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REGULATION OF CANINE RENAL VESICLE P_i TRANSPORT BY GROWTH HORMONE AND PARATHYROID HORMONE

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Summary

Renal phosphate (P_i) reabsorption is increased by growth hormone (GH) and decreased by parathyroid hormone (PTH). Na^+-stimulated P_i transport across the brush border membrane of the proximal tubule is the initial step in the process of P_i reabsorption. To determine whether changes in P_i reabsorption induced by GH or PTH are accompanied by changes in brush border membrane Na^+-gradient-stimulated P_i transport, we examined the effect of in vivo GH and PTH administration and thyroparathyroidectomy on P_i transport by isolated brush border membrane vesicles prepared from canine kidney. In experiments in which the effect of PTH administration was examined, the same animal provided the control kidney (before PTH administration) and the experimental kidney (after PTH administration). The Na^+-gradient P_i overshoot in vesicles isolated from normal, GH-treated and thyroparathyroidectomized dogs was increased after in vivo PTH administration. GH administration and thyroparathyroidectomy increased the height of the overshoot compared to normal. PTH administration decreased the apparent V value by 44% in vesicles from normal animals. The apparent V value was increased, compared to normal, by GH (34%) and thyroparathyroidectomy (57%). PTH administration decreased the apparent V in both the latter groups. GH administration to thyroparathyroidectomized dogs further increased the apparent V. Changes in the apparent V paralleled changes in P_i reabsorption in vivo induced by experimental manipulations. We conclude that changes in renal P_i reabsorp-

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Abbreviation: SDS, sodium dodecyl sulfate; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

tion induced by GH were like those induced by PTH, accompanied by changes in the Na^+-stimulated P_i transport system in the renal brush border membrane, and that the effect of PTH on vesicular P_i transport in GH-treated dogs did not differ from the effect on vesicles from normal animals.

Introduction

Administration of growth hormone (GH) to man and experimental animals results in increased renal reabsorption of phosphate (P_i) [1—4]. Parathyroid hormone (PTH) decreases renal reabsorption of P_i by inhibiting P_i transport in the proximal and distal renal tubule [5]. PTH decreases renal P_i reabsorption in GH-treated animals [4]. GH increases P_i reabsorption in parathyroidectomized animals [4]. The exact mechanisms by which GH and PTH effect renal reabsorption of P_i are unknown. However, it has been demonstrated in experimental animals that in vivo administration of PTH or parathyroidectomy induces changes in the Na^+-stimulated P_i transport of brush border membrane vesicles isolated from the kidneys of those animals [6—11]. These changes in P_i transport of brush border membrane vesicles parallel the changes in P_i transport which occur in vivo in response to the above manipulations.

Previous studies have characterized the transport of P_i by brush border membrane vesicles and the effects of PTH administration or parathyroidectomy on this transport. The present studies extend these observations to include the effect of GH administration in vivo on P_i transport by isolated renal brush border membranes and the effects of PTH administration in vivo or parathyroidectomy on isolated P_i transport by brush border membranes of dogs previously given GH. We used the dog as an experimental animal because its serum P_i is similar to that of man and because its large kidney size enable us to isolate sufficient brush border membranes to perform experiments from both a control kidney (prior to PTH administration) and an experimental kidney (after PTH administration) originating from the same animal in experiments where the PTH effect was studied. The use of the dog also enabled us to measure readily alterations in in vivo P_i reabsorption induced by experimental manipulations and to compare them with alterations in isolated brush border membrane Na^+-stimulated P_i transport. We have confirmed previously reported observations made in normal, PTH-treated and parathyroidectomized animals to validate our experimental model. Some of our findings have been reported in abstract form [12,13].

Methods

Mongrel dogs, weighing 14—25 kg and fed a high-protein Purina Dog Chow (Ralston Purina, St. Louis, MO), were anesthesized with pentobarbitol (0.12 g/kg, given slowly intravenously) and ventilated mechanically through an endotracheal tube. A jugular vein catheter was placed for obtaining blood samples and for infusion of solutions. A urinary bladder cathether was placed for collection of urine. Baseline collections for determinations of endogenous creatinine and P_i clearances were obtained after which a kidney was removed.

After the first nephrectomy (left kidney) in experiments for assessing the effect of PTH, a second baseline clearance period was obtained from the now single experimental kidney. Then, when indicated, bovine parathyroid hormone (b-PTH 1-84) (Inolex, Park Forest, IL, 1500 U/mg in a rat hypercalcemic assay) was administered intravenously at a dose of 2 μg/kg of body weight. 30 min after administration of PTH, a 15 min clearance period was performed, followed immediately by right nephrectomy.

After each nephrectomy, the renal artery was immediately catheterized and 75—100 ml of ice-cold saline were infused through the arterial catheter, and the kidneys were placed in ice.

Three groups of dogs with at least three animals in each group were studied as follows: 1, normal; 2, pretreated with intramuscular injections of GH (1.2 I.U./injection per day) for 3 days prior to study; 3, total thyroparathyroidectomy 48—60 h prior to study. (Thyroid supplement of 60 mg was given at 12 and 36 h following surgery). An additional dog was studied before and after administration of oxidized b-PTH 1-84 [14] to rule out nonspecific effects of the peptide on the kidney.

Vesicles of membranes from the brush border of the kidney were isolated by a $CaCl_2$-precipitation technique described previously in detail [15,16]. The enrichment of the membrane preparation was evaluated randomly by using specific enzyme markers [17] and electron microscopy. Protein concentrations were determined using a standard procedure [18] using bovine serum albumin as the reference protein.

Uptake of $H_3^{32}PO_4$ ($^{32}P_i$) by the vesicles was measured using a Millipore-filtration technique [19,20], using 0.65 μm Millipore filters. Incubations were terminated by the rapid addition of cold 154 mM NaCl, 5 mM arsenate and 1 mM Hepes-Tris, pH 7.5, to the reaction medium. Retention of $^{32}P_i$ on the filter was determined on a liquid scintillation spectrometer. Values for the nonspecific retention of radioactivity on the filters (0.005–0.02% of the total radioactivity in the incubation mixture) were subtracted from the value of the incubation samples. All incubations were carried out in triplicate with freshly prepared membrane vesicles. The results are expressed as ±S.E. All experiments were performed on at least three separate occasions using at least nine separate incubation mixtures except where indicated.

Analysis of variance was utilized to examine differences among experimental and control data unless otherwise noted. Duncan's multiple range test [21] was utilized to determine which sample means were significantly different from the others and individual differences examined using the least-square means.

$^{32}P_i$, D-[2-^{3}H(n)]glucose (18.1 Ci/mmol) and ^{22}NaCl were obtained from New England Nuclear, Boston, MA. Other chemicals were of the highest purity available from commercial sources. Highly purified human GH was obtained as the kind gift of Dr. William H. Daughaday and Dr. James R. Gavin, III. All solutions were filtered through 0.45 μm Millipore filters prior to use [22].

Plasma and urine levels of creatinine and phosphorus were determined by using previously described methods [23]. Calcium was determined using atomic absorption spectrophotometry. In vivo P_i reabsorption (T_{P_i}) was determined from the differences between the filtered load of phosphorus and the absolute urinary excretion of P_i.

Results

Biochemical characterization of renal brush border membrane vesicles

The enzyme content of membrane vesicles from the brush border as compared to crude homogenates was not enriched for mitochondrial, endoplasmic reticulum, or basolateral membrane enzyme markers [17]. Enrichment for alkaline phosphatase and trehalase, both brush border membrane markers, was 9—12-fold. PTH, GH administration and thyroparathyroidectomy did not influence brush border enzyme enrichment or contamination of brush border membranes with mitochondrial, lysozomal, endoplasmic reticulum, or basolateral membrane markers, in agreement with findings reported previously [6].

Na^+-gradient-dependent uptake of P_i

The uptake of 25 μM $^{32}P_i$ by renal brush border membrane vesicles as a function of time of incubation is illustrated in Fig. 1. In the presence of a 100 mM KCl gradient (extravesicular > intravesicular), the initial rate of uptake was low and steady-state levels (approx. 90 pmol P_i/mg protein) were reached in 90 min. In the presence of a 100 mM NaCl gradient (extravesicular > intravesicular), the uptake of P_i was markedly stimulated. The initial (20 s) rate was approx. 350 pmol/mg of membrane protein with the NaCl gradient as compared to 15 pmol/mg of membrane protein with the KCl gradient. Na^+-gradient-dependent accumulation of P_i peaked at 1 min. Thereafter, the amount of P_i in the vesicles decreased, indicating efflux. The 90 and 120 min levels of P_i uptake in the presence of the NaCl gradient were the same, approx. 170 pmol/mg of protein, suggesting that a steady-state was established. Steady-state uptake of 25 μM D-[2-^{3}H(n)]glucose under the same conditions using the same brush border membrane preparations was 25.3 ± 0.3 pmol/mg of membrane protein. At the peak of the Na^+-gradient-dependent P_i 'overshoot' (1 min), the accumulation of P_i was about 6-times the final steady-state value. This finding suggested that the imposition of a large extravesicular-to-intravesicular

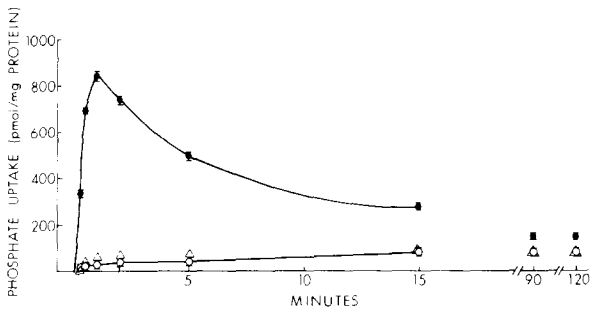

Fig. 1. The time course of uptake of 25 μM $^{32}P_i$ by renal brush border membrane vesicles. When uptake was measured in the presence of an extravesicular to intravesicular gradient of NaCl (●) or KCl (○), the vesicles were preloaded with 300 mM buffered mannitol and incubation initiated with the addition of a medium containing P_i and 100 mM salt replacing mannitol osmotically. When uptake was measured in the presence of 100 mM NaCl but in the absence of an Na^+ gradient (△), the membrane vesicles were preloaded with 100 mM mannitol, 100 mM NaCl and 5 mM Hepes-Tris (pH 7.5), and incubation begun with the addition of the same medium containing $^{32}P_i$. Data are reported as the mean ±S.E.

Na^+ electrochemical graident provided the driving force to effect the transient movement of P_i into the vesicles against its concentration gradient. When the membrane vesicles were preloaded with 100 mM NaCl and the uptake of P_i determined with 100 mM NaCl in the extravesicular medium (Fig. 1), the overshoot was abolished. This result indicated that it was not the concentration of Na^+ per se but the electrochemical gradient that was crucial in energizing uphill transport of P_i into the brush border membrane vesicles.

It was found (data not shown) that the initial rate of P_i uptake under these experimental conditions was proportional to the concentration of brush border membrane protein over the range used. Also, the uptake of P_i in the presence of an NaCl or KCl gradient was a linear function of time for at least 30 s of incubation. Thus, in subsequent experiments, the uptake of 20 s was used to estimate the initial unidirectional rate of uptake of P_i.

The initial rate of P_i uptake was approx. 23-times greater in the presence of an NaCl gradient than in the presence of a KCl gradient. These results eliminated the presence of a Cl^- gradient as a factor which might have accounted for the observed stimulation with the NaCl electrochemical gradient. In addition, they indicated that an Na^+ chemical gradient rather than a K^+ chemical gradient was necessary to generate an overshoot. It is likely that the Na^+ electrical gradient, however, also supported the concentrative uptake of P_i [15].

The question as to whether the uptake of P_i by the membranes represented transport into membrane vesicles, binding to the membrane, or both, was examined by determining the effect of extravesicular medium osmolality on $^{32}P_i$ uptake and by experiments measuring exchange diffusion.

In the experiments shown in Fig. 2, intravesicular space was decreased by increasing the osmolality of the extravesicular medium with sucrose, a relatively impermeable solute which is not hydrolyzed in the kidney [24]. When sucrose was added at time zero (Fig. 2A), uptake of 25 μM P_i was found to be inversely proportional to the osmolality of the extravesicular medium from 0.3 to 0.86 M, at incubation times of 20 s, 1 min and 90 min and thus directly related to intravesicular space. Extrapolation of Fig. 2A to infinite extravesicular osmolality (dashed line) demonstrated no uptake that was

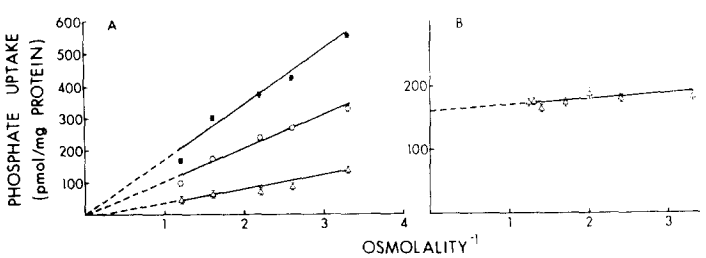

Fig. 2. Effect of osmolality of the medium on the uptake of 25 M $^{32}P_i$ by brush border membrane vesicles. Membrane vesicles were preloaded with 300 mM buffered mannitol and 100 mM NaCl-stimulated uptake measured after 20 s (○), 1 min (●) and 90 min (△) of incubation. The extravesicular medium osmolality was adjusted with sucrose. Sucrose was added at time zero (A) or at 1 min of incubation (B). Data are reported as mean ±S.E.

independent of intravesicular space, which would represent P_i binding to vesicles.

When sucrose was added after 1 min of incubation and P_i uptake determined 90 min later (Fig. 2B), uptake of 25 μM P_i was to a considerable extent independent of the osmolality of the extravesicular medium. Extrapolation of the regression (dashed line) to infinite osmolality of the extravesicular medium demonstrated that 85% of P_i uptake at steady state was independent of intravesicular space, indicating probable P_i binding to vesicles. An identical experiment which determined the effect of sucrose added after 1 min of incubation on D-glucose uptake 90 min later showed less than 10% binding (data not shown), demonstrating that the findings illustrated in Fig. 2B were not an artifact of the experimental techniques such as might be expected if addition of sucrose at 1 min of incubation resulted in lysis of membrane vesicles.

Trichloroacetic acid precipitation of brush border membrane protein incubated for 90 min revealed no membrane phosphorylation. No protein band stained in an SDS-polyacrylamide gel of the brush border membrane preparation exhibited a positive autoradiogram and no lipid spots of an extract of brush border membrane lipid had $^{32}P_i$ detectable above the background on thin-layer chromatography.

Evidence that a portion of the P_i uptake by brush border membranes at early times of incubation represented transport into membrane vesicles rather than binding was obtained from experiments measuring $^{32}P_i$ transport into mem-

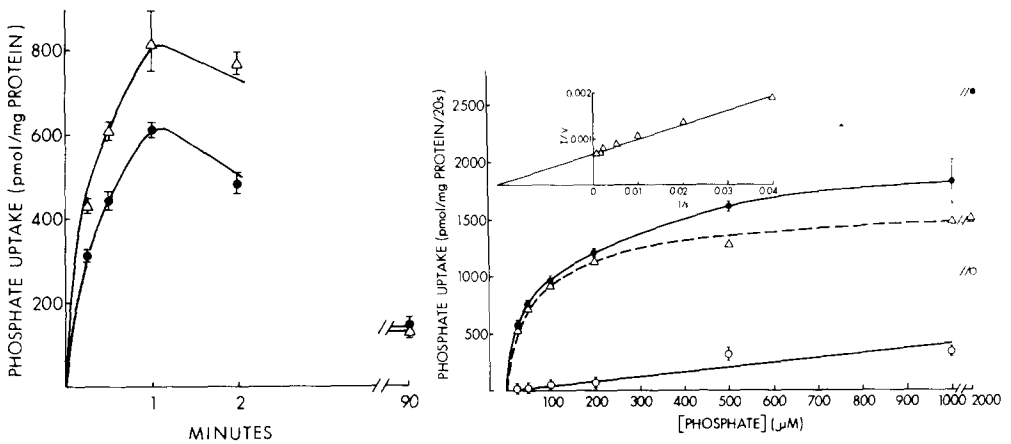

Fig. 3. Effect of preloading membrane vesicles with P_i on the uptake of $^{32}P_i$. Membrane vesicles were preloaded with 125 μM unlabeled P_i in 300 mM buffered mannitol (△) or with the mannitol medium in the absence of P_i (●). The uptake of 25 mM $^{32}P_i$ by these vesicles was determined in the presence of an initial 100 mM Na^+ gradient. Data are reported as the mean ±S.E. The two uptake curves differed significantly at times 15 s, 30 s, 1 min and 2 min. At these times preloaded uptake > non-preloaded uptake; $P < 0.0001$.

Fig. 4. The relationship between P_i concentration and the Na^+-gradient-dependent and independent initial rates of P_i uptake. Incubations were for 20 s in the presence of 100 mM NaCl (●) or KCl (○) replacing the buffered mannitol isosmotically at the initiation of the incubation. The dashed line (△- - - - -△) represents the net Na^+-gradient-dependent uptake obtained by subtracting the uptake in the presence of NaCl, at each concentration of P_i. The inset shows a double-reciprocal plot of the net Na^+-gradient-dependent transport system. Data are reported as the mean ±S.E.

brane vesicles preloaded with 125 μM unlabelled P_i in buffered mannitol or with the mannitol medium in the absence of P_i (Fig. 3). The uptakes of 25 μM $^{32}P_i$ by these vesicles were compared in the presence of an initial 100 ml NaCl gradient. Membrane vesicles preloaded with unlabeled P_i had greater uptakes of $^{32}P_i$ during the first 2 min of uptake relative to those that were not preloaded. At steady state (90 min) the amounts taken up were identical. This finding, consistent with a demonstration of accelerated exchange diffusion, provides evidence that at least the difference between P_i uptake observed under non-preloaded and preloaded conditions is representative of carrier-mediated transport. If P_i uptake were all binding, pre-incubation with unlabeled P_i would have occupied binding sites and, if anything, would have inhibited the uptake of $^{32}P_i$.

Apparent kinetics of the Na^+-gradient-dependent uptake of P_i

The effect of different concentrations of P_i on the initial rate (20 s) of transport in the presence of a 100 mM Na^+ or K^+ electrochemical gradient is shown in Fig. 4. In the presence of the K^+ gradient, the rate of P_i uptake increased linearly with increasing P_i concentration throughout the range from 10 μM to 2 mM. Thus, the Na^+-independent transport system for P_i appeared not to saturate. In contrast, in the presence of the Na^+ gradient, the relationship between P_i concentration and rate of uptake was nonlinear, providing evidence for saturability. At higher P_i concentrations, however, uptake was more linearly related to P_i concentration, proceeding with a pattern similar to that found with the K^+ gradient. If at each P_i concentration the Na^+-free uptake was substracted from the uptake measured in the presence of an Na^+ gradient, a curve (Fig. 4, dashed line) was obtained which described a completely saturable net Na^+-gradient-dependent P_i transport process. This system was saturated at about 1 mM P_i. Uptake at 20 s of 100 μM $^{22}NaCl$ by membrane vesicles did not vary significantly over the P_i concentration range of 10 μM to 2 mM (data not shown). A double-reciprocal plot of the initial rate of the 100 mM net Na^+-gradient-dependent uptake as a function of P_i concentration (Fig. 4, inset) describes a linear relationship with a calculated apparent K_m value of 51 μM and an apparent V value of 1603 μM P_i/mg of membrane protein per 20 s.

Effects of PTH and GH on transport properties of brush border membrane vesicles

Na^+-gradient-dependent uptakes of 25 μM P_i over time and double-reciprocal plots of P_i uptake calculated as described above (insets) are illustrated in Figs. 5—7 for: (a) brush border membrane vesicles isolated from normal dogs, and the same animals given PTH (Fig. 5); (b) brush border membrane vesicles isolated from GH-treated dogs and the same animals given PTH (Fig. 6); and (c) brush border membrane vesicles isolated from thyroparathyroidectomized dogs and the same animals given PTH (Fig. 7). Physiologic data from intact animals showing plasma Ca^{2+} and P_i levels, creatinine clearance and tubular reabsorption values of P_i prior to first nephrectomy, after first nephrectomy prior to PTH administration, and after PTH administration are displayed in Table I. GH administration resulted in hyperphosphatemia. Thyroparathyroid-

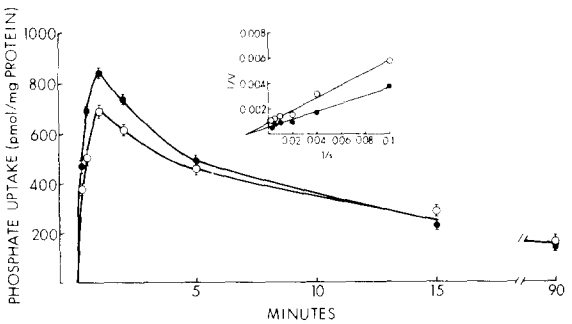

Fig. 5. The time course of the Na^+-gradient-dependent uptake of 25 μM $^{32}P_i$ by renal brush border membrane vesicles isolated from normal dogs (•) and normal dogs given PTH in vivo (○). Data are reported as the mean ±S.E. The inset shows a double-reciprocal plot of the net Na^+-gradient-dependent transport system in vesicles isolated from normal dogs (•) and normal dogs treated with PTH in vivo (○). Experiments were performed as outlined in Figs. 1 and 4. Normal overshoot peak > PTH; $P < 0.0005$.

ectomy reduced plasma calcium and increased plasma P_i. Every dog responded to PTH administration with a decrease in tubular reabsorption of P_i. Administration of oxidized PTH did not change tubular readsorption of P_i (data not shown).

Data comparing apparent K_m and apparent V values obtained from multiple double-reciprocal plots performed as described above are presented in Table II. Initial rates (20 s) of P_i uptake in those experiments with an initial extravesicular > intravesicular K^+ gradient did not vary significantly (data not shown). Table II also lists T_{P_i} for experimental and control animals, a measure of in vivo P_i reabsorption.

Figs. 5—7 demonstrate that PTH administered in vivo decreased the Na^+-gradient-dependent P_i overshoot in vesicles isolated from normal, GH-treated and thyroparathyroidectomized dogs. Steady-state (90 min) levels of P_i uptake were identical among pairs. The peak of the overshoot was significantly higher in membranes isolated from thyroparathyroidectomized dogs than in membranes isolated from normal dogs. The peak of the overshoot was

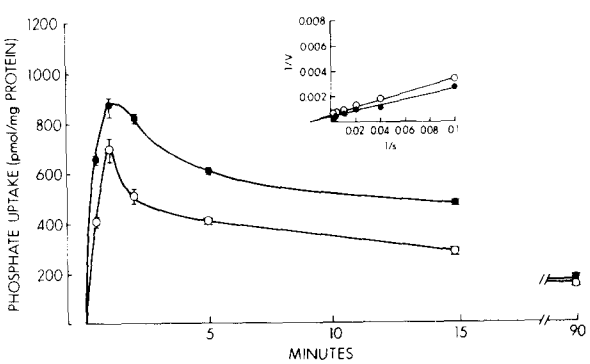

Fig. 6. The time course of the Na^+-gradient-dependent uptake of 25 μM $^{32}P_i$ by renal brush border membrane vesicles isolated from GH-treated dogs (•) and GH-treated dogs given PTH in vivo (○). Data are reported as the mean ±S.E. The inset shows a double-reciprocal plot of the net Na^+-gradient-dependent transport system in vesicles isolated from GH-treated dogs (•) and GH-treated dogs given PTH in vivo (○). Experiments were performed as outlined in Figs. 1 and 4. GH-treated overshoot peak > GH plus PTH; $P < 0.0001$.

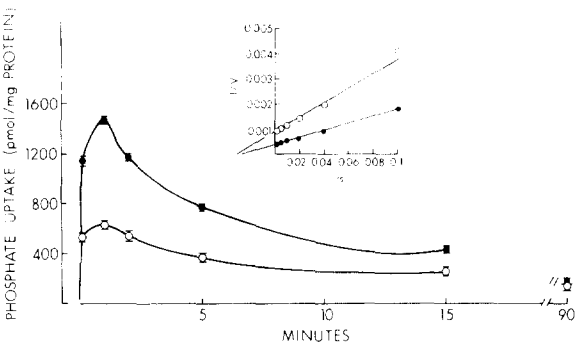

Fig. 7. The time course of the Na^+-gradient-dependent uptake of 25 μM $^{32}P_i$ by renal brush border membrane vesicles isolated from thyroparathyroidectomized dogs (●) and thyroparathyroidectomized dogs given PTH in vivo (○). Data are reported as the mean ±S.E. The inset shows a double-reciprocal plot of the net Na^+-gradient-dependent transport system in vesicles isolated from thyroparathyroidectomized dogs (●) and thyroparathyroidectomized dogs treated with PTH in vivo (○). Experiments were performed as outlined in Figs. 1 and 4. Thyroparathyroidectomized overshoot > thyroparathyroidectomized plus PTH; $P < 0.0001$.

significantly higher in membranes isolated from GH-treated dogs than in membranes isolated from normal dogs. Administration of oxidized PTH had no effect on the time course of P_i uptake measured using vesicles isolated from the second kidney of a normal dog (data not shown).

TABLE I

PLASMA Ca^{2+} and P_i, CREATININE CLEARANCE, AND TUBULAR REABSORPTION OF P_i IN EXPERIMENTAL ANIMALS

Plasma Ca^{2+} and P_i, creatinine clearance (Ccr) and tubular reabsorption of P_i (TRP) were measured in normal, GH-treated and thyroparathyroidectomized dogs: (a) after anesthesia prior to the first nephrectomy; (b) following the first nephrectomy prior to the administration of PTH; and (c) 30 min following administration of PTH. Data are reported as mean ± S.E. for three animals in each group. A paired Student's *t*-test was performed to analyze statistical differences.

Animals	Ca^{2+} (mg/100 ml)	P_i (mg/100 ml)	Ccr (ml/min)	TRP (%)
Normal				
a	9.7 ± 0.8	4.2 ± 0.2	74 ± 5	92 ± 4
b	9.4 ± 0.2	3.8 ± 0.4	38 ± 4	88 ± 2 [a]
c	9.4 ± 0.1	3.9 ± 0.1	37 ± 3	64 ± 1
GH-treated				
a	9.5 ± 0.3	6.3 ± 0.3	77 ± 6	92 ± 2
b	9.5 ± 0.3	5.8 ± 0.4	39 ± 3	83 ± 2 [b]
c	9.2 ± 0.4	5.4 ± 0.3	40 ± 3	74 ± 1
Thyroparathyroidectomized				
a	6.3 ± 0.3	5.3 ± 0.4	88 ± 2	98 ± 5
b	5.6 ± 0.4	4.7 ± 0.4	50 ± 1	95 ± 1 [c]
c	5.3 ± 0.4	4.6 ± 0.4	41 ± 2	76 ± 2
Thyroparathyroidectomized GH				
a	6.8 ± 0.5	5.5 ± 0.2	72 ± 8	98 ± 1

[a] Normal > normal + PTH ($P < 0.05$).
[b] GH-treated > GH-treated + PTH ($P < 0.05$).
[c] Thyroparathyroidectomized > thyroparathyroidectomized + PTH ($P < 0.01$).

TABLE II

EFFECT OF EXPERIMENTAL MANIPULATIONS ON K_m AND V FOR P_i UPTAKE BY BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM NORMAL, GH-TREATED AND THYROPARATHYROIDECTOMIZED DOGS, AND ON T_{P_i}

Double-reciprocal plots of the Na^+-gradient-dependent P_i transport system were obtained as detailed in Fig. 4. The P_i concentration of the incubation media was varied from 0.01 to 0.5 mM. Apparent K_m and apparent V values were calculated from regression lines obtained from least-squares analysis in Lineweaver-Burk plots (regression coefficients = 0.97—0.99). Six different concentrations of P_i were used and the transport experiments were performed in triplicate on each of at least three different occasions using kidneys from different animals. Data are reported as mean ± S.E. N values for analysis of variance were calculated based on the number of kidneys. T_{P_i} was calculated as described in Methods.

Animals (n)	K_m[h] (μM)	V (pmol/20 s per mg protein)	T_{P_i} (mg P_i/min)
Normal (9)	51 ± 2	1585 ± 20	1.1 ± 0.1
Normal + PTH (3)	45 ± 3	892 ± 68 [a]	0.92 ± 0.07 [a]
GH-treated (3)	52 ± 1	2124 ± 99 [b]	2.2 ± 0.2 [b]
GH-treated + PTH (3)	48 ± 4	1388 ± 104 [c]	1.5 ± 0.2 [c]
Thyroparathyroidectomized (3)	43 ± 4	2490 ± 139 [f]	2.4 ± 0.1 [f]
Thyroparathyroidectomized + PTH (3)	55 ± 8	1259 ± 68 [g]	1.5 ± 0.5 [g]
Thyroparathyroidectomized + GH (3)	34 ± 13	3018 ± 160 [d,e]	2.7 ± 0.5 [d,e]

[a] Normal > normal + PTH (V, $P < 0.001$; T_{P_i}, $P < 0.1$).

[b] GH-treated > normal (V, $P < 0.005$; T_{P_i}, $P < 0.005$).

[c] GH-treated > GH-treated + PTH (V, $P < 0.005$; T_{P_i}, $P < 0.05$).

[d] Thyroparathyroidectomized + GH > GH-treated (V, $P < 0.0005$, T_{P_i}, $P < 0.05$).

[e] Thyroparathyroidectomized + GH > thyroparathyroidectomized (V, $P < 0.02$; T_{P_i}, $P < 0.15$).

[f] Thyroparathyroidectomized > normal (V, $P < 0.0001$; T_{P_i}, $P < 0.001$).

[g] Thyroparathyroidectomized > thyroparathyroidectomized + PTH (V, $P < 0.0001$; $P < 0.02$).

[h] No difference among groups.

Figs. 5—7 (insets) demonstrate that PTH administration decreased the apparent V for P_i transport by vesicles isolated from normal, GH-treated and thyroparathyroidectomized dogs. Table II further demonstrates this finding. Administration of PTH in vivo to normal dogs decreased the apparent V by approx. 45% compared to that observed prior to PTH administration. Oxidized PTH did not change the apparent V (data not shown). Administration of GH in vivo increased the apparent V by approx. 34% compared to normal. Thyroparathyroidectomy increased the apparent V 57% compared to normal. Administration of GH to thyroparathyroidectomized dogs increased the apparent V by 21% compared to thyroparathyroidectomy alone.

Data comparing in vivo P_i reabsorption (T_{P_i}) among groups of animals were qualitatively in agreement with apparent V values for isolated brush border membrane Na^+-stimulated P_i transport.

To distinguish effects of in vivo GH or PTH administration or parathyroidectomy on Na^+-stimulated P_i transport in isolated brush border membrane vesicles from possible effects on Na^+ permeability resulting in nonspecific effects on Na^+-stimulated P_i transport, the effects of these in vivo manipulations on Na^+-stimulated D-glucose transport in brush border membrane vesicles were examined [6—11]. It has been previously shown that initial rates of Na^+-stimulated D-glucose transport into brush border membrane vesicles were not altered by PTH administration in vivo, or parathyroidectomy in experimental

TABLE III

INITIAL AND STEADY-STATE D-[2-^{3}H(n)]GLUCOSE UPTAKES IN EXPERIMENTAL ANIMALS

Initial (20 s) and steady-state (90 min) uptakes of D-[2-^{3}H(n)]glucose in the presence of an initial 100 mM NaCl gradient were determined in experimental animals. Data are reported as mean ± S.E. Values are expressed as pmol/mg protein.

Animals	Na^+-stimulated D-[2-^{3}H(n)]glucose uptake	
	20 s	90 min
Normal	162 ± 3	29.2 + 1
Normal + PTH	154 ± 4	27.0 ± 0.5
GH-treated	168 ± 5	28.4 ± 0.5
GH-treated + PTH	167 ± 2	25.0 ± 0.3
Thyroparathyroidectomized	153 ± 6	23.0 ± 4
Thyroparathyroidectomized + PTH	161 ± 8	27.2 ± 3
Thyroparathyroidectomized + GH	153 ± 9	23.1 ± 4

animals [6,8—11]. Our results (Table III) are in agreement with these previous reports, and suggest that the observed effects of the in vivo manipulations on isolated brush border membrane Na^+-stimulated P_i transport do not result from alterations in Na^+ permeability. Administration of GH to dogs in vivo likewise did not alter Na^+-stimulated D-glucose transport. D-Glucose uptake at 20 s and 90 min in the presence of an initial 100 mM KCl gradient did not vary significantly among groups (data not shown).

Steady-state levels of the Na^+-stimulated $^{32}P_i$ uptake were similar in vesicles isolated from normal, PTH- and GH-treated, and thyroparathyroidectomized dogs (Figs. 5—7). Steady-state levels of Na^+-stimulated D-[2-^{3}H(n)]glucose uptake were also similar among vesicles isolated from each of these groups (Table III).

Discussion

Administration of human GH to man increases renal reabsorption of P_i and increases the Tm phosphate (tubular maximum for P_i reabsorption) [1—3]. PTH lowers the increase in Tm phosphate due to GH administration to dogs, and it has been suggested that the antiphosphaturic effect of GH is mediated through inhibition of PTH activity [4]. The antiphosphaturic effect of GH has, however, been observed in thyroparathyroidectomized dogs [4], suggesting that this is not the case.

The present studies correlate known in vivo effects of GH administration on renal transport of P_i with effects on transport of P_i in isolated membrane vesicles from the renal brush border of dogs. They also evaluate the interaction of GH and PTH as regards their effects on renal transport of P_i in vivo and on P_i transport in isolated vesicles. Administration of GH to dogs increased P_i transport in vivo and in vitro. The changes in P_i reabsorption in vivo were qualitatively in agreement with alterations in brush border membrane apparent V values for Na^+-stimulated P_i transport. The effect of GH on Na^+-stimulated P_i transport into vesicles was probably independent of effects GH may have had on Na^+ transport per se [25] because the Na^+-stimulated transport of D-glucose into vesicles isolated from GH-treated dogs was not increased compared to normal.

PTH administration to GH-treated dogs decreased P_i transport in vivo and in vitro. The apparent V for Na^+-stimulated P_i transport in brush border membrane vesicles isolated from GH-treated thyroparathyroidectomized dogs was increased compared to that observed in vesicles isolated from thyroparathyroidectomized dogs. These findings are consistent with the hypothesis that the effect of GH on the renal reabsorption of P_i is at least partially independent of PTH [4].

The Na^+-P_i cotransport system in vesicles from the renal brush border membrane has been previously characterized [26]. An observation which has been made and has remained unexplained is the finding that the steady-state or equilibrium level of P_i uptake into brush border membrane vesicles measured using a given concentration of extravesicular medium P_i is 5—10-times greater than the steady-state uptakes of D-glucose [10,27], measured using the same extravesicular concentration of the latter substrates. We observed approx. 7-fold greater uptake of P_i into brush border membrane vesicles at 90 min of incubation than D-glucose using the same brush border membrane preparation.

Fig. 2A demonstrates that decreased intravesicular space resulted in decreased brush border membrane P_i uptake at 20 s, 1 min and 90 min of incubation when sucrose was added to brush border membrane vesicles at time zero. Under these conditions, P_i uptake at 20 s and 1 min (peak uptake) was decreased by increasing extravesicular osmolality. If, however, sucrose was added to the extravesicular medium at time 1 min, which represents the peak of the P_i overshoot (Fig. 2B), extrapolation of P_i uptake after 90 min more of incubation to infinite osmolality indicated that 85% of the P_i uptake was not responsive to shrinkage of the intravesicular space, and most likely represented binding. This extent of P_i binding to vesicles at steady state explains the observation that 90 min P_i uptake is very much higher than that observed for other solutes. A possible explanation for these findings is that when vesicles shrink in response to increasing osmolarity, binding sites become unavailable and binding is inhibited.

We attempted to characterize the nature of the steady-state P_i binding to brush border membrane vesicles. No evidence for phosphorylation of membrane protein resulting from intrinsic protein kinase activity [26] or $^{32}P_i$ incorporation into lipid extracts of brush border membranes was detected.

The steady-state vesicular P_i uptakes under all experimental conditions were similar, suggesting that P_i binding characteristics of brush border membrane vesicles were not altered by experimental manipulations of animals. Binding of P_i to membrane vesicles may be representative of an event which occurs in vivo relating to transport of P_i across the intact brush border membrane or it may represent an experimental artifact of the membrane vesicle experimental system. At the present time, our data do not permit further speculation.

In these studies, we have used apparent K_m and apparent V values for net Na^+-stimulated P_i transport to compare brush border membrane P_i transport under several experimental conditions. Apparent K_m and V values were calculated based on an estimate of initial rates of P_i transport by brush border membrane vesicles in the presence of an initial extravesicular to intravesicu-

larly directed NaCl gradient. Effects of experimental manipulations on Na^+ flux which may alter Na^+-stimulated P_i transport by membrane vesicles were excluded by demonstration of a lack of effect of experimental manipulations on Na^+-stimulated D-glucose uptake by brush border membrane vesicles. This has been a previously utilized technique [6,8].

Na^+-linked reabsorption of solutes in vivo across the proximal tubular brush border membrane most likely occurs accompanied with an Na^+ flux across the membrane into the proximal tubular cell. The Na^+ flux is maintained by active extrusion of Na^+ from the proximal tubular cell by an oubain-sensitive (Na^+ + K^+)-ATPase located at the basolateral membrane [17]. Measurement of Na^+-stimulated transport of P_i into isolated brush border membrane vesicles associated with the dissipation of a Na^+ gradient across the membrane rather than under Na^+ pre-equilibrated conditions is an attempt to simulate in vivo conditions.

Hopfer [28,29] has pointed out that kinetic parameters, calculated as described above, fail to account for dissipation of the Na^+ gradient as a function of increasingly higher solute concentrations utilized in substrate velocity determinations and has suggested that an equilibrium exchange technique should be utilized to determine kinetic parameters for solute transport in the brush border membrane system. This technique employs measurements of times necessary for tracer radioactively labeled solute transport to equal a given percentage of equilibrium quantities of radioactively labeled solute transported across brush border membranes pre-equilibrated with solute and Na^+ [28]. Recently, the advantage of using equilibrium exchange over initial influx kinetics for Na^+-dependent D-glucose transport in kidney brush border membrane vesicles has been questioned [30].

Where solute uptake is comprised of transport of solute into an intravesicular space as well as binding of solute to membranes, as is the case with P_i in the brush border membrane system, it is clearly not possible to quantitate solute transport in terms of equilibrium solute transport and the technique of equilibrium exchange cannot be utilized.

Hilden and Sacktor [31] have demonstrated D-glucose-stimulated $^{22}Na^+$ uptake by brush border membrane vesicles utilizing millimolar quantities of D-glucose. No discernable effect of D-glucose on $^{22}Na^+$ uptake was demonstrable, however, using micromolar quantities of D-glucose (Chernoff, A., unpublished data), and we have not been able to demonstrate an effect of P_i on $^{22}Na^+$ uptake in brush border membrane vesicles at the concentrations of P_i used in substrate velocity experiments. No doubt, such effects exist at micromolar D-glucose and P_i concentrations, but they are probably small and would not be expected to dissipate significantly the Na^+ gradient.

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