Effects of Glucose and Parathyroid Hormone on the Renal Handling of Myoinositol by Isolated Perfused Dog Kidneys

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ABSTRACT The effects of glucose and parathyroid hormone (PTH) on the transport and metabolism of myoinositol (MI) and [2-^{3}H]MI were studied in isolated perfused dog kidneys. Studies during perfusion of kidneys with normal and elevated glucose concentrations demonstrated that under normal conditions the isolated kidney reabsorbed 94.7±0.2% of the filtered MI, and the renal production of ^{3}H-metabolities of MI was 117.9 ±6% of the filtered MI load. This indicated that entry of MI into tubular cells by reabsorption was not the sole pathway for entry into the pool of MI within the kidney undergoing catabolism. High glucose perfusate decreased MI reabsorption to 68.6±4.7% and thus decreased delivery of [2-^{3}H]MI into the catabolic pool from the reabsorptive pathway. In the high glucose experiments, the rate of [2-^{3}H]MI catabolism exceeded [2-^{3}H]MI reabsorption by the same fraction as in normal glucose experiments, which indicates that high glucose did not affect nonreabsorptive access of MI to the catabolic site.

In contrast to the effects of glucose, PTH administration resulted in an increase in perfusate MI concentration and a decrease in the perfusate [2-^{3}H]MI specific activity. Concomitantly, urinary MI and [2-^{3}H]MI concentrations were increased, again with a decrease in [2-^{3}H]MI specific activity. These results indicate that PTH caused a release of MI into the urine (not the same as decreased MI reabsorption, which would not affect urinary [^{3}H]MI specific activity) and into the perfusate of the isolated kidneys. These effects on MI release were about coincidental with the increase in urinary cyclic 3′,5′-AMP after PTH and preceded the peak phosphaturic effect of PTH. There was no detectable effect of PTH on MI synthesis from glucose as a source of the MI released into the urine and perfusate. However, PTH temporarily halted accumulation of tritiated MI catabolites. There was no effect of inactivated PTH on urinary cyclic 3′,5′-AMP or on MI transport, which indicates that the PTH effect on MI handling was a specific hormonal effect. These studies clarify the renal metabolism of MI, and they demonstrate heretofore unknown effects of PTH on the renal handling and metabolism of MI. The effects of PTH on renal MI metabolism have important implications in renal carbohydrate metabolism and phospholipid turnover.

INTRODUCTION

The kidney occupies a unique role in myoinositol (MI)[1] metabolism by virtue of high intracellular MI levels and its active tubular transport and subsequent metabolism of MI. MI enters the glomerular filtrate freely, and reabsorption is nearly complete under normal conditions (1–2). Fractional excretion of MI is increased by elevated tubular fluid glucose, and brisk inosituria occurs in uncontrolled diabetes mellitus (1–2). Phlorizin administration inhibits both MI and glucose reabsorption, which further strengthens the hypothesis that the two substances are competitive for the same reabsorptive pathway (2).

The concentration of free MI within the renal cortical cells is much higher than in plasma (3). This concentration gradient is maintained by an active transport system (3–5). The free MI within the renal tubular

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[1] *Abbreviations used in this paper*: bPTH, bovine parathyroid hormone; GFR, glomerular filtration rate; MI, myoinositol; PI, phosphatidylinositol; PTH, parathyroid hormone; TRP, tubular reabsorption of phosphate.

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cell can either be metabolized by way of the glucuronate-xyulose pathway to glucose and other intermediates (5), or it can be incorporated into lipid components of cell membranes as phosphoinositides (6). Although the phosphoinositides are quantitatively minor components of phospholipid membranes, these compounds have attracted recent attention because of their possible role in cellular secretory and transport processes. Phosphatidylinositol (PI) turnover is increased during secretory activity of the thyroid (7), adrenal medulla (8), exocrine pancreas (9–11), and islets of Langerhans (12). Recently, it has been reported by Lo et al. (13) that parathyroid hormone increased incorporation of [^{32}P]phosphate into phosphoinositides in renal cortical slices.

In these investigations we have characterized the renal clearance and metabolism of MI in the isolated dog kidney. We have also compared the effects of hyperglycemia, known to affect MI tubular reabsorption, and parathyroid hormone, reported to affect PI turnover, on the renal handling of MI. The results show that whereas both hyperglycemia and parathyroid hormone produce inosituria, the effects are through different mechanisms. In addition, parathyroid hormone induces an inhibition of MI catabolism. The possible implications of these findings are discussed.

METHODS

Kidney perfusion. Mongrel dogs, weighing 18–27 kg, were fed standard dog chow until 16 h before the study and had free access to water. Kidneys were removed from the dogs under Nembutal (Abbott Laboratories, North Chicago, Ill.) anesthesia. The renal arteries and ureters were cannulated, and the kidneys were placed in a Waters MOX 100 TM perfusion apparatus (Waters Instruments Inc., Rochester, Minn.) that was modified to function at 37°C. Each kidney was perfused with 900 ml of heparinized dog blood which was diluted with 100 ml of a modified Ringer's solution in a recycling system (14). Creatinine and paraminohippurate were added to the perfusate to bring their concentrations to 7 mg/dl and 1–2 mg/dl, respectively.

Less than 5 min elapsed between excision, cannulation, and reinstitution of arterial blood flow. Regardless of flow, systolic blood pressure was maintained between 110 and 120 mm Hg, and diastolic pressure was determined by the vascular resistance of the kidney. 10–20 min after renal perfusion was started, blood flow rose and remained stable during the 120-min study. Urinary electrolyte and glucose losses were replaced by a continuous infusion into the venous pool of 0.045 N NaCl that contained creatinine paraminohippurate, phosphate, and glucose.

Protocol of study. Perfusions of both kidneys from the donor dog allowed paired experiments to be performed. The perfusate glucose concentration of one kidney was adjusted to 120 mg/dl (normal glucose), and for the other kidney the perfusate glucose was adjusted to 330 mg/dl (high glucose). After each kidney stabilized on the perfusion machine (40 min), control studies were made during three to four 10-min periods; then parathyroid hormone (PTH) was administered to both kidneys, and the effects of MI handling were observed during three or four 10-min periods.

Infusions. Because preliminary studies revealed that MI concentrations fell steadily during perfusion to levels below normal physiologic plasma levels (15), MI was added to the perfusate of some of the studies to be reported. In these studies, a bolus of MI (5–6 mg) and [2-^{3}H]MI (9–11 dpm × 10^4) was given between 15 and 28 min after the initiation of perfusion. Thereafter, a mixture that contained MI and [2-^{3}H]MI was infused at a rate of 0.15 mg/min and 2.6–2.9 dpm × 10^5/min, respectively, by a calibrated Harvard syringe pump (Harvard Apparatus Co., Inc., Millis, Mass.) into the arterial line proximal to the arterial sampling port.

In three nonpaired studies, [2-^{3}H]glucose (New England Nuclear, Boston, Mass.) was added to the perfusate as a bolus with a sustaining infusion. Total glucose concentrations were adjusted by adding glucose to the perfusate to maintain the desired level. MI was not infused in these studies so that synthesis of MI from glucose would be favored.

Highly purified bovine PTH (bPTH) 1–84 (900–1500 U/mg in a rat bioassay) was obtained from the Inolex Corp., Biomedical Div., Glenwood, Ill. 25 or 50 μg of bPTH 1-84 powder was dissolved in 1.0 ml of plasma immediately before administration and given as a bolus into the renal arterial perfusion line proximal to a 5-ml arterial mixing chamber connected directly to the renal arterial catheter. In three experiments, bPTH 1–84 from the same lots used in other experiments reported in this paper were oxidized by reconstituting lyophilized PTH in 50 μl 0.15 M acetic acid and 60 μl of 30% H_2O_2, shaking for 60 min at 37°C, and then lyophilized. Reconstitution of oxidized PTH for each experiment was as for untreated bPTH 1–84.

Sources of MI. [2-^{3}H]MI (17 Ci/mmol sp act) and [U-^{14}C]MI (215 mCi/mm) was purchased from New England Nuclear. Chromatography on Whatman #1 chromatography paper (Whatman Inc., Clifton, N. J.) in acetone:water (4:1 vol/vol), and phenol:water (4:1 vol/vol) established the purity of >97% for both preparations. Crystalline MI was purchased from Sigma Chemical Co., St. Louis, Mo., and used for preparing gas-liquid chromatography standards, primer, and constant infusion solutions.

Radioactivity determinations. The following measurements of radioactivity were made. (*a*) Total radioactivity: 200 μl of plasma or 100 μl of urine was added to the scintillation vial to which was added 10 ml of Instagel (Packard Instrument Co., Inc., Downers Grove, Ill.). (*b*) Nonvolatile radioactivity: 200 μl of plasma or 100 μl of urine was lyophilized in scintillation vials. The residues were redissolved in the same volumes of water before adding 10 ml of Instagel. (*c*) Plasma MI radioactivity: 3 ml of 0.15 M $BaSO_4$ and 0.9 ml of $ZnSO_4$ were added to 2 ml of plasma with mixing. After centrifugation, 2.6 ml of the supernatant solution was lyophilized. The residue was redissolved in 1 ml of H_2O. The protein-free supernate of plasma was added to a 1.2 × 4-cm column of a mixed bed resin (AG 501-X8 [D]) (Bio-Rad Laboratories, Richmond, Calif.) and eluted with distilled water. The first 12 ml of effluent was collected and lyophilized. The dried residue was then dissolved in 200 μl of water to which 10 ml of Instagel was added. Recovery of MI from plasma was monitored by adding [U-^{14}C]MI to control plasma samples, which were then carried through the same procedure. Three of these recovery samples were run for every 10 experimental samples. Under the conditions employed, the AG 501-X8 (D) column effectively removed all charged metabolites of MI and 95% of glucose radioactivity that was added to the columns for test purposes. (*d*) Urine MI radioactivity: 1 ml of urine was acidified with 150 μl of concentrated HCl. A control urine, to which 1,500 dpm of [2-^{3}H]MI was added, was used to determine column recovery. The acidified urine was added to a 1.2 × 5-column of AG 501-X8 (D) and followed by distilled water (1 ml/

min). 9 ml of effluent was collected and lyophilized in a scintillation vial. The residue was dissolved in 100 μl of distilled H_2O, and 10 ml of Instagel was added. This is a modification of the method of Clements and Rhoten (16) and removes urea as well as charged metabolites of inositol.

Radioactivity was measured in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc.). An automatic external standard was used to determine quenching. The percentage of efficiency for ^3H at each automatic external standard ratio was determined with scintillation vials that contained 200 μl of plasma or 100 μl of urine and variable amounts of acetone.

Gas-liquid chromatography methods. Measurement of MI by gas chromatography: A protein-free filtrate of plasma was prepared as described above, and an aliquot was lyophilized to dryness. The residue was dissolved in 100 μl of silylation reagent prepared by mixing equal parts of *N-O-bis* (trimethylsilyl)-trifiluoroacetamide with 10% trimethylchlorosilane and pyridine.

For urine measurements, the lyophilized residue from the column effluent described above representing 1 ml of urine was dissolved in 100 μl of the silylation reagent.

Gas chromatography was carried out by the method of Hipps et al. (17) except that the absorbent was 3% methyl polysiloxy gum on Gas-Chrom Q (Applied Science Labs, Inc., State College, Pa.). The helium flow rate was 48 ml/min. The temperature of the column was 165°C. The inositol in plasma and urine samples was quantitated by comparison of peak heights produced by 5- to 10-μl aliquots of the silylated sample with inositol standards.

Paper chromatography. To determine possible stimulation of inositol synthesis from [2-^{3}H]glucose, a protein-free filtrate of plasma was prepared (1 ml of plasma, 3.5 ml of 0.174 M barium hydroxide, and 3.5 ml of 0.174 M zinc sulfate). 150 μl of this filtrate was spotted on to Whatman 3 MM paper (Whatman Inc.) and the chromatogram was developed in a descending system with acetone:H_2O (4:1 vol/vol). The positions of glucose and MI standards were identified after staining with alkaline silver nitrate (18). Unstained paper strips which contained the plasma filtrates were cut in 1-cm-wide sections, added to scintillation vials, and 10 ml of Instagel was added before counting.

Other analytic methods. The following analytic methods were employed. (*a*) Creatinine by the Jaffe reaction (19) and adapted for the Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.); (*b*) paraminohippurate by the method of Harvey and Brothers (20) as adapted for the Technicon Autoanalyzer; (*c*) phosphate by the methods of Kraml (21) and Hurst (22) as adapted for the Technicon Autoanalyzer; (*d*) calcium by an atomic absorption spectrophotometer (model 503, Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.); (*e*) urinary and plasma sodium and potassium by flame photometry (model 143, Instrumentation Laboratory, Inc., Lexington, Mass.); (*f*) urinary cyclic AMP by a modification of the method of Steiner et al. (23); and (*g*) glucose was measured by a glucose oxidase method (Beckman Instruments, Inc., Irvine, Calif.).

Calculations. Standard formulas were used to calculate glomerular filtration rate (GFR), renal plasma flow, and tubular reabsorption of phosphate (TRP). To determine the filtered load of [2-^{3}H]MI, linear regression equations of the arterial [2-^{3}H]MI were calculated and the concentration for the midpoint of the urine collection period determined. The equations were: normal glycemic [2-^{3}H]MI (disintegrations per minute per milliliter = $-77.4\,t$ (minutes) + 14,567; and hyperglycemic [2-^{3}H]MI (disintegrations per minute per milliliter) = $-62.3\,t$ (minutes) + 16,173. The correlation coefficient for the normal glycemic kidneys was 0.921, and for the hyperglycemic kidneys it was 0.873.

In calculations of [2-^{3}H]MI metabolism between 70 and 90 min, the appearance of ^3H in perfusate metabolites and the urinary excretion of ^3H-metabolites was expressed as a percentage of the filtered load of MI. The total renal production of ^3H-metabolites (perfusate and urine) over the [2-^{3}H]MI reabsorbed is a minimal estimate of the renal MI metabolite production independent of glomerular filtration and reabsorption of MI.

Results are presented as mean±SE. Significance was determined by Student's paired *t* test.

RESULTS

Renal handling of MI—effects of high glucose perfusate. GFR, effective renal plasma flow, fractional excretion of Na, and TRP were stable during three control periods in paired perfusions of eight kidneys. The mean of three determinations for each renal function is listed for each kidney in Table I. The normal and hyperglycemic kidneys did not differ significantly with respect to these parameters except for a reduced base-line TRP in the hyperglycemic kidneys (Table I). The GFR and effective renal plasma flow were 20.4 and 162.2 ml/min, respectively. The mean filtrate fraction (GFR per effective renal plasma flow) for eight kidneys

TABLE I
Renal Function for Four Kidney Pairs

	Normal glucose	High glucose
Creatinine clearance, *ml/min*	21.7	21.9
	19.1	16.0
	25.7	13.9
	25.2	19.9
	22.9±1.5	18.0±1.8
Effective renal plasma flow, *ml/min*	113.4	145.3
	162.5	108.0
	268.7	103.8
	211.3	184.3
	188.9±33	135.4±18.8
Fractional excretion of Na, %	2.35	2.20
	3.71	2.69
	3.11	3.47
	1.58	0.15
	2.68±0.65	2.13±0.07
TRP, %*	96.6	86.9
	92.8	92.7
	86.8	78.0
	93.5	86.7
	92.2±2.2	86.1±3.0

* For a period of 40–80 min.

in four paired experiments was 0.13, similar to that found by others (24) under the conditions of these experiments. Mean arterial glucose concentrations in normal and hyperglycemic perfusates were 153.7±10 and 315.9±7.1 mg/dl, respectively. Arterial plasma glucose concentrations remained stable throughout all studies except in one hyperglycemic kidney in which the perfusate glucose concentration fell and glycosuria ceased during the last three urine collection periods. The fractional excretion of Na had a mean value of 2.41%, and the mean control TRP was 89.2%.

TABLE II
Renal Handling of [2-^{3}H]MI in Normal and High Glucose Perfusate

	Normal glucose	High glucose
MI		
Filtered load, *dpm/min* × 10^{-3}	158.7	253.9
	221.8	256.4
	157.0	221.0
	146.7	128.6
	171.1±17.1	215.0±29.9
Reabsorbed, *% of the FL**	94.3	56.2
	95.1	66.8
	94.7	75.5
	94.6	76.1
	94.7±0.2	68.6±4.7‡
MI		
(*a*) Excreted, *% of the FL of MI*	10.4	11.1
	20.4	14.2
	26.1	23.3
	9.0	32.1
	16.5±4.0	20.2±4.7
(*b*) Added to perfusate, *% of the FL of MI*	105.5	85.9
	114.9	57.0
	81.6	65.8
	103.7	72.1
	101.4±7.0	70.2±6.1§
Total renal production of MI metabolites (*a* + *b*), *% of the FL of MI*	115.9	97.0
	135.3	71.2
	107.7	89.1
	112.7	104.2
	117.9±6.0	90.4±7.1
Renal MI metabolite production in excess of reabsorbed MI, *% of the FL of MI*	21.6	41.8
	40.2	4.4
	13.0	13.6
	18.1	28.1
	23.2±5.9	22.0±2.3

* FL, filtered load.
‡ $P < 0.05$.
§ $P < 0.01$.

The renal handling of [2-^{3}H]MI in four pairs of kidney perfusions is shown in Table II. The kidneys with normal glucose perfusate reabsorbed a much higher fraction of the filtered MI than kidneys perfused with high glucose (94.7%±0.2 vs. 68.6±4.7, $P < 0.01$). Kidneys with normal glucose perfusate also released more [2-^{3}H]MI metabolites into the renal perfusate. Total renal production rate of tritiated metabolites, expressed as a percentage of the filtered load of [2-^{3}H]MI, significantly exceeded the quantity of [2-^{3}H]MI reabsorbed for both the normal and high glucose kidneys. This indicates an access of [2-^{3}H]MI to renal catabolic sites independent of glomerular filtration. Also, the greater rate of tritiated metabolite production in the normal glucose kidney can be explained solely by a greater rate of MI transport from the tubular lumen into the catabolic pool. Thus, an inhibitory effect of high glucose perfusate on nonluminal membrane MI transport was not found.

Effects of PTH. Fig. 1 shows the results of an experiment in which physiologic MI concentrations were not maintained in the renal perfusate. Both arterial and venous MI concentrations decreased rapidly during control periods. 42 min after initiation of kidney perfusion, a single bolus of 100 μg bPTH was administered followed by 1 μg/min constant infusion. An increase in venous MI and reversal of the arterial-venous difference occurred 26 min later. Arterial and venous MI concentrations increased until 90 min of perfusion then fell as the renal arterial-venous difference for MI was reestablished.

The venous concentrations of MI and [2-^{3}H]MI in

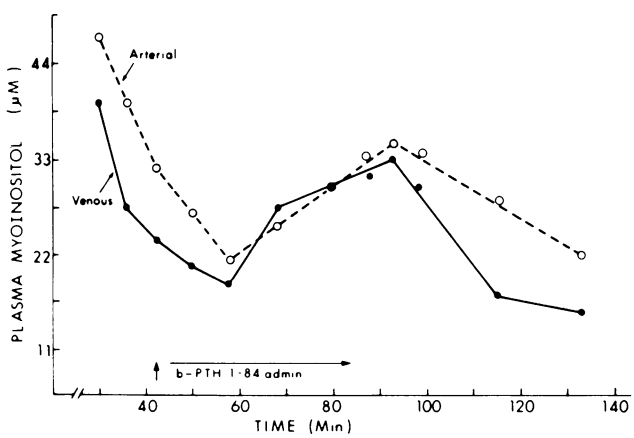

FIGURE 1 Perfusate MI concentrations from an isolated kidney not receiving added MI. Arrows indicate bolus and sustaining infusions of b-PTH 1-84. Admin, administration.

four kidney pairs perfused with normal glucose in one kidney and with high glucose in the other are shown in Fig. 2. The venous perfusate concentration of MI decreased slightly during control periods (40–80 min) and then rose abruptly: 14.78 μM in the venous perfusate of the normal glucose kidneys ($P < 0.01$) after PTH administration at 80 min. The mean rise in perfusate MI was greater in the kidneys perfused with high glucose, but the variance was greater so that the difference was not significant. The higher perfusate MI levels in the high glucose kidneys is explained by the decreased rate of MI metabolism shown in Table II because renal function and the MI infusion rates were alike in all kidney pairs. It should be noted that in one high glucose kidney there was an unexplained fall in perfusate glucose, which may have contributed to the degree of variance in the perfusate MI concentrations of the high glucose kidneys. Perfusate [2-^{3}H]MI concentrations remained relatively constant throughout the period of observation. The mean disintegrations per minute per milliliter in the high glucose kidneys were higher in the period from 60 to 100 min, which again demonstrates a reduced clearance of MI in the high glucose kidneys.

The addition of ^{3}H-metabolites of MI to the perfusate pool for the eight kidneys is shown in Fig. 3. During the three control periods and first experimental-period

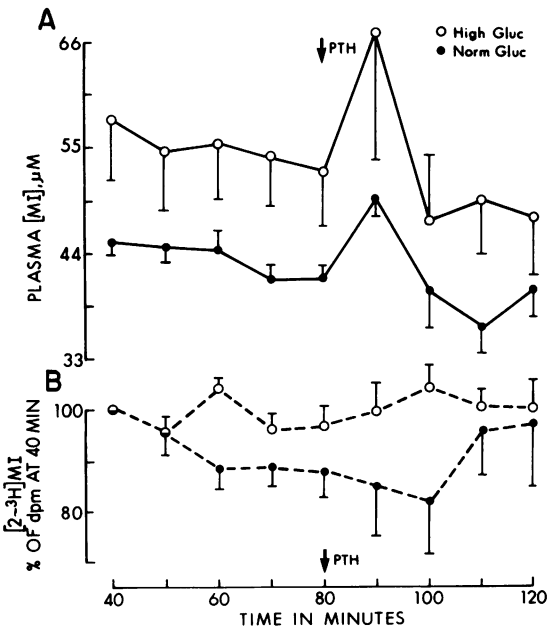

FIGURE 2 Upper panel: mean±SEM perfusate MI concentrations from four pairs of kidneys receiving sustaining infusions of cold and tritiated MI to sustain physiologic perfusate MI levels. Lower panel: venous perfusate [2-^{3}H]MI levels. Arrows in both panels indicate bolus administration of 25 μg b-PTH 1-84. Gluc, glucose.

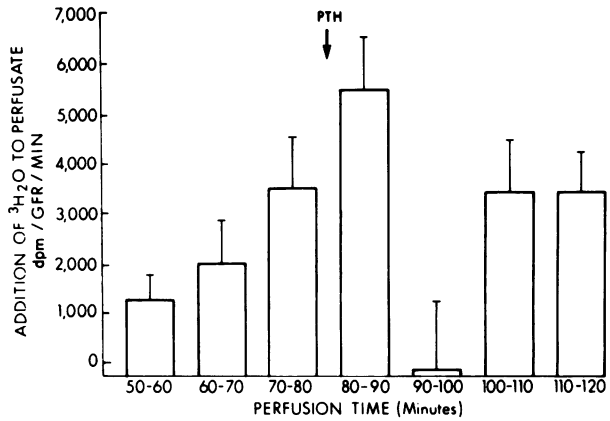

FIGURE 3 The mean±SEM addition of ^{3}H-metabolites of MI to venous perfusate, mostly as ^3H_2O, during 10-min periods of perfusion in the eight kidneys studied as low and high glucose pairs.

post-PTH injection there was a steadily increasing addition of ^{3}H-metabolites to the perfusate. 10 min after PTH was added to the perfusate, the addition of ^{3}H-metabolites to the perfusate virtually ceased. During the following 20-min interval, the addition of ^{3}H-metabolites to the perfusate pool returned toward the pre-PTH levels.

The urinary excretion of cyclic AMP, inorganic phosphate, MI, and [2-^{3}H]MI expressed as the percentage of the mean pre-PTH value is shown in Table III. The effects of PTH on the excretion of cyclic AMP and the TRP were not different for normal and high glucose perfused kidneys and were, therefore, averaged together. Cyclic AMP excretion was stable during the control urinary periods but increased to 445% of control levels during the first period after PTH administration. This effect was short lived, and the excretion of cyclic AMP returned to base line 10 min after PTH injection. There was no significant change in the tubular reabsorption of phosphate during the control periods. However, after PTH administration there was a significant ($P < 0.01$) fall in TRP by the first collection period. This decrease in TRP continued during the remaining collection periods with the nadir (66.4%) occurring during the third and final post-PTH collection period.

The urinary excretion of MI and [2-^{3}H]MI was calculated in Table III for four normal and three high glucose perfused kidneys. The fourth high glucose kidney was not included in these calculations as the rapidly decreasing glucose concentrations in the high glucose kidney altered the MI reabsorptive capacity, masking any PTH effect. The urinary excretion of MI and [2-^{3}H]MI was relatively constant during the control periods between 55 and 80 min. After PTH administration there was a rapid increase ($P < 0.05$) in the ex-

TABLE III
*Effect of PTH on Urinary Cyclic AMP, TRP, MI, and [2-^{3}H]MI**

		Time of perfusion (min)				
	Kidneys	55–70	70–80	PTH ↓ 80–90	90–100	100–115
	n					
Cyclic AMP	8	111±17	96±12	445±99	150±58	90±46
TRP	8	100±1	98±1	85±3	75±3	66±3
MI						
Normal glucose	3	112±7	88±7	515±281	259±74	220±115
High glucose	3	73±18	126±18	292±112	211±112	—
[2-^{3}H]MI						
Normal glucose	3	99±12	101±12	199±63	226±62	207±51
High glucose	3	108±2	95±3	153±27	155±19	132±14

* Data is expressed as the percentage of mean for a period of 55–80 min.

cretion of both [2-^{3}H]MI and MI in the kidneys perfused with normal glucose concentrations. In the kidneys perfused with high glucose, the excretion of MI was also increased significantly ($P < 0.05$) by PTH. In five of the eight kidneys, the maximal increase in excretion of the tritiated tracer occurred during the first collection period after PTh administration. However, the maximal increase in MI excretion occurred during the second collection period in three of the eight kidneys. Urinary specific activity of [2-^{3}H]MI decreased significantly ($P < 0.05$) during the first experimental period after PTH administration, but returned to control levels thereafter for both normal and high glucose perfused kidneys.

Control experiments with oxidized PTH. In three experiments oxidized PTH was added to one kidney's perfusate while the paired kidney received intact PTH (Fig. 4). No significant change in perfusate MI was observed with oxidized PTH, whereas the intact PTH induced the characteristic rise in perfusate MI.

Conversion of [2-^{3}H]glucose into MI. In three experiments [2-^{3}H]glucose was added to the renal arterial perfusate. The specific activity of ^{3}H in the arterial perfusate of one of the three experiments is shown in disintegrations per minute per milligram glucose at the top of Fig. 5. MI was not added to the perfusate, and, as in the experiment shown in Fig. 1, the perfusate MI dropped to low levels during control periods. After a bolus injection of bPTH 1-84 there was a rise in venous perfusate MI. However, none of the infused ^{3}H was detected in MI as determined by paper chromatography of each plasma sample. This is portrayed on the lower panel of Fig. 5. The results of the other two kidneys were similar.

A representative paper chromatographic profile of the ^{3}H radioactivity in the perfusate at 110 min of the experiments is shown in Fig. 6. No significant ^{3}H counts appeared in the MI region of the paper strips of any plasma sample.

DISCUSSION

These studies in paired perfused dog kidneys showed the extensive tubular reabsorption of MI previously observed in other systems (1, 2). Elevation of perfusate glucose resulted in the expected decrease in renal MI reabsorption. The experimental design also allowed us to measure separately the appearance of metabolites of [2-^{3}H]MI in effluent perfusate and in urine. We were

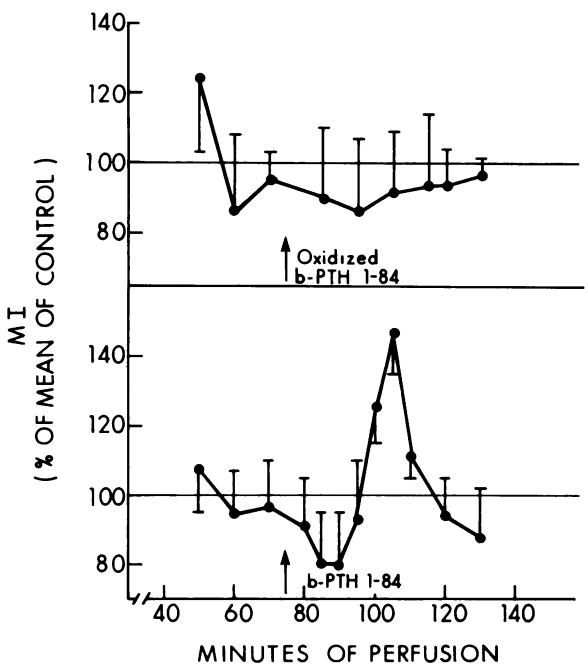

FIGURE 4 Upper panel: venous perfusate MI levels in three kidneys given bolus injections of oxidized b-PTH 1-84 expressed as percentage of the mean of three control perfusate samples. Lower panel: venous perfusate MI levels in three kidneys given bolus injections of biologically active b-PTH 1-84.

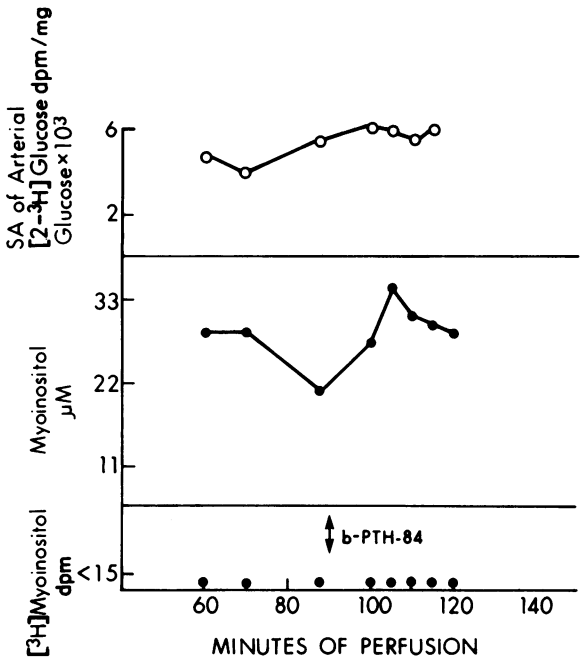

FIGURE 5 Upper panel: specific activity (SA) of [2-^{3}H]glucose in arterial perfusate in a perfused kidney receiving a [2-^{3}H]glucose infusion. Middle panel: venous perfusate MI concentration. Lower panel: absence of detectable ^{3}H counts in MI isolated by paper chromatography.

able to show that the total metabolism of MI exceeded the amount of MI reabsorbed from the glomerular filtrate, which indicates that MI has an additional access to the catabolic pool, probably by entering from the antiluminal surface of the cell. This is supported by previous experiments demonstrating transport of MI by the antiluminal membrane of dog kidneys (25). Glucose reduced the formation of MI metabolites, but this effect was mainly because it decreased tubular reabsorption.

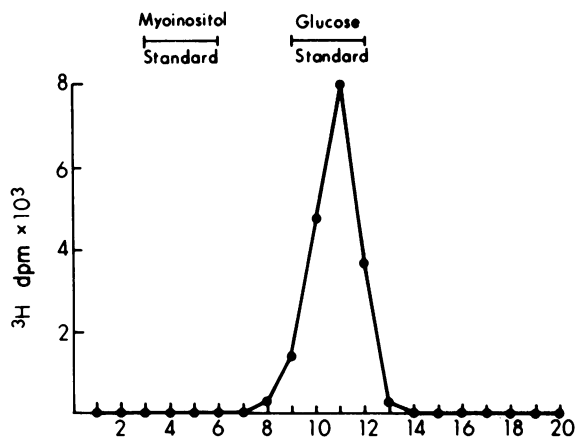

FIGURE 6 Paper chromatographic profiles of the ^{3}H counts in a perfusate sample from one of the kidneys infused with [2-^{3}H]glucose.

The amount of MI which entered the catabolic pool from the antiluminal surface was little decreased. Thus, the effect of high glucose on MI metabolism is mediated through decreasing delivery to the catabolic pool through tubular reabsorption. In contrast to the glucose effect on MI transport, PTH led to both inosituria and release of MI into the renal venous perfusate. That the inosituria seen after PTH represented more than a decrease in reabsorption is indicated by the observed decrease in urinary [2-^{3}H]MI specific activity. Thus, in those experiments with a continuing infusion of [2-^{3}H]-MI, the fall in urinary and perfusate specific activity after PTH probably indicates the incomplete mixing of the [2-^{3}H]MI with the intracellular MI pool from which PTH stimulated release. PTH also induced the expected decrease in the TRP and increased urinary cyclic 3′,5′-AMP. High glucose perfusate had little effect on the release of MI into the perfusate after PTH but appeared to reduce the magnitude of MI release into the urine.

Because of the observed differences between the effects of high glucose perfusate and of PTH, it is likely that they have distinct effects on MI metabolism. Glucose appears to inhibit mainly the reabsorption of MI from the glomerular filtrate by mechanisms that remain to be elucidated. The effect may be through competition for a common luminal membrane transporter because both glucose and MI transport are inhibitable by phlorizin (2). However, glucose appeared not to affect nonreabsorptive entry of MI into the catabolic site of the kidney. On the other hand, PTH led to release of MI into the perfusate and urine, and acutely inhibited the

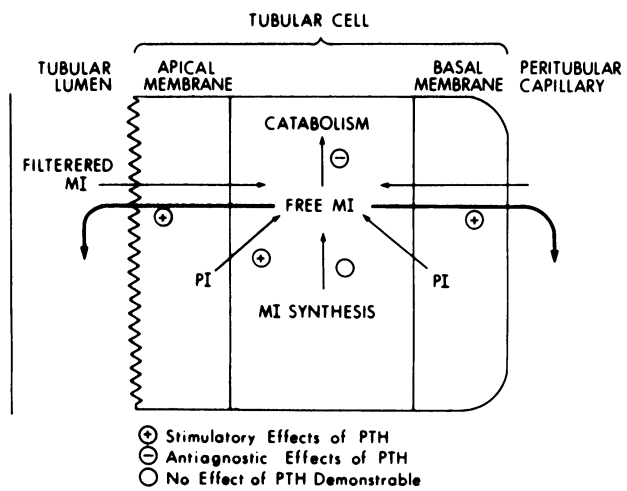

FIGURE 7 Conceptual treatment of the PTH effects on renal tubular cell MI transport and metabolism. PTH causes a release of MI into the urine and renal venous perfusate. It probably stimulates PI turnover, releasing MI into the free MI pool (12). It stops the accumulation of MI metabolites in the renal perfusate, reflecting decreased catabolism, and it appears not to affect MI synthesis.

accumulation of ^{3}H-metabolites of MI. There are several possible mechanisms for the observed actions of PTH (Fig. 7). The first is that PTH induced an increase in MI synthesis from glucose. This would produce an elevation of tissue-free MI levels, favoring an outward movement of MI from the cell into urine and perfusate. The isotopic experiments with [2-^{3}H]glucose make this possibility unlikely because we were unable to detect any significant incorporation of glucose either before or after PTH exposure. While MI synthesis undoubtedly occurs in the kidney (26), its magnitude is small compared with the observed changes in MI turnover in the kidneys studied.

The second possibility is a stimulation of phosphoinositide hydrolysis and subsequent accumulation of free MI within the cell favoring its movement outward. This possibility appears to be particularly attractive in that a hydrolysis of phosphoinositides has been observed in other tissues after hormonal or metabolic activation (7–12). The MI released by the renal cell could arise directly from the cell membranes or by raising the concentration of intracellular MI. However, while stimulation of phosphoinositides may have occurred after PTH, it probably cannot explain the abrupt cessation of [2-^{3}H]MI catabolite accumulation illustrated in Fig. 3 because the phosphoinositide pool of the kidney is much smaller than that of free MI (27). Complete hydrolysis of the lipid-bound MI would only lead to a small decrease in free MI specific activity and could not explain the decrease in ^{3}H in MI metabolites.

The observed cessation of [^{3}H]MI catabolic accumulation after PTH administration suggests that PTH may have inhibited MI catabolism. MI is catabolized through the glucuronate-xyulose pathway to the pentose cycle and to glucose (5). It is possible that PTH-induced changes in carbohydrate metabolism could have raised the concentration of intermediates of the glucuronic acid pathway and thereby inhibited MI catabolism. Thus, the possible pathways involved in producing PTH-induced release of MI from the kidney are several. It remains to be determined to what extent the observed changes in MI metabolism are related to other effects of PTH on the kidney.

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