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Na^+–Ca^{2+} exchange and calcium permeability in canine basolateral membrane vesicles: The effects of dibutyryl cAMP and specific inhibitors

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The role of dibutyryl 3',5'-cyclic adenosine monophosphate (dibutyryl cAMP) as putative second messenger for parathyroid hormone (PTH) in regulating canine proximal tubular basolateral membrane Na^+–Ca^{2+} exchange and passive calcium permeability was assessed, as was the nature of this passive calcium permeability. Dibutyryl cAMP (50 mg) infused in vivo over 30 min increased fractional phosphate excretion from $4.9 \pm 1.8\%$ to $20.5 \pm 4.6\%$, $P < 0.05$, $n = 6$, but had no effect on either passive Ca^{2+} efflux or sodium-stimulated Ca^{2+} efflux from Ca^{2+}-preloaded basolateral membrane vesicles (BLMV). Both of these mechanisms have been previously shown to be stimulated by PTH. Further studies were performed to investigate the mechanism of the passive calcium flux. Calcium uptake by BLMV was blocked by lanthanum (La^{3+}) but not by the calcium-channel blocker verapamil. La^{3+} blocked efflux of Ca^{2+} from preloaded vesicles when it was placed in the external solution. This La^{3+}-blockable efflux was larger in potassium equivalent BLMV prepared from normal dogs than in BLMV prepared from thyroparathyroidectomized dogs. Benzamil produced 50% inhibition of sodium-stimulated Ca^{2+} uptake at 250 μM whereas neither amiloride nor diltiazem achieved 50% inhibition at the maximal doses studied. Benzamil, 1 mM, had no effect on passive calcium efflux and neither did the substitution of sucrose for potassium, which has been shown to affect Ca^{2+}–Ca^{2+} exchange by the Na^+–Ca^{2+} exchanger. This suggests that the calcium flux under potassium equivalent conditions was not mediated by Ca^{2+}–Ca^{2+} exchange by the Na^+–Ca^{2+} exchanger. These results demonstrate that the basolateral membrane of proximal tubular cells possesses both a Na^+–Ca^{2+} exchanger inhibitable by benzamil and a passive calcium permeability not inhibited by benzamil nor by verapamil but by La^{3+}. Neither of these two mechanisms of calcium flux was affected by dibutyryl cAMP whereas both have been shown to be stimulated by PTH.

Introduction

The nature of Ca^{2+} reabsorption by the proximal tubule is unclear [1]. Evidence previously reviewed [2] has suggested that at least a portion of the Ca^{2+} reabsorption may be active. For active transcellular Ca^{2+} resorption to occur there must be an apical Ca^{2+} entry step and a basolateral exit step from the tubular cell. Khalifa et al. [3] have investigated the nature of a possible brush-border entry step and its modulation by parathyroid hormone (PTH). Basolateral membrane Ca^{2+}-ATPase and Na^+–Ca^{2+} exchange activities [2,4,5] have been identified, either or both of which may constitute the Ca^{2+} exit step. We and others have

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demonstrated that the Na^+–Ca^{2+} exchanger possesses properties similar to the antiporter found in other membranes and it is modulated by PTH [2,5]. The Na^+–Ca^{2+} exchange activity is diminished in the thyroparathyroidectomized state and partially restored by infusion of PTH to animals prior to nephrectomy and preparation of basolateral membrane vesicles (BLMV). We also demonstrated in canine BLMV that passive Ca^{2+} permeability in the absence of a sodium gradient was diminished in the thyroparathyroidectomized state and it was increased by PTH infusion [2].

The aims of these studies were to determine whether bibutyryl cAMP would mimic the effect of PTH on these fluxes and to more closely define the nature of the passive Ca^{2+} flux.

Methods

Female mongrel dogs weighing 15–20 kg fed Purine dog chose (Ralston Purina, St. Louis, MO) were thyroparathyroidectomized under phenobarbitone anaesthesia, 25 mg/kg, 48 h prior to study and nephrectomy. This procedure was omitted for control dogs. For clearance studies the dogs were anaesthetized with phenobarbitone, an external jugular catheter inserted, the bladder catheterized and the abdominal cavity opened. An infusion of 0.9% saline at 0.8 ml/min was started and continued throughout the clearance period. Blood samples were taken before and after a 30 min urine collection after which the first kidney was removed. A second clearance study was performed in the same way after the first nephrectomy. Upon completion of the second clearance period an infusion of 50 mg dibutyryl cAMP in 20 ml of 0.9% saline at 0.8 ml/min was started and a third clearance study was performed 30 min after completion of the dibutyryl cAMP infusion. Plasma and urine calcium, phosphorus, creatinine and sodium levels were determined by using previously described methods [6].

The second kidney was removed after the dibutyryl cAMP infusion. This methodology follows the protocol previously described for the investigation of the action of PTH on BLMV [2].

BLMV were prepared as described in detail previously [2]. The kidney was perfused with ice-cold 0.9% saline after removal and the renal cortical tissue removed, diced and homogenized. After a low-speed spin in 250 mM sucrose, 20 mM Tris base and 1 mM EDTA to remove fragments from the homogenate a further centrifugation at 24000 $\times g$ was performed. The pellet was resuspended and mixed with Percoll (Pharmacia, Uppsala, Sweden) and centrifuged to produce a density gradient. The fraction enriched in BLMV was removed and washed by further centrifugation in the buffer used for the experiment. The enzyme enrichments and estimates of sidedness have been described previously [2].

To investigate the effect of La^{2+} and verapamil on Ca^{2+} uptake, BLMV from normal dogs were prepared in150 mM KCl, 20 mM Tris, 20 mM Hepes (pH 7.5) and aliquoted in 10 μl samples on ice. After equilibration at 30 °C, 110 μl of 150 mM KCl, 20 mM Hepes, 110 μM $^{45}CaCl_2$ (pH 7.5) and varying concentrations of $LaCl_3$ or verapamil were mixed and the reaction was stopped after 10 s by addition of ice-cold 150 mM KCl, 20 mM Tris, 20 mM Hepes, 1 mM EGTA (pH 7.4) and rapid filtration through 0.65 μm filters (Millipore Corp.). Radioactivity trapped on the filter was measured with a scintillation spectrometer (Hewlett-Packard model 460-CD, Downers Grove, IL). All experimental points were performed in triplicate and background Ca^{2+} binding to the filter alone was subtracted. Maximum uptake (0% inhibition) was the uptake of $^{45}Ca^{2+}$ in the absence of any inhibitor and minimum uptake (100% inhibition) was taken as the background binding of calcium to the filter alone.

The effect of La^{2+} on Ca^{2+} efflux was investigated using BLMV from thyroparathyroidectomized dogs prepared in 150 mM KCl, 5 mM Hepes (pH 7.3). The vesicles, 10 μl, were preincubated at 30 °C with 40 μl of 150 mM KCl, 5 mM Hepes, 100 μM $^{45}CaCl_2$ for 30 min. Efflux was initiated by 10-fold volume dilution into either: (i) 150 mM KCl, 5 mM Hepes (pH 7.3); (ii) 150 mM KCl, 5 mM Hepes, 0.5 mM $LaCl_3$ (pH 7.3): (iii) 150 mM NaCl, 5 mM Hepes (pH 7.3); (iv) 150 mM NaCl, 5 mM Hepes, 0.5 mM $LaCl_3$ (pH 7.3). After mixing the reaction was stopped by the addition at set times of 150 mM KCl, 5 mM Hepes, 0.5 mM $LaCl_3$ (pH 7.3) and rapid filtration.

The effect of amiloride, benzamil and diltiazem on sodium-dependent calcium flux was measured in BLMV from normal dogs prepared in 150 mM NaCl, 20 mM Tris, 20 mM Hepes (pH 7.5) and divided into 10 μl aliquots on ice. After preincubation at 30°C, 100 μl of uptake solution containing either 150 mM NaCl, 20 mM Tris, 20 mM Hepes, 100 μM $^{45}CaCl_2$ (pH 7.5) or 150 mM KCl, 20 mM Tris, 20 mM Hepes, 100 μM $^{45}CaCl_2$ (pH 7.5) and varying concentrations of inhibitors were added. Uptake was terminated after 10 s by the addition of ice-cold 150 mM KCl, 20 mM Tris, 20 mM Hepes, 2 mM EGTA (pH 7.5) and rapid filtration.

Sodium-dependent uptake was assessed as the difference between $^{45}CaCl_2$ uptake with no sodium gradient (NaCl uptake solution) and the outwardly directed sodium gradient (KCl uptake solution). The effect of benzamil on Ca^{2+} efflux was measured using BLMV prepared from normal dogs and the final buffer contained either 150 mM KCl, 20 mM Tris, 20 mM Hepes (pH 7.5) or 300 mM sucrose, 20 mM Tris, 20 mM Hepes (pH 7.5). The BLMV, 10 μl, were preincubated at 30°C in either 150 mM KCl, 20 mM Tris, 20 mM Hepes, 40 μM $^{45}CaCl_2$ (pH 7.5) or 300 mM sucrose, 20 mM Tris, 20 mM Hepes, 40 μM $^{45}CaCl_2$ (pH 7.5). Efflux was initiated by the addition of either: (i) 150 mM KCl, 20 mM Tris, 20 mM Hepes, 1 mM EGTA ± 1 mM benzamil (pH 7.5); or (ii) 300 mM sucrose, 20 mM Tris, 20 mM Hepes, 1 mM EGTA ± 1 mM benzamil (pH 7.5). Efflux was terminated by the addition of ice-cold 150 mM KCl, 20 mM Tris, 20 mM Hepes, 1 mM EGTA (pH 7.5) or 300 mM sucrose, 20 mM Tris, 20 mM Hepes, 1 mM EGTA (pH 7.5).

Analysis of the results was by paired Student's *t* test for dibutyryl cAMP–thyroparathyroidectomy vs. thyroparathyroidectomy experiments and unpaired Student's *t* test for comparison of normal vs. manipulated. All BLMV experimental points were performed in triplicate and the results described represent the mean of at least three experiments.

$^{45}CaCl_2$ (14–17 Ci/g Ca^{2+}) was obtained from New England Nuclear, Amiloride and benzamil were donated by Merck, Sharp and Dohme, West Point, PA. Diltiazem was donated by Marion Laboratories, Kansas City, MO and dibutyryl cAMP was obtained from Sigma Chemical, St. Louis, MO. Verapamil was obtained from Knoll Pharmaceutical, Whippany, NJ. All other chemicals were of the highest purity available from commercial sources. All solutions were filtered through 0.45 μm Millipore filters on the day of experiment prior to use.

Results

The effects of dibutyryl cAMP on renal calcium and phosphate clearance are shown in Fig. 1. The protocol used for these experiments was identical to that previously used for PTH [2]. The dose of dibutyryl cAMP was the same as that used by Agus et al. [7] to mimic the effects of PTH on the proximal tubule. The data show that an infusion of dibutyryl cAMP, produces significant phosphaturia. This effect was not due to the removal of the control kidney since the fractional excretion of phosphate did not rise following unilateral nephrectomy, dibutyryl cAMP in this dose produces the same phosphaturic effect as the 2 μg/kg dose of PTH used in the previous studies [6]. The phosphaturia was associated with a significant fall

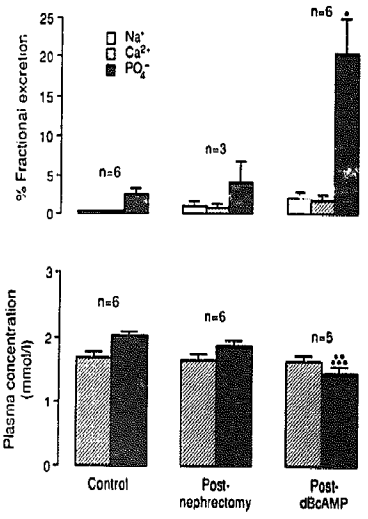

Fig. 1. The effect of dibutyryl cAMP (dBcAMP) infusion on the fractional excretion of sodium, calcium and phosphate and plasma calcium and phosphate. The clearance studies were performed as described in Methods. * $P < 0.05$ compared with post-nephrectomy, ** $P < 0.001$ compared with control, *** $P < 0.002$ compared with post-nephrectomy.

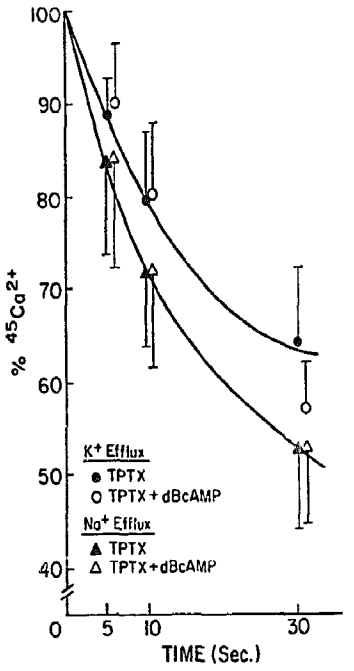

Fig. 2. The effect of dibutyryl cAMP (dBcAMP) on $^{45}Ca^{2+}$ efflux from BLMV exposed to symmetrical KCl solutions or to sodium gradients directed from out to in. BLMV were prepared from kidneys of thyroparathyroidectomized (TPTX) animals (●, ▲) and thyroparathyroidectomized animals administered dibutyryl cAMP (○, △) using a buffer of 150 mM KCl, 20 mM Tris, 20 mM Hepes (pH 7.5) in the final vesicular loading steps as described in Methods. BLMV, 10 μl, were preincubated at 30 °C with 40 μl of 150 mM KCl, 20 mM Tris, 20 mM Hepes, 100 μM $^{45}CaCl_2$ (pH 7.5) for 30 min and calcium efflux was initiated by dilution with 150 mM KCl, 20 mM Tris, 20 mM Hepes, 1 mM EGTA (pH 7.5) (○, ●) or 150 mM NaCl, 20 mM Tris, 20 mM Hepes, 1 mM EGTA (pH 7.5) (△, ▲). Efflux was terminated by addition of ice-cold stop solution containing either 150 mM KCl or NaCl, 20 mM Tris, 20 mM Hepes (pH 7.5), depending on the solutions used to initiate efflux followed by rapid filtration as described in Methods.

in plasma phosphate concentration. There was no significant effect on plasma calcium.

The effect of dibutyryl cAMP on $^{45}Ca^{2+}$ efflux is shown in Fig. 2. The experimental methods used for this study were the same as those previously utilized to demonstrate the action of PTH on Ca^{2+} efflux from BLMV [2] except that the sodium gradient effect was maximized by the use of 150 mM NaCl in the efflux solution instead of 100 mM NaCl. There was no significant difference in the sodium-stimulated calcium efflux between BLMV prepared from the control kidney and BLMV from the kidney removed following dibutyryl cAMP infusion. These results indicate that in a setting where dibutyryl cAMP mimics the effect of PTH on phosphate transport [7,8] it did not reproduce the effect of PTH on the Na^+-Ca^{2+} exchange activity. Furthermore, passive calcium fluxes in conditions of potassium equivalency were not affected by dibutyryl cAMP infusion. Again this is in contrast to the results observed following PTH infusion which increased calcium permeability of the membrane [2]. Thus the action of PTH on Na^+-Ca^{2+} exchange and the passive calcium flux must be either direct or via a second messenger other than cAMP as has been demonstrated for the stimulation of renal phoshoinositide metabolism [9] and gluconeogenesis [10]. The inhibition of $^{45}Ca^{2+}$ uptake by verapamil and lanthanum is shown in Fig. 3. Verapamil in the range of concentrations known to inhibit calcium channels [11] did not inhibit $^{45}Ca^{2+}$ uptake by the BLMV. These experiments were performed under conditions such that transmembrane voltage was minimal. Thus they did not address the presence or absence of a verapamil effect in the presence of a membrane potential, although this was also absent in the BLMV when the calcium permeability was demonstrated. Lanthanum inhibited calcium

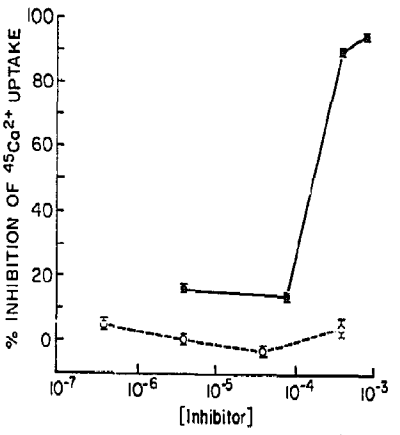

Fig. 3. Lanthanum and verapamil inhibition of $^{45}Ca^{2+}$ uptake. BLMV were prepared as described in Methods. $^{45}Ca^{2+}$ uptake in the presence of varying concentrations of (●) $LaCl_3$ or (○) verapamil is expressed as percent inhibition compared to $^{45}Ca^{2+}$ uptake in the absence of either inhibitor.

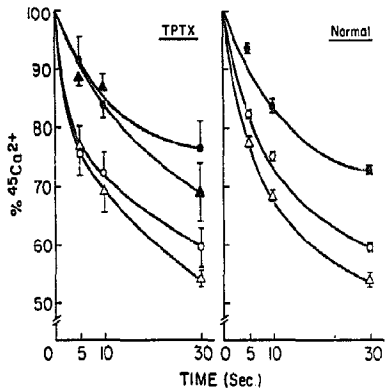

Fig. 4. Inhibition of $^{45}Ca^{2+}$ efflux by lanthanum. BLMV from thyroparathyroidectomized (TPTX) dogs were prepared and preincubated as described in the methods. Efflux was initiated by dilution into: (○) 150 mM KCl, 5 mM Hepes (pH 7.3), (●) 150 mM KCl, 5 mM Hepes, 0.5 mM $LaCl_3$ (pH 7.3), (△) 150 mM NaCl, 5 mM Hepes (pH 7.3), (▲) 150 mM NaCl, 5 mM Hepes, 0.5 mM $LaCl_3$ (pH 7.3). Data previously reported [2] showing similar studies in BLMV prepared from kidneys of normal animals are shown on the right for purposes of comparison.

uptake in a concentration-dependent manner. Under potassium equivalent conditions 'passive' calcium flux is inhibitable by lanthanum but not by the calcium-channel blocker verapamil.

Inhibition of $^{45}Ca^{2+}$ efflux in thyroparathyroidectomized BLMV using lanthanum is shown in Fig. 4. Under potassium equivalent conditions for the BLMV, 0.5 mM lanthanum inhibited passive calcium efflux. The data from BLMV prepared from normal dogs demonstrate a significant sodium-stimulated calcium efflux at 5 and 10 s of incubation. The effect of thyroparathyroidectomy on sodium-stimulated calcium efflux redocuments the modulation of Na^+–Ca^{2+} exchange activity by PTH previously reported [2]. Higher concentrations of lanthanum more completely inhibited passive $^{45}Na^+$ efflux and uptake but were difficult to maintain in solution using the pH and buffer conditions similar to our previously described experiments. The activity of the Na^+–Ca^{2+} exchanger did not appear to be completely inhibited by lanthanum. The effect of lanthanum on the Na^+–Ca^{2+} exchanger in other tissues is variable as discussed by Eisner and Lederer [12].

The effect of amiloride, benzamil and diltiazem on Na^+–Ca^{2+} exchange is shown in Fig. 5. Benzamil inhibited Na^+–Ca^{2+} exchange in BLMV from canine proximal tubule with an apparent IC_{50} of 250 μM between the values found for the IC_{50} of benzamil on sodium-dependent calcium exchange in sarcolemmal vesicles from guinea pig heart described by Siegl et al. [13], 120 μM, and that described for the IC_{50} of the bovine heart Na^+–Ca^{2+} exchanger, 400 μM [14]. Amiloride inhibited sodium-dependent calcium exchange in this preparation but the potency was lower than for benzamil, in keeping with the IC_{50} reported by Siegl et al. for guinea pig sarcolemma [13] but with a lower potency than reported by Schellenberg et al. [15] for rat cerebral cortex plasmalemma-enriched vesicles. Diltiazem, a calcium-channel blocker, which has also been reported to have an inhibitory effect on sodium-induced calcium release by isolated rabbit heart mitochondria [16], produced some inhibition at high concentrations but was less potent than amiloride or benzamil at the highest concentration used. It is possible that diltiazem may inhibit Na^+–Ca^{2+} exchange significantly at higher but not pharmacologically useful concentrations.

The effect of benzamil on passive calcium efflux is shown in Fig. 6. Benzamil had no significant inhibitory effect on passive calcium efflux at the early time points nor was there an enhance-

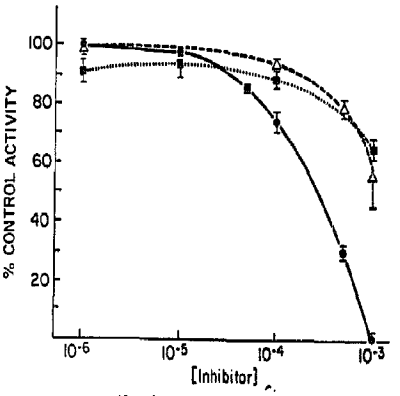

Fig. 5. Inhibition of $^{45}Ca^{2+}$ uptake by amiloride, benzamil and diltiazem. BLMV prepared in 150 mM NaCl, 20 mM Tris, 20 mM Hepes (pH 7.5) were mixed with solutions containing either NaCl or KCl plus varying concentrations of inhibitors. Results are expressed as percent of sodium gradient-stimulated uptake activity in the presence of (△) amiloride, (●) benzamil and (▩) diltiazem as described in the text.

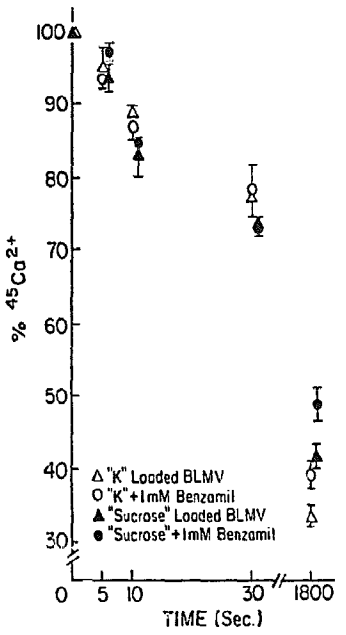

Fig. 6. Inhibition of $^{45}Ca^{2+}$ efflux by benzamil. BLMV were prepared in (Δ, ○) KCl, or (▲, ●) sucrose and preincubated with $^{45}Ca^{2+}$ as described in Methods. Efflux was initiated by addition of: (Δ) 150 mM KCl, 20 mM Tris, 20 mM Hepes, 1 mM EGTA (pH 7.5); (○) 150 mM KCl, 20 mM Tris, 20 mM Hepes, 1 mM EGTA, 1 mM benzamil (pH 7.5); (▲) 300 mM sucrose, 20 mM Tris, 20 mM Hepes, 1 mM EGTA (pH 7.5); or (●) 300 mM sucrose, 20 mM Tris, 20 mM Hepes, 1 mM EGTA, 1 mM benzamil (pH 7.5). Efflux was initiated by addition of stop solution and rapid filtration as described in the text.

ment of passive calcium flux in BLMV prepared in KCl compared to those prepared in sucrose. However at the later time points, 30 min, these effects were present. Slaughter et al. [17] have demonstrated that in bovine cardiac sarcolemmal vesicles there can be significant Ca^{2+}–Ca^{2+} exchange, presumably through the Na^+–Ca^{2+} exchange mechanism. This Ca^{2+}–Ca^{2+} exchange was potentiated by the presence of potassium on both sides of the membrane although it did not seem to involve co-transport of the cation when ^{86}Rb fluxes were measured. The lack of potentiation of calcium efflux from BLMV prepared in potassium-containing solutions versus sucrose at the early time points and the lack of effect of benzamil demonstrate the absence of any Ca^{2+}–Ca^{2+} exchange through the Na^+–Ca^{2+} exchanger during the early time points which have been used for the kinetic analyses [1,4]. However at 30 min there was enhancement of calcium efflux by the presence of potassium versus sucrose, and that efflux was partially inhibitable by benzamil. This suggests that Ca^{2+}–Ca^{2+} exchange does occur through the Na^+–Ca^{2+} exchange mechanism.

Discussion

Most of the effects of PTH on the proximal tubular cell have been shown to be mimicked by cAMP analogues indicating a role for it as a second messenger for PTH. The presence of a PTH-stimulated adenylate cyclase [18] system supports this mediation. Effects of PTH have, however, been demonstrated which are not mediated by cyclic nucleotides [9,10]. Another possible second messenger for PTH in the proximal tubular cell is a rise in cytosolic calcium and it has been shown that PTH induces a rise in cytosolic calcium in suspended and individual proximal tubular cells [19,20].

The protocol used in the experiments described in this paper for dibutyryl cAMP produced the same phosphaturic effect as the dose of PTH used in previous studies [8]. The experimental design was identical to that used to demonstrate the effect of PTH on passive calcium flux and Na^+–Ca^{2+} exchange [2]. The PTH-infused kidney showed increased Na^+–Ca^{2+} exchange and passive Ca^{2+} flux in each pair of kidneys examined in our previous study. Hanai et al. [21] have demonstrated PTH stimulation of sodium-dependent calcium efflux in whole freshly isolated rat renal cortical cells. They also found that dibutyryl cAMP mimicked the action of PTH in this preparation [21] but this group has not repeated their original experiments on BLMV using dibutyryl cAMP. The interpretation of calcium changes in whole renal cells in response to changes in external sodium is a more complex procedure than calcium flux changes in BLMV in response to sodium gradients. Snowdowne and Borle [22] have shown that in the LLC-MK_2 renal cell line a sodium-free medium stimulates calcium efflux [22]. We have not been able to reproduce the effect of dibutyryl cAMP on sodium-stimulated calcium efflux in cultured canine proximal tubules (Hruska, K.A.,

unpublished results) and this may represent a difference between the rat and canine kidney. Fraser et al. [23] have shown that there is a Na^+–Ca^{2+} exchanger in rat synaptosomes and have further reported in abstract form that PTH will activate the synaptosome Na^+–Ca^{2+} exchanger in vitro by a mechanism independent of cAMP production [24]. In the experiments reported in this paper it was impossible to demonstrate a difference between control and dibutyryl cAMP-infused kidneys. No other paper has directly addressed this problem and interpretation of data from other preparations is difficult, although the synaptosome vesicle data would appear to find a similar effect independent of cAMP on the Na^+–Ca^{2+} exchanger.

The nature of the coupling of the PTH receptor binding to activation of Na^+–Ca^{2+} exchange and increase in passive Ca^{2+} permeability remains unclear, although recent work in other cells would suggest that inositol trisphosphate-mediated changes in cytosolic Ca^{2+} and/or phospholipid-stimulated protein kinases could be candidates [25]. It has recently been shown that PTH both stimulates release of inositol trisphosphate by proximal tubular cells [26] and causes a rise in cytosolic calcium concentration [19]. The data presented here show that passive Ca^{2+} flux seen in the BLMV is not inhibited by calcium-channel blockers nor Na^+–Ca^{2+} exchange inhibitors but is inhibitable by lanthanum. This does not exclude the presence of calcium-channel blocker-inhibitable Ca^{2+} in other conditions, although calcium channels have not been demonstrated in proximal tubular cells. Unfortunately in concentrations often used with proximal tubular cells, calcium-channel blockers have a variety of other effects [11]. Work in the platelet [27] has demonstrated a Ca^{2+} permeability that is inhibitable by low concentrations of lanthanum but not verapamil. This calcium permeability seemed to correlate with the presence of glycoproteins IIb and IIIa [28]. Thus a Ca^{2+} permeability which is not a classic voltage-dependent calcium channel but is lanthanum-inhibitable has been described in another system. The changes in passive Ca^{2+} permeability produced by PTH [2] but not by dibutyryl cAMP could be alterations in a similar glycoprotein. Irvine and Moor [29,30] have shown that micro-injection of inositol 1,3,4,5-tetrakisphosphate in the presence of inositol 2,4,5-trisphosphate causes activation of sea urchin eggs by a mechanism which is dependent on external calcium. Kuno and Gardner [31] have shown that inositol 1,4,5-trisphosphate activates voltage-insensitive calcium channels in human T-lymphocytes. A mechanism similar to these in the proximal tubular basolateral membrane Ca^{2+} permeability would be compatible with the findings described in this paper.

The demonstration of inhibition of the Na^+–Ca^{2+} exchanger in BLMV by similar concentrations of benzamil confirms the exchanger's similarity to the exchanger found in other systems. It also raise the possibility of the investigation of the importance of the Na^+–Ca^{2+} exchanger in the intact cell by use of this specific inhibitor. Preliminary data suggest [32] that benzamil inhibits transepithelial Ca^{2+} flux and causes Ca^{2+}-dependent cell toxicity.

The importance of the Na^+–Ca^{2+} exchanger and passive Ca^{2+} flux depends on the presence of transcellular calcium transport as to whether they represent cell homeostatic mechanisms or a means of producing and modulating active Ca^{2+} reabsorption. One criticism of the importance of transcellular transport often made [33] has been the difficulty of having a varying apical to basolateral calcium gradient within the cell, although various models have been proposed [1] invoking calcium-binding proteins within the cell. Wide variations in cytosolic Ca^{2+} concentrations to achieve Ca^{2+} reabsorption might be expected to cause disarray in the many proximal tubular metabolic functions dependent on cytosolic calcium. A further argument against transcellular Ca^{2+} fluxes could be inferred from the work reviewed by Berridge and Irvine [25] where it has been shown that the endoplasmic reticulum can actively buffer the ambient cytosolic (or cytosolic and external medium when permeabilized) Ca^{2+} within a narrow range. This would make the presence of a calcium gradient within the cell unlikely. However the single-lumen endoplasmic reticulum acting as a calcium-buffering organelle may represent a means of magnifying a calcium gradient by taking up Ca^{2+} at the apical side and releasing it at the basolateral side (Fig. 7). Thus fluctuations in Ca^{2+} flux would be confined to a compartment,

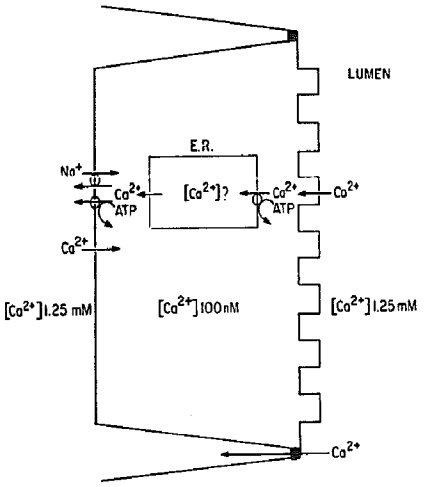

Fig. 7. Potential calcium transport mechanisms in the proximal tubule.

the endoplasmic reticulum, which within the hepatocyte has been found to contain 5–10 mmol/kg dry weight of calcium [34].

The model of Ca^{2+} transport shown in Fig. 7 would integrate the observations made in this paper with the other published data into a mechanism for transcellular calcium transport. Apical calcium entry into the cytosol would be locally offset by uptake into the endoplasmic reticulum. This would create an intra-endoplasmic reticulum concentration gradient favouring movement of Ca^{2+} away from the apical surface within the endoplasmic reticulum. Efflux of calcium at the basolateral surface by the Ca^{2+}-ATPase or Na^{+}–Ca^{2+} exchanger of the cell would be locally buffered by the release of Ca^{2+} from the endoplasmic reticulum, thus favouring any gradient within the endoplasmic reticulum. However increased basolateral calcium permeability to Ca^{2+} in the absence of any increase in calcium efflux rate would lead to the endoplasmic reticulum uptake of Ca^{2+} and inhibit a transcellular calcium flux. Thus PTH would decrease active calcium reabsorption of Ca^{2+} in the proximal tubule by increasing basolateral Ca^{2+} permeability in spite of its ability to stimulate the Na^{+}–Ca^{2+} exchanger. These experiments demonstrate that this effect, however, is not mediated by cAMP. Each of the steps in this model of calcium flux across the cell have been described in various cells or subcellular systems [2–4,25]. This model requires further investigation but no new mechanisms need be postulated to achieve transcellular calcium reabsorption in the proximal tubule. In the proximal tubular cell this mechanism for transcellular calcium transport is inhibited by PTH acting through an increase in basolateral membrane calcium permeability, in keeping with the data which show that PTH causes a decrease in proximal tubular calcium reabsorption.

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