The inhibition of parathyroid-hormone actions on gluconeogenesis and phosphate transport by 3-mercaptopycolinate in rabbit renal proximal tubules

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By using a glucose microassay and the technique for isolated renal-tubule perfusion *in vitro*, the addition of 3-mercaptopycolinate, a gluconeogenesis inhibitor which inhibits phosphoenolpyruvate carboxykinase specifically, was found to abolish the effects of parathyroid hormone on gluconeogenesis and phosphate-transport rate in isolated rabbit renal proximal straight tubules, suggesting that these parathyroid-hormone actions may share some unknown, yet 3-mercaptopycolinate-inhibitable, intracellular processes.

INTRODUCTION

In renal proximal tubules, it has been noted that gluconeogenesis and phosphate reabsorption rate are, in general, affected in opposite directions by a number of metabolic and hormonal perturbations. For example, metabolic acidosis, starvation, PTH and glucocorticoids enhance glucose production and inhibit phosphate reabsorption, whereas phosphate depletion, urinary obstruction and insulin inhibit glucose production and enhance phosphate reabsorption. The findings that NAD^+ inhibits phosphate uptake by isolated brush-border membrane vesicles of renal proximal tubules *in vitro* [1], and that gluconeogenesis positively correlates with the NAD^+ content of the renal cortex [2], led to the proposal that gluconeogenesis may regulate phosphate reabsorption in the renal proximal tubule through changes in cytosolic $[\text{NAD}^+]$ [1]. Subsequent findings that PTH increases the renal-cortex $[\text{NAD}^+]/[\text{NADH}]$ ratio [3], and that treatment *in vivo* with nicotinamide increases cellular NAD^+ content and restores the phosphaturic effect of PTH during phosphate depletion [4], led Berndt *et al.* [3, 4] to suggest further that PTH may inhibit phosphate reabsorption by an increased cytosolic $[\text{NAD}^+]$ through its stimulatory effect on gluconeogenesis.

The validity of such a proposition has been questioned, however. The inhibitory effect of NAD^+ on phosphate uptake by the brush-border membrane vesicles was found to be an artifact [5–7], and phosphate reabsorption across the renal proximal tubule was also found to be dissociable from the cytosolic redox potential [8, 9] and glucose production rate [8, 10–12]. Although these observations dispute the proposed regulatory role of gluconeogenesis on phosphate transport function, they still do not explain the apparent reciprocal relationship between these two events in general, and their interaction in response to PTH in particular.

The aim of the present study was to examine the possible interaction between PTH actions on gluconeogenesis and phosphate reabsorption in isolated rabbit renal proximal straight tubules by using 3-MP, a specific gluconeogenesis inhibitor [13].

MATERIALS AND METHODS

Animals

Male New Zealand White rabbits, weighing 1.5–2.0 kg, were fed on standard rabbit chow (Universal Feed, Colton, CA, U.S.A.) *ad libitum*, with free access to tap water for drinking.

Chemicals

Radioisotopes were purchased from New England Nuclear, Boston, MA, U.S.A.; fatty acid-poor bovine serum albumin was from Calbiochem, La Jolla, CA, U.S.A.; and synthetic bovine PTH (1–34-peptide) was from Peninsula Laboratories, San Carlos, CA, U.S.A. All other chemicals of analytical grade were obtained from Sigma, St. Louis, MO, U.S.A. 3-MP was generously given by Smith, Kline and French Laboratories, Philadelphia, PA, U.S.A.

Measurements of glucose production rates

Because of the heterogeneous response to PTH in different segments of renal proximal tubule from rabbit kidney [14], the glucose production rate was measured in isolated proximal straight tubules by a glucose microassay, as was reported previously [15, 12]. In brief, after the kidney was perfused through the renal artery with Krebs–Ringer bicarbonate solution to wash out the blood completely, the proximal straight tubules of the superficial nephrons were dissected freehand under the dissecting microscope. Tubules were pooled into different samples, and the total length of the tubule segments in each sample was measured under the microscope through a camera lucida. The tubules of each sample, in 100 μl of Krebs–Ringer bicarbonate containing 5 mM- α -oxoglutarate (pH 7.4), were incubated for 60 min under O_2/CO_2 (19:1) at 37 °C in a shaking water bath (50 oscillations/min). The tubules were then removed under the microscope, and the glucose produced and released in the remaining incubation medium was measured in replicate, by a method described by Chick & Like [16]. In brief, glucose was phosphorylated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ by hexokinase. The unchanged $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and ^{32}P -labelled

Abbreviations used: PTH, parathyroid hormone (parathyrin); 3-MP, 3-mercaptopycolinate.

contaminants were removed by charcoal adsorption, acid treatment and ammonium molybdate precipitation. The remaining glucose 6- ^{32}P phosphate was counted for radioactivity by a liquid-scintillation counter. The results were expressed as pmol of glucose produced/h per mm of tubule length.

Measurements of phosphate flux across proximal straight tubules

Proximal straight tubules of superficial nephrons were isolated and perfused *in vitro*, by using conventional methods as reported previously in detail [17]. The perfusate was an artificial solution designed to simulate late-proximal tubular fluid, containing (mM): NaCl 140, NaHCO_3 5, KCl 5, MgSO_4 1.2, NaH_2PO_4 2, CaCl_2 1.5, pH 6.7. The bath solution was similar to the incubation medium used for gluconeogenesis studies, containing (mM): NaCl 125, NaHCO_3 25, KCl 5, MgSO_4 1.2, NaH_2PO_4 2, α -oxoglutarate 5, pH 7.4, under O_2/CO_2 (19:1) at 37 °C, except for the presence of 6% (w/v) fatty-acid-poor bovine serum albumin. Albumin, which was omitted in gluconeogenesis studies because of its interference with the glucose assay, was added to the bath medium to generate an oncotic gradient required for tubule fluid reabsorption. The CaCl_2 concentration in the bath medium for tubule perfusion studies was adjusted so as to give the Ca^{2+} concentration, as measured by a flow-through Ca electrode, similar to that used for gluconeogenesis studies, i.e. 1 mM. The perfusate and bath were made iso-osmolar with NaCl.

Some 20–30 min of equilibration were allowed after the addition of testing agents to the bath solution before the first sample collection. Unidirectional lumen-to-bath phosphate flux (J_p) was determined by adding $\text{H}_3^{32}\text{PO}_4$ (5 $\mu\text{Ci/ml}$) as well as extensively dialysed [^3H]methoxyinulin (10 $\mu\text{Ci/ml}$) as an impermeant volume marker to the perfusate. The ^{32}P and ^3H in the perfusate and the samples of fluid collected from the tubule were determined by a liquid-scintillation counter. Results are expressed as the mean of four to five timed collections of tubular fluid. J_p was calculated as:

$$J_p = \frac{V_i C_i - V_o C_o}{L} \times \frac{P_i}{C_i}$$

where V_i is the perfusion rate (nl/min), V_o the collection rate (nl/min), C_i the ^{32}P concentration in the perfusate (c.p.m./nl), C_o the ^{32}P concentration in the collected samples (c.p.m./nl), L the tubule length (mm), and P_i the

inorganic phosphate concentration in the perfusate (pmol/nl).

Statistics

Data are expressed as means $\pm$ S.E.M. and analysed with Student's *t* tests for paired or unpaired data, as appropriate.

RESULTS

Gluconeogenesis in isolated rabbit renal proximal straight tubules

In rabbit renal proximal straight tubules, PTH (1 unit/ml) as well as 8-bromo cyclic AMP (0.1 mM) stimulated the rate of glucose production from α -oxoglutarate (Table 1). The addition of 3-MP (1 mM) alone inhibited the glucose production rate, and abolished the stimulatory effects of PTH and 8-bromo cyclic AMP (Table 1).

J_p across the isolated perfused rabbit renal proximal straight tubules

As shown in Table 2, in isolated perfused rabbit renal proximal straight tubules, PTH (1 unit/ml) in the bath medium caused significant inhibition of J_p . The addition of 3-MP (1 mM) to the bath medium alone did not affect J_p , but prevented the inhibitory effect of PTH. Similar results were also obtained with 8-bromo cyclic AMP (0.1 mM).

Since the bath medium used in these studies did not contain glucose, the inhibition of gluconeogenesis by 3-MP may deplete the intracellular glucose in those renal proximal straight tubules studied. If glucose is required for PTH to inhibit J_p , the depletion of intracellular glucose may account for the failure of PTH to inhibit J_p . To test such a possibility, the effect of 3-MP on the inhibition of J_p by PTH was studied with 5 mM-glucose added to the bath medium. As shown in Table 2, when glucose was present in the bath medium, the addition of 3-MP (1 mM) alone did not alter J_p , but continued to prevent the inhibitory effect of PTH (1 unit/ml) on J_p .

DISCUSSION

The mechanism whereby PTH inhibits phosphate reabsorption in the renal proximal tubule has been studied extensively. Since the hormone stimulates cyclic AMP formation in the renal proximal tubule and the

Table 1. Effects of bovine PTH (1–34-peptide) (1 unit/ml), 8-bromo cyclic AMP (0.1 mM) and 3-MP (1 mM) on the glucose production rate from α -oxoglutarate (5 mM) in isolated rabbit renal proximal straight tubules

* $P < 0.05$, ** $P < 0.01$ as compared with control. Values are means $\pm$ S.E.M.

Glucose production rate (pmol/h per mm)									
Control	PTH	Control	3-MP	3-MP + PTH	Control	Cyclic AMP	Control	3-MP	3-MP + cyclic AMP
7.34 ± 0.91 (<i>n</i> = 7)	9.66* ± 0.74 (<i>n</i> = 7)	7.85 ± 0.77 (<i>n</i> = 6)	1.40 ± 0.32 (<i>n</i> = 6)	1.29** ± 0.29 (<i>n</i> = 6)	7.76 ± 1.21 (<i>n</i> = 7)	10.81* ± 1.47 (<i>n</i> = 7)	9.52 ± 1.05 (<i>n</i> = 6)	1.90** ± 0.37 (<i>n</i> = 6)	2.17** ± 0.53 (<i>n</i> = 6)

Table 2. Effects of bovine PTH (1–34-peptide) (1 unit/ml), 8-bromo cyclic AMP (0.1 mM) and 3-MP (1 mM) on the unidirectional lumen-to-bath phosphate flux (J_p) across the isolated perfused rabbit renal proximal straight tubules, with or without glucose (5 mM) in the bath medium* $P < 0.05$ as compared with control. Values are means $\pm$ S.E.M.

J_p (pmol/min per mm)												
Without glucose in the bath							With glucose in the bath					
Control	PTH	Control	3-MP	3-MP + PTH	Control	Cyclic AMP	Control	3-MP	3-MP + cyclic AMP	Control	3-MP	3-MP + PTH
2.09 ± 0.41 ($n = 6$)	1.39* ± 0.28 ($n = 6$)	1.61 ± 0.27 ($n = 8$)	1.59 ± 0.26 ($n = 8$)	2.14 ± 0.40 ($n = 8$)	2.14 ± 0.44 ($n = 5$)	1.44* ± 0.55 ($n = 5$)	1.96 ± 0.26 ($n = 7$)	2.12 ± 0.26 ($n = 7$)	1.97 ± 0.29 ($n = 7$)	1.91 ± 0.43 ($n = 6$)	1.32 ± 0.29 ($n = 6$)	1.70 ± 0.31 ($n = 6$)

administration of cyclic AMP inhibits phosphate reabsorption, it has been generally accepted that cyclic AMP serves as the intracellular mediator for PTH action. The mechanism whereby intracellular cyclic AMP inhibits phosphate reabsorption, however, remains unclear. The recent proposition that gluconeogenesis may interact with phosphate reabsorption in renal proximal tubules [1], although shown to be unlikely in subsequent studies [8, 12], raised the possibility of an interaction between the effects of PTH on these two functions in the renal proximal tubule. In order to examine such a possibility, the effects of 3-MP on these hormonal actions in the isolated rabbit renal proximal straight tubules were studied.

The present observation that 3-MP significantly inhibited glucose production without affecting phosphate absorption in isolated rabbit renal proximal straight tubules is similar to a previous report [12], and negates the possibility that gluconeogenesis can causally affect phosphate reabsorption in the renal proximal tubule. In the presence of 3-MP, however, PTH failed to inhibit the phosphate absorption. The fact that 3-MP also prevented the inhibitory effect of 8-bromo cyclic AMP on phosphate absorption would indicate that the effect of 3-MP is, at least in part, at steps after cyclic AMP formation, and suggests the existence of a mutual interaction between these two hormonal actions. It is possible that PTH actions in both gluconeogenesis and phosphate transport involve a common intracellular pathway which may be inhibited by 3-MP. It is also possible that changes in the intermediate metabolism introduced by 3-MP may interfere with the inhibitory effect of PTH on phosphate transport in renal proximal tubules. Among these metabolic intermediates, the effect of 3-MP on the intracellular glucose concentration is apparent. To test whether or not glucose is required for the action of PTH on phosphate transport in renal proximal tubules, the effect of 3-MP was studied in isolated perfused proximal straight tubules bathed in a medium containing 5 mM-glucose. It was found that, in the presence of glucose in the bath medium, 3-MP continued to abolish the inhibitory effect of PTH on phosphate absorption. These results may suggest therefore that the depletion of intracellular glucose content alone cannot account for the mechanism whereby 3-MP abolishes PTH action on phosphate absorption. Although

glucose is likely to enter the proximal tubular cells through the basolateral membrane, the actual concentrations of intracellular glucose and related metabolites in the proximal straight tubules could not be monitored in these studies, owing to the apparent technical limitation. A definitive conclusion therefore awaits further studies.

In summary, the present studies demonstrate that, in rabbit renal proximal straight tubules, the PTH actions on both gluconeogenesis and phosphate reabsorption are abolished by 3-MP, and suggest that these hormonal actions may share some unknown, yet 3-MP-inhibitable, intracellular process(es).

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