inwardly directed electrochemical gradient, and these results were obtained with similar solutions in either group. The transport rates were obtained by single sample analysis in the absence of inhibitors. The results are expressed as mean ± S.E.

![Fig. 1. The effect of PTH on calcium uptake in renal BBMV. BBMV were prepared and suspended as described under "Experimental Procedures" from control kidneys, removed before administration of PTH, or from kidneys removed 30 min after administration of bovine PTH 1-84, 4.5 μg/kg, body weight. Uptake of Ca2+ at 20 s (estimate of initial rates) and 90 min (steady state uptake) was determined as described under "Experimental Procedures." P, uptake in BBMV from kidneys exposed to PTH in vivo was greater than in BBMV from control (c) kidneys; p < 0.01 at 20 s and p < 0.05 at 90 min.](https://www.jbc.org/doi/10.1074/jbc.144010119)
directed chemical potential for Ca\(^{2+}\). In previous studies (1, 3) using similar experimental conditions Ca\(^{2+}\) uptake proceeded over time reaching a steady level of uptake between 80-90 min of incubation. Ca\(^{2+}\) uptake also increased as the Ca\(^{2+}\) concentration in the solution external to the BBMV was increased without showing a definite tendency to saturate. In BBMV from PTH-treated kidneys these characteristics of Ca\(^{2+}\) uptake were not qualitatively altered (data not shown). These results are in agreement with other reports of Ca\(^{2+}\) uptake in renal and intestinal brush border membrane vesicles (1, 3, 21).

Ca\(^{2+}\) uptake in the experimental setting employed in the above studies may have represented either binding of Ca\(^{2+}\) to the membrane or movement of Ca\(^{2+}\) into the vesicular space in association with other ions. We have previously shown (3) that Ca\(^{2+}\) uptake in BBMV prepared exactly as those from control kidneys reported above represented largely binding to the exterior face of the BBMV followed rapidly by distribution within the membrane and to the interior face of the membrane. Thus, in the present experiments, we also sought to determine the nature of the Ca\(^{2+}\) uptake in BBMV from kidneys exposed to PTH.

An effect of PTH on the volume of the vesicular space might explain the stimulatory effects of PTH. Steady state glucose uptake has been used to estimate the intravesicular volume of BBMV since there is little binding of glucose to the membrane other than its carrier protein (18, 22). We have previously reported that PTH does not affect Na\(^+-\)dependent glucose uptake has been used to estimate the intravesicular volume of BBMV (3). The chelation of Ca\(^{2+}\) at 90 min of incubation, Ca\(^{2+}\) chelation by EGTA in isotonic stop solutions decreased Ca\(^{2+}\) uptake by only 37-46%. In comparison, Ca\(^{2+}\) chelation at 90 min of incubation, when EGTA was added to hypotonic lysis stop solutions, resulted in 60-65% reduction in Ca\(^{2+}\) uptake. These results were similar in BBMV from both control and PTH-treated kidneys. Thus, the general sequence of Ca\(^{2+}\) uptake in BBMV appeared to represent binding to the exterior vesicular face where it was accessible for chelation by EGTA in isotonic solution. This was followed by rapid distribution of Ca\(^{2+}\) within the membrane including appearance on its interior face supported by the increased availability of Ca\(^{2+}\) for chelation.

A decrease in Ca\(^{2+}\) uptake in BBMV from PTH-treated kidneys occurred in a manner similar to the ionophore.

**Effects of Hypotonic Lysis and EGTA Treatment**—Lysis of BBMV, associated with stopping Ca\(^{2+}\) uptake, would be expected to release unbound Ca\(^{2+}\) accumulated within the vesicular space. As shown in Fig. 3, only small amounts of the Ca\(^{2+}\) accumulated by BBMV (either control or experimental) were released by hypotonic lysis. In similar experiments for Na\(^+-\)dependent glucose uptake, release was 75% of uptake (data not shown) similar to previous results (3). The chelation of Ca\(^{2+}\) bound to the exterior face of BBMV (23-25), by addition of EGTA to isotonic solutions used for stopping Ca\(^{2+}\) uptake, decreased Ca\(^{2+}\) uptake by 70-80% at 20 s of incubation. However, after 90 min of incubation, Ca\(^{2+}\) chelation by EGTA in isotonic stop solutions decreased Ca\(^{2+}\) uptake by only 37-46%. In comparison, Ca\(^{2+}\) chelation at 90 min of incubation, when EGTA was added to hypotonic lysis stop solutions, resulted in 60-65% reduction in Ca\(^{2+}\) uptake. These results were similar in BBMV from both control and PTH-treated kidneys. Thus, the general sequence of Ca\(^{2+}\) uptake in BBMV appeared to represent binding to the exterior vesicular face where it was accessible for chelation by EGTA in isotonic solution. This was followed by rapid distribution of Ca\(^{2+}\) within the membrane including appearance on its interior face supported by the increased availability of Ca\(^{2+}\) for chelation.

![Fig. 2. Effects of the Ca\(^{2+}\) ionophore (A23187) on Ca\(^{2+}\) uptake in BBMV. Ca\(^{2+}\) uptake was determined as described under Experimental Procedures in BBMV from control kidneys and kidneys exposed to PTH in vivo. Data from five paired experiments are shown for Ca\(^{2+}\) uptake in BBMV from control kidneys without (C) or with (C+I) the addition of 1 x 10\(^{-5}\) M A23187, and in BBMV from PTH-treated kidneys without (P) and with (P+I) the addition of the ionophore. The numbers on the left of each data set indicate the number assigned to the experiment. The numbers in parentheses on the right of each data set are the increase in Ca\(^{2+}\) uptake induced by the ionophore. The mean Ca\(^{2+}\) uptake ± S.E. in the BBMV from control and PTH-treated kidneys and the mean increase ± S.E. in Ca\(^{2+}\) uptake induced by the ionophore are shown at the bottom of each data set. Ca\(^{2+}\) uptake was significantly increased in BBMV from PTH-treated kidneys (P) compared to control BBMV (C), p < 0.05. The Ca ionophore was added to solutions used to initiate 45CaCl\(_2\) uptake.](http://www.jbc.org/)

**Table 1**

*Estimated vesicular volumes and steady state Ca\(^{2+}\) spaces*

| Estimated vesicular volume | Ca\(^{2+}\) uptake at steady state (observed/equilibrium) |
|---------------------------|----------------------------------------------------------|
| C                         | PTH                                                      |
| 2.6 ± 0.2                 | 2.8 ± 0.2                                                |
| 15.1 ± 0.6                | 18.5 ± 0.7                                               |

*Experimental Procedures.* See text for discussion of calculations. Results are presented as mean ± S.E., n = 5.
lation by EGTA in the presence of hypotonic lysis. PTH did not appear to alter the general sequence of binding and distribution within and to the opposite face of the membrane.

Studies were also performed to analyze the effects of decreasing intravesicular volume on the stimulation of Ca\(^{2+}\) uptake by PTH. Intravesicular volume was decreased by increasing the osmolality of the incubation medium in the range from 108-1500 mosm/kg with an impermeant solute (sucrose) (3, 4). Ca\(^{2+}\) uptake at 20 s of incubation in BBMV prepared from kidneys exposed to PTH exhibited a similar lack of sensitivity to increasing external osmolalities as Ca\(^{2+}\) uptake in BBMV from control kidneys (data not shown) (3). These data further indicated that the major portion of Ca\(^{2+}\) uptake in BBMV from kidneys exposed to PTH in vivo represented binding of Ca\(^{2+}\) to the membrane.

**Effects of BBMV Phosphorylation and Gentamicin**—Since the stimulation of Ca\(^{2+}\) uptake in BBMV by PTH appeared to represent mainly an increase in membrane Ca\(^{2+}\) binding, further studies were performed to analyze the nature of the Ca\(^{2+}\) binding sites. In previous studies, binding of Ca\(^{2+}\) to BBMV was increased by membrane phosphorylation in vitro using exposure of BBMV to small concentrations of ATP to produce membrane phosphorylation (3). The effect of membrane phosphorylation on Ca\(^{2+}\) uptake was reproduced in the studies reported here as shown in the left side of Fig. 4. The stimulation of Ca\(^{2+}\) uptake was associated with an increase in the membrane content of anionic phospholipids which bind calcium (3). As shown in Fig. 4, gentamicin, which binds to anionic phospholipids, inhibited the stimulatory effect of phosphorylation of Ca\(^{2+}\) uptake. We previously suggested that this represented competition between Ca\(^{2+}\) and gentamicin for the binding sites produced by phosphorylation. Since PTH produces a stimulation of protein phosphorylation in BBMV (9, 26, 27) and an increase in the content of anionic phospholipids in renal cortical tubular segments (10), the effects of ATP-induced phosphorylation on Ca\(^{2+}\) uptake in BBMV from kidneys exposed to PTH were studied.

Exposure of BBMV prepared from kidneys of dogs treated with PTH to ATP failed to further stimulate Ca\(^{2+}\) uptake (Fig. 4, right). This is compatible with a stimulation of BBM phosphorylation in vivo since this would impair the ability of ATP to stimulate phosphorylation in vitro and thus limit the effect of ATP on Ca\(^{2+}\) uptake. Also, as shown in Fig. 4, the addition of gentamicin to BBMV from kidneys exposed to PTH returned Ca\(^{2+}\) uptake to levels seen in BBMV from control kidneys, suggesting that the stimulatory effects of PTH were similar to ATP in vitro. Since both gentamicin and Ca\(^{2+}\) bind to negatively charged phospholipids, reversal of the stimulation produced by PTH may have represented competition between Ca\(^{2+}\) and gentamicin for the binding sites produced by PTH treatment. Alternatively, gentamicin may have acted to reverse the effect of PTH by stimulating degradation of the binding sites produced by the PTH.

To analyze if PTH treatment affected the BBMV in terms of phospholipid content, the studies shown in Table II were...
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Fig. 5. Autoradiography of a thin layer chromatogram showing the effects of gentamicin on the turnover of phosphorylated phospholipids in renal cortical BBMV. BBMV from kidneys exposed to PTH were phosphorylated as previously described (3) by addition of (γ-32P)ATP (1-2 μCi/0.1 mg of BBMV protein) to hypotonic solutions containing 10 mM tetrabutylammonium sulfate. Following extraction of BBMV lipids and separation by TLC as described under "Experimental Procedures," the TLC plate was exposed to radiographic film (Kodak XRP-1, Eastman) for 48 h. The major phosphorylated products in the lipid extract were PA, TPI, and DPI (3). In control samples performed. They indicate that PTH administration increased the content of PA and of MPI in BBMV from kidneys exposed to PTH in vivo. These anionic phospholipids are known to bind Ca²⁺ and gentamicin avidly (28-32). The increase in the levels of these phospholipids correlated with the stimulation of Ca²⁺ binding by PTH administration and the inhibition of effect of PTH by gentamicin. These data also demonstrate that the previously reported effects of PTH on PA and MPI phospholipid metabolism in renal cortical tubules (10, 33, 34) are reflected in the BBM, while the effects of PTH on renal tubular polyphosphoinositide content did not reach statistically significant levels in the BBMV.

As shown in Fig. 5 and Table III, gentamicin added to BBMV prepared from kidneys exposed to PTH caused an increase in [32P]-labeled phospholipids after exposure to PTH and a tendency for TPI content to increase at the expense of MPI content. The data in Fig. 5 and Table III indicated that gentamicin tended to increase the phosphorylation of MPI producing DPI and TPI. This would tend to increase available Ca²⁺-binding sites, not decrease them. This data tends to favor the possibility that the effect of gentamicin on Ca²⁺ uptake was due to competition for binding sites produced by PTH rather than through stimulation of binding site degradation.

Effects of PTH on Ca²⁺ Loss from BBMV—To further analyze Ca²⁺ movement in BBMV from kidneys exposed to PTH, loss of Ca²⁺ from BBMV pre-equilibrated with Ca²⁺ was analyzed as previously described (3). BBMV isolated from

![Diagram](http://www.jbc.org/Downloaded)
kidneys exposed to PTH in vivo had significantly higher initial rates of Ca\(^{2+}\) loss than BBMV of control kidneys (Fig. 6). However, at steady state (30 min) slightly greater amounts of Ca\(^{2+}\) were retained in BBMV isolated from kidneys exposed to PTH. Since Ca\(^{2+}\) retained by BBMV at steady state far exceeded expected retention of Ca\(^{2+}\) at equilibrium, and since addition of the Ca\(^{2+}\) ionophore had little effect on the Ca\(^{2+}\) retained by the BBMV, most of the Ca\(^{2+}\) retained by BBMV at steady state probably represented Ca\(^{2+}\) bound to the membranes. The addition of gentamicin to BBMV isolated from kidneys exposed to PTH reversed the increase in Ca\(^{2+}\) retained by the BBMV at steady state to levels seen in the BBMV from control kidneys. This again is compatible with an effect of gentamicin competing for anionic binding sites with Ca\(^{2+}\), and the effect of PTH being to increase these binding sites.

**DISCUSSION**

PTH is an important regulator of renal tubular transport processes, especially for phosphorus and calcium transport. The biochemical mechanisms through which PTH affects the transport of solutes are largely unknown. In the case of Pi, PTH decreases transport in both the proximal and distal renal tubule. A sodium-dependent Pi carrier is located in the BBM, and it is responsible for uphill transport of Pi into the renal tubular cell. We and others have demonstrated that PTH, administered to dogs, inhibits the rate of Na\(^{+}\)-dependent Pi transport in BBMV isolated from kidneys exposed to PTH in vivo (4, 7–9). The inhibition by PTH of this active step has been shown to be related to biochemical modification of the BBM. We have demonstrated that cAMP-dependent protein phosphorylation of BBMV stimulated by PTH is associated with a decrease in the rate of Na\(^{+}\)-dependent Pi transport (9). The suggestion arose, therefore, that stimulation of membrane phosphorylation may be a general mechanism through which PTH affects cellular physiology.

The data presented in this manuscript show that BBMV isolated from kidneys exposed to PTH in vivo exhibit greater rates of Ca\(^{2+}\) uptake than BBMV from control kidneys. This effect was observed in isolated BBMV exposed to a chemical potential for Ca\(^{2+}\) as the driving force for uptake. In this situation, most of Ca\(^{2+}\) uptake represents binding of Ca\(^{2+}\) to the membrane. PTH treatment did not alter the nature of the Ca\(^{2+}\) uptake, but it stimulated the early rates of uptake and the steady state level of Ca\(^{2+}\) within the BBMV.

The possibility that the effect of PTH on Ca\(^{2+}\) uptake in BBMV was due to phosphorylation of the BBMV was supported by three sets of data. First, if PTH stimulated phosphorylation of the BBM in vivo, then they might be less susceptible to phosphorylation in vitro. In fact, phosphorylation of BBMV from kidneys exposed to PTH in vivo failed to increase Ca\(^{2+}\) uptake while phosphorylation was stimulatory in BBMV from control kidneys. Second, addition of gentamicin, which binds to anionic phospholipids, to BBMV from PTH-treated kidneys decreased Ca\(^{2+}\) uptake to the levels observed in BBMV from the control kidneys. Third, in renal tubular segments, PTH stimulated production of phosphorylated membrane phospholipids, PA, DPI, and MPI (10, 33, 34), and the effects on membrane PA were manifested in the BBMV. These data and the effect of PTH on protein phosphorylation (9) indicate that membrane phosphorylation may be a general mechanism by which PTH affects cellular physiology. We also demonstrate that PTH may increase Ca\(^{2+}\) binding to the BBM of proximal renal tubular cells by increasing the membrane content of PA and MPI. The lack of an effect on PTH of the polyphosphoinositide metabolism, which was observed in renal tubular segments, may be operative at a location within the cell other than the BBM.

The relationship between PTH-stimulated Ca\(^{2+}\) binding in BBMV and transcellular Ca\(^{2+}\) transport is unknown. Ca\(^{2+}\) binding may contribute to transcellular movement of Ca\(^{2+}\) if, after translocation of bound Ca\(^{2+}\) to the interior membrane face, a mechanism is initiated for moving Ca\(^{2+}\) from the membrane to cellular compartments involved in transcellular movement. At the present time, no direct evidence for this potential mechanism in Ca\(^{2+}\) transport is known. However, both phosphorylation of the membrane and an increase in the membrane-bound Ca\(^{2+}\) would be expected to have dramatic effects on the physiologic properties of the membrane. An effect of PTH on the negative charge density and the Ca\(^{2+}\) bound to the BBM may affect the function of ion channels and carrier proteins within the membrane. Direct analysis of the effect of phosphorylation on membrane stability or fluidity will provide insight into this possibility.

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