The influence of orthophosphate on the renal handling of inorganic pyrophosphate in man and dog

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Summary
1. The urinary excretion of inorganic pyrophosphate \((\text{PP}_1)\), a known inhibitor of the growth and aggregation of crystals of calcium phosphate and calcium oxalate, increases after ingestion of orthophosphate \((\text{Pi})\). This effect may contribute to the apparent ability of oral phosphate to reduce the formation of urinary stones in man. This paper is a study of the mechanism by which \(\text{Pi}\) increases \(\text{PP}_1\) excretion, investigated by renal clearance techniques in man and renal arterial infusion in dogs. \(\text{PP}_1\) in plasma was measured by an isotope-dilution method after ion-exchange chromatography.

2. The mean renal clearance of endogenous \(\text{PP}_1\) in ten men was \(7.9 \pm 1.7 \text{ ml/min}\), and the mean ratio of \(\text{PP}_1\) clearance to creatinine clearance was \(0.08 \pm 0.02 \text{ (SE)}\). The oral ingestion of \(\text{Pi}\) increased the urinary excretion and renal clearance of \(\text{PP}_1\) about threefold, without significantly changing its concentration in plasma.

3. In dogs, the infusion of \(\text{Pi}\) into one renal artery caused a greater increase in urinary \(\text{PP}_1\) from the infused than from the non-infused kidney, an effect that could be accentuated by simultaneous intravenous infusion of \(\text{PP}_1\). In dogs, only \(1-3\%\) of an injected or infused dose of \(\text{PP}_1\) appeared intact in the urine, regardless of whether it was infused into the systemic or renal circulation.

4. These results suggest that \(\text{Pi}\) has a direct effect on the kidney to increase the excretion of \(\text{PP}_1\). It is possible that \(\text{Pi}\) either interferes with tubular reab-

Introduction
Low concentrations of inorganic pyrophosphate \((\text{PP}_1)\) inhibit the formation of crystals of calcium phosphate (Fleisch, Russell & Straumann, 1966) and the growth and aggregation (Fleisch & Monod, 1973; Robertson, Peacock & Nordin, 1973) of crystals of calcium oxalate \textit{in vitro}. Inorganic pyrophosphate may therefore be a physiological regulator of biological calcification (Russell & Fleisch, 1975) and the \(\text{PP}_1\) in urine may help to prevent renal stones composed of calcium phosphate or oxalate (Russell & Fleisch, 1969, 1973). Excretion of \(\text{PP}_1\) may be lower than normal in the urine of stone-formers (Lewis, Thomas & Tomita, 1966; Russell & Hodgkinson, 1966; Sommer-Tsilenis, Fenner, Kallistratos & Timmermann, 1967; O’Brien, Uhlemann & McIntosh, 1967). Moreover, the marked increase in the renal excretion of \(\text{PP}_1\) that follows ingestion of orthophosphate \((\text{Pi})\) (Fleisch, Bisaz & Care, 1964) may contribute towards the apparent ability of phosphate supplements to reduce the rate of production of renal calculi in man (Thomas & Miller, 1967; Horn, 1967; Howard, 1962; Smith, Thomas & Arnaud, 1973). Since the mechanism by which \(\text{Pi}\) causes this increase in the urinary excretion of \(\text{PP}_1\) is unknown, we decided to study the effect of \(\text{Pi}\) on the renal clearance of \(\text{PP}_1\) in man. We have also infused sorption of \(\text{PP}_1\), perhaps by competing for a common tubular transport mechanism, or that \(\text{Pi}\) diminishes the intrarenal hydrolysis of \(\text{PP}_1\).

Key words: phosphate, pyrophosphate, renal clearance, urinary stones.
into single renal arteries of dogs, in order to see whether P_i directly influences the renal handling of PP_i.

Methods

Renal clearance studies in man

Ten men convalescing from allergic pulmonary disease in Davos consented to take part. None had a history of renal or bone disease. The effects of a single oral dose of P_i and of continued dosage with P_i were studied in five patients for each procedure.

The single-dose study was performed by giving 24 mmol of P (as 16-8 mmol of KH_2PO_4 plus 7-2 mmol of Na_2HPO_4·2H_2O) at 09.00 hours to fasting subjects. Urine samples were collected every 30 min and refrigerated until analysis. Urine flow was augmented by 200 ml of unsweetened drink every 30 min. Venous blood samples were taken at 08.30, 10.00 and 11.30 hours.

In the continuous dosage study, clearances were measured essentially as above in the fasting state and then at 12.00 hours the subject received two capsules of the phosphate mixture orally, followed by one capsule every 3 h until the following morning at 09.00 hours. Each capsule contained 5-4 mmol of P and the total phosphorus intake in this form was therefore 48 mmol. The P_i was given as a mixture of KH_2PO_4 (33-6 mmol/day) and Na_2HPO_4·2H_2O (14-4 mmol/day). On the second day, the procedure for clearances was similar to that on the first day but no further phosphate was given after 09.00 hours. The diet was otherwise similar on each day.

Renal arterial infusion studies in dogs

Seven mongrel dogs, weight range 13-5-22 kg., were used. Anaesthesia was induced with intravenous pentobarbital (Nembutal, 20-30 mg/kg) and maintained either with a halothane/O_2 gas mixture or by constant infusion of pentobarbital (15-90 mg/h). The femoral artery was catheterized for blood sampling and a femoral or brachial vein was catheterized for an intravenous infusion of inulin (12 mg/min), p-aminohippuric acid (2-06 μmol/min) and creatinine (34 μmol/min). These substances were dissolved in NaCl soln. (155 mmol/l) infused at 6 ml/min through a multichannel constant-infusion pump. In most experiments, 10% (w/v) mannitol replaced some of the NaCl soln. on a volume-for-volume basis so that the dog received 0-5-1-5 mmol of mannitol/min. A priming injection of inulin (60 mg/kg), p-aminohippuric acid (5-2 μmol/kg) and creatinine (27 μmol/kg) was given. The ureters were separately catheterized, either by a transvesical extraperitoneal approach or at laparotomy. After the animals had been given 10 000 units of heparin intravenously, the renal artery was catheterized by a technique described previously (Russell & Fleisch, 1968).

Infusion through the catheter in the renal artery was done with an LKB single-channel constant-infusion pump. During the control periods, NaCl soln. (155 mmol/l) was infused at 1 ml/min. During the test periods, solutions of P_i (161 μmol/ml), dissolved in water, or PP_i (various concentrations: see the Figures), dissolved in NaCl soln., were infused at the same rate.

In order to eliminate traces of PP_i from the P_i solutions to be infused, the latter were prepared by boiling solutions of orthophosphoric acid for 1 h before adjusting the pH to 7-4 with NaOH. The sodium phosphate solutions were stored frozen and their PP_i content was measured before use by the same isotope-dilution method as was used for the determination of PP_i in plasma. This procedure was successful in reducing the PP_i content to 0-009% of the total P.

Analytical methods

The following methods were used: PP_i in urine (Fleisch & Bisaz, 1963) and plasma (Russell, Bisaz, Donath, Morgan & Fleisch, 1971), by ion-exchange chromatography; P_i, by reduction of phosphomolybdate complex with ascorbic acid (Bisaz, Russell & Fleisch, 1968); calcium, by atomic absorption spectrophotometry (Bisaz et al., 1968); creatinine (Bonsnes & Taussky, 1945), inulin (Heyrovsky, 1956) and p-aminohippurate (Bratton & Marshall, 1939), by established methods.

All doses, concentrations and excretion rates of PP_i and P_i are expressed in terms of phosphorus. Clearances of PP_i and P_i were estimated assuming each was 100% ultrafiltrable, and that of calcium assuming it was 60% ultrafiltrable. Previous studies with PP_i suggest that less than 20% is bound to plasma proteins (Russell et al., 1971).

Results

Effect of feeding with phosphate on the renal excretion of pyrophosphate in man

The mean renal clearance of PP_i in ten men was
Renal handling of pyrophosphate

7.9 ± 1.7 (SE) ml/min, and the mean clearance ratio of PP1 to creatinine was 0.08 ± 0.02 (SE). The response to increased intake of P1 is shown in Fig. 1 and Tables 1 and 2.

After ingestion of a single dose of 24 mmol of P1, the urinary excretion of both P1 and PP1 increased within 30 min and the maximum was obtained after 14–2 h (Fig. 1). The increase was about fourfold for P1 and about threefold for PP1. The renal clearance of both P1 and PP1 rose more than twofold. The clearance of creatinine decreased slightly and that of calcium markedly.

Table 1 shows that P1 in plasma significantly increased by about 30% (P<0.02) but that the PP1 in plasma did not change significantly (P>0.7). There were significant increases in the PP1/creatinine and P1/creatinine clearance ratios.

Similar results were obtained when P1 was given over 21 h instead of in one dose (Table 2). Again, P1 induced an increase in the excretion of both P1 and
Table 1. Effect of a single oral dose of 48 nmol of orthophosphate (P1) on plasma concentrations and clearance ratios of PPI and P1 in five men during the 1 day experiment

See the text for details. Values shown are mean results±se. ** Difference from 08.00-09.00 hours period significant at less than the 2% level.

<table>
<thead>
<tr>
<th>Before P1</th>
<th>After P1 (given at 09.00 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine: 08.00-09.00 hours Plasma: 08.30 hours</td>
<td>Urine: 09.30-10.30 hours Plasma: 10.00 hours</td>
</tr>
<tr>
<td>Plasma PPI (μmol/l)</td>
<td>2.97±0.54</td>
</tr>
<tr>
<td>Plasma P1 (mmol/l)</td>
<td>1.22±0.06</td>
</tr>
<tr>
<td>PPI clearance/creatinine clearance</td>
<td>0.09±0.03</td>
</tr>
<tr>
<td>P1 clearance/creatinine clearance</td>
<td>0.10±0.02</td>
</tr>
</tbody>
</table>

Table 2. Effect of ingestion of P1 on plasma concentrations, renal clearances, and on renal clearance ratios (to creatinine) of PPI, P1, and calcium in five men during the 2 day experiment

See the text for details. Values are mean results±SEM. The phosphate supplement was 1.5 g of P given over the preceding 21 h. ** Difference between day 1 and 2 significant at less than the 2% level.

<table>
<thead>
<tr>
<th>Day 1: before phosphate supplement</th>
<th>Day 2: after phosphate supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PPI (μmol/l)</td>
<td>3.08±0.19</td>
</tr>
<tr>
<td>Plasma P1 (mmol/l)</td>
<td>1.18±0.06</td>
</tr>
<tr>
<td>Plasma Ca (mmol/l)</td>
<td>2.50±0.10</td>
</tr>
<tr>
<td>Clearance PPI (ml/min)</td>
<td>6.6±1.3</td>
</tr>
<tr>
<td>Clearance P1 (ml/min)</td>
<td>12.1±1.7</td>
</tr>
<tr>
<td>Clearance Ca (ml/min)</td>
<td>0.77±0.06</td>
</tr>
<tr>
<td>PPI clearance/creatinine clearance</td>
<td>0.069±0.016</td>
</tr>
<tr>
<td>P1 clearance/creatinine clearance</td>
<td>0.124±0.015</td>
</tr>
<tr>
<td>Calcium clearance/creatinine clearance</td>
<td>0.008±0.001</td>
</tr>
</tbody>
</table>

PPI, and the renal clearance of both substances increased. The concentration of P1 in plasma again increased but the concentration of PPI did not increase significantly. The clearance of creatinine was unaltered.

Renal arterial infusion studies in dogs

Effect of renal arterial infusion of P1 on the excretion of PPI of endogenous origin. In each of the seven dogs studied, infusions of P1 into one renal artery at a rate of 161 μmol of P/min over 50-60 min induced a marked rise in the excretion of both PPI and P1. The changes in urine PPI and P1 were both more rapid and greater from the infused than from the non-infused kidney. Figs. 2, 3 and 4 show the results from three of the seven dogs. Similar but smaller effects were seen during the infusion of 65 μmol of P1/min. The responses to infusion of P1 were seen within 10 min of starting the infusion and could not be attributed to corresponding changes in glomerular filtration rate or renal plasma flow as assessed by the renal clearance of inulin and p-aminohippurate respectively.

The increase in urine PPI in response to renal arterial infusion of P1 was shown to be due to PPI itself, since the "PPI" peaks on the ion-exchange chromatographic runs could be removed by prior hydrolysis of the urine either in hot acid or with yeast inorganic pyrophosphatase.

Fig. 2 and Fig. 3 show that the effect of P1 on urine PPI cannot be attributed to trace amounts of PPI in the P1 infusion solution. Isotope-dilution analysis had shown that the infusion solution of P1 used in these experiments contained 0.009% of the total P in the form of PPI. Infusion of PPI at an equivalent rate, i.e. 7.25 nmol of PPI/min, into the left kidney caused no change in PPI excretion from either kidney (Fig. 3), whereas the infusion of P1 itself at 161 μmol/min increased the excretion of PPI from the infused kidney approximately twofold. Infusion of PPI at a rate ten times higher (72.5 nmol/min) into the left kidney also had a negligible effect on the urinary excretion of PPI, in one experiment (Fig. 3), but in the other induced an appreciable rise in PPI excretion (Fig. 2). Only infusion of PP1 at a rate of 0.8 μmol/min, i.e. at a concentration more...
Renal handling of pyrophosphate

Fig. 2. Effect of infusions of \( P_1 \) (A, 161 \( \mu \)mol/min) and \( PP_1 \) (B, 72.5 nmol/min and C, 0.8 \( \mu \)mol/min) into the left renal artery of a dog (21.0 kg) on urinary excretion of \( PP_1 \) (stippled columns) and \( P_1 \) (open columns) from right and left kidneys. The high rates of excretion of \( PP_1 \) (marked by arrow heads, values off the scale) from the left kidney from 4 h to 4 h 10 min, from 4 h 10 min to 4 h 20 min and from 5 h 50 min to 6 h were 15, 23 and 24 nmol of \( PP_1 \)/min respectively. The individual (left and right) renal clearances of inulin \( (C_{\text{inulin}}) \) and the plasma \( P_1 \) are also shown.

than 100 times the amount present in the \( P_1 \) solution, caused a marked rise in \( PP_1 \) excretion from the infused but not from the non-infused kidney (Fig. 2).

Effect on urine \( PP_1 \) of intravenous injection and infusion of \( PP_1 \), and the response to renal arterial infusion of \( P_1 \) during intravenous infusion of \( PP_1 \). In the experiment shown in Fig. 3, 6 h after beginning the experiment a priming solution containing 80 \( \mu \)mol of \( PP_1 \) was injected intravenously and this was followed by a continuous intravenous infusion of \( PP_1 \) at 1 \( \mu \)mol/min. The plasma concentration of \( PP_1 \) was not measured in these experiments because of the haemodynamic consequences of withdrawing the large volumes of blood necessary for the analysis. However, other studies in dogs (Jung, Russell, Bisaz, Morgan & Fleisch, 1970) have shown that the plasma concentration of \( PP_1 \) is directly related to the rate of infusion and rises to reach a new plateau concentration about 2 \( \mu \)mol/l higher than the previous concentration for each \( \mu \)mol of \( PP_1 \) infused/min. The plasma
concentration of PP₁ would therefore have been expected to double in this experiment. In spite of this, only very little of the infused PP₁ appeared in the urine. Thus, in the first 10 min after the priming dose of 80 μmol of PP₁, only 1·2 μmol of PP₁ appeared in the urine. After 20 min the total excretion rate was about 0·015 μmol/min above the pre-infusion rate, meaning that less than 2% of the amount infused was being excreted in the urine.

Fig. 4 illustrates similar results in another dog which also received PP₁ intravenously. In this case, considerably less than 1% of the injected or infused PP₁ appeared intact in the urine. Even infusion of PP₁ directly into the renal artery was not accompanied by a much higher rate of excretion. In the dog shown in Fig. 2, the maximum rate of excretion of PP₁ infused at 0·8 μmol/min in the left renal artery was 2·9%. 
Renal handling of pyrophosphate

441

PPi (20 pmol, i.v.)

PPi (2.42 pmol/min i.v.)

Right

0 5 10

0 5 10

P1 (nmol/min)

161 pmol/min

PPi

Left

0 5 10

0 5 10

Plasma P1 (nmol/l)

0 10 20

0 10 20

Creat. (mg/dl)

0 10 20

0 10 20

Time (h)

0 10 20

0 10 20

Fig. 4. Effect of infusion of P1 (161 pmol/min) into the left renal artery of a dog (13.5 kg) on the urinary excretion of PPi (stippled columns) and P1 (open columns) before and during a systemic intravenous infusion of PPi at 2.42 pmol/min. A priming intravenous injection of 20 pmol of PPi was given at 3 h.

In dogs receiving systemic infusions of PPi (Fig. 3 and Fig. 4), the response to renal arterial infusion of P1 (161 pmol/min) was much more striking than in the animals not receiving PPi intravenously. The increment in urine PPi was now several times greater than before the systemic infusion of PPi was started. The response from the infused kidney was again greater than from the non-infused kidney. In contrast to the effect on urine PPi, the rise in urine P1 was less than before, probably because, with successive renal arterial infusions of P1 at 161 pmol/min, glomerular filtration diminished (see the changes in inulin in Fig. 4).

Discussion

In the present studies in man, an average of only about 8% of the presumed filtered load of PPi was excreted in the urine under fasting conditions. The remaining 92% of the PPi was therefore either reabsorbed and/or hydrolysed in the kidney.

When the intake of P1 was increased, the percentage of excreted PPi increased from an average of 8% to 29% of the presumed filtered load. The concentration of PPi in plasma, however, did not change significantly or consistently after the ingestion of P1, in spite of the large increase in urinary PPi. Attempts to reduce the abnormally high plasma PPi in hypophosphatasia by feeding with P1 (Bongiovanni, Album, Root, Hope, Marino & Spencer, 1968) is therefore unlikely to be successful. Since oral P1 increases urinary PPi with little change in plasma PPi, there is probably a direct effect of P1 on the renal handling of PPi. The renal arterial infusion studies in dogs support this.

In all dogs studied, the infusion of P1 into one renal artery caused a greater increase in urinary PPi from the infused than from the non-infused control kidney, suggesting that P1 directly affects the renal handling of PPi. The effect of renal arterial infusion of P1 on urinary PPi could not be attributed to trace amounts of PPi in the infusion solutions, nor did it seem likely to be due to the stimulation of secretion of parathyroid hormone, which occurs during P1 infusion, since this would have been expected to produce an equal effect on each kidney. Secretion of parathyroid hormone may, however, account for part of the fall observed in calcium clearance in the human studies (Fig. 1).

The observation that the renal effect of P1 on PPi excretion was greater when the animal was receiving exogenous PPi intravenously suggests that the effect is not due to increased renal synthesis of PPi induced by phosphate, but that P1 in some way alters the handling by the kidney of PPi delivered to it via the blood. The most likely explanations are that P1 either diminishes the tubular reabsorption of PPi, or diminishes its intrarenal hydrolysis. Either mechanism could explain most of the known facts about the renal handling of PPi. Thus there is a close and direct relation between urinary PPi and P1 under a variety of circumstances (Fleisch & Bisaz, 1963; Russell & Hodgkinson, 1966; Russell & Fleisch, 1973), even though there is no chemical interconversion of the two in urine (Russell, Wadstrom, Lindstedt, Care, Bisaz & Fleisch, 1969). If PPi and P1 did share a common renal tubular transport mechanism for reabsorption, then the amount of PPi reabsorbed
would be inversely related to tubular concentrations of $P_i$, which would therefore, in turn, be directly related to the amount of $PP_i$ in urine. However, there is at present no direct evidence for a common tubular transport mechanism for $PP_i$ and $P_i$, so that an effect of $P_i$ on $PP_i$ hydrolysis is also possible.

It does seem that at least some intrarenal hydrolysis of $PP_i$ to $P_i$ takes place. Thus, after intravenous injections of $^{32}P$-labelled $PP_i$, it can be shown that at least part of the $^{32}P$-labelled $P_i$ appearing in urine probably arises from hydrolysis of $[^{32}P]PP_i$ to $[^{32}P]P_i$ in the kidney (Jung et al., 1970). $P_i$ is known to be a competitive inhibitor of alkaline phosphatases (Ferney & Walker, 1967), enzymes which are now recognized to be pyrophosphatases. $P_i$ might therefore enhance the urinary excretion of $PP_i$ by inhibiting pyrophosphatases within the kidney. If these enzymes were active on the luminal surfaces of the tubular cells, where they can be demonstrated histochemically, then the tubular concentration of $P_i$ could directly influence the amount of $PP_i$ appearing in urine.

It is worth noting that after intravenous injection or infusion of $PP_i$, only a very small amount of the $PP_i$ was excreted into the urine. Studies on the disappearance of small amounts of $[^{32}P]PP_i$ injected intravenously in dogs have shown that plasma $PP_i$ is subject to a high rate of metabolic turnover and confirm that urinary excretion constitutes only a minor route for the disappearance of $PP_i$ from plasma (Jung et al., 1970). In view of this high turnover, it is unlikely that the small amounts of $PP_i$ that could be derived from resorption of bone could comprise a major portion of the $PP_i$ excreted daily in urine, as was at one time thought (Avioli, McDonald & Singer, 1965; Avioli, McDonald, Hennenman & Lee, 1966). The major part of the $PP_i$ in body fluids is more likely to arise as a by-product of the many biosynthetic reactions that produce $PP_i$ during pyrophosphorylysis of nucleotide triphosphates (Kornberg, 1962).

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References


