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Restoration of a Phosphaturic Response to Parathyroid Hormone in the Immature Rat

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ABSTRACT. Recent studies have shown that immature rats display a diminished sensitivity to the phosphaturic effects of parathyroid hormone (PTH), and that the responsiveness to PTH increases with age. The attenuated phosphaturia may reflect an inability of the neonate to respond to the hormone because of functional immaturity of the developing kidney. Alternatively, PTH may actually inhibit tubular phosphate reabsorption in the neonate but, due to other phosphate conservation mechanisms, no phosphaturia occurs. Our objective was to determine whether a phosphaturic response to PTH would be elicited in immature rats during infusion of moderate amounts of phosphate (Pi). Clearance experiments were performed on 26 acutely thyroparathyroidectomized immature Wistar rats (3–5 wk of age) fed a normal Pi diet (0.63%). In response to infusion of either Pi (1 μmol/min·100 g) (group I) or PTH (8.3 ng/min·100 g) (group II) alone, the fractional excretion of phosphate rose minimally (from 0.01 ± 0.01% to 4.9 ± 1.9% and from 0.12 ± 0.12% to 2.9 ± 1.4% for groups I and II, respectively). However, when Pi and PTH were combined either Pi first followed by PTH (group III) or PTH first followed by Pi (group IV), the fractional excretion of Pi rose dramatically (from 0.01 ± 0.01 to 21.8 ± 3.5% and from 0.04 ± 0.04 to 27.7 ± 3.3% for groups III and IV, respectively). A significant increase in urinary cAMP excretion occurred during infusion of PTH even when Pi excretion was minimal, but there was no further increase in urinary cAMP during the combined infusion of Pi and PTH. These results indicate that a phosphaturic response to PTH can be elicited in immature rats during infusion of moderate amounts of phosphate, and that the phosphaturia is dissociated from the increase in urinary excretion of cAMP. Thus, the attenuated phosphaturic response to PTH seen in developing rats is not due to functional immaturity of the kidney, but rather could be attributed to other factors that promote Pi reabsorption and prevent the phosphaturic effect from being expressed. (*Pediatr Res* 26: 54–57, 1989)

Abbreviations

PTH, parathyroid hormone
TPTX, thyroparathyroidectomized
Pi, phosphate

The need for young, immature animals to maintain positive phosphate balance for growth is well recognized. This process may be facilitated by several adaptations in the developing kidney. Previous studies have shown that immature animals (1, 2) and newborn infants (3) have a relatively higher maximal rate of tubular phosphate reabsorption compared to adults. In addition, the neonate displays a relative hyporesponsiveness to stimuli which are normally phosphaturic in the adult, such as phosphate loading (2) and PTH (1, 4, 5). Indeed, we recently reported that immature, acutely TPTX rats were less sensitive to the phosphaturic effects of PTH, and that the phosphaturic response increased progressively with age (6). Furthermore, this attenuated response was not associated with a diminished urinary excretion of cAMP, suggesting that the blunted response to the phosphaturic effects of PTH in the neonate is dissociated from the generation of cAMP. What remains unclear is whether the diminished sensitivity to the phosphaturic effects of PTH in the neonate represents a true adaptation as a consequence of the high metabolic demand for phosphate in a growing animal, or whether it is simply a reflection of a functionally immature nephron that is unable to respond to the hormone.

The purpose of these experiments was to further examine the mechanism by which developing animals resist the effects of PTH. Specifically, the objective was to determine whether a phosphaturic response could be elicited in immature rats during simultaneous infusion of moderate amounts of phosphate, *i.e.* which elevate the filtered load of phosphate without overwhelming the renal phosphate transport capacity. Such a manuever has been shown to restore a phosphaturic response to PTH in adult animals that were phosphate deprived (7), another model of renal resistance to PTH. If in the presence of phosphate infusion a phosphaturic response to PTH is observed in immature rats, then this would suggest that the blunted response to PTH in these animals is not due to functional immaturity of the kidney, but rather to other factors serving to promote phosphate retention.

MATERIALS AND METHODS

Clearance experiments were performed on 26 male Wistar rats at 3–5 wk of age (94 ± 3 g). The animals were fed normal rat Chow (Ralston Purina, St. Louis, MO) containing 0.63% Pi. All the animals were given food and water *ad libitum* and care was taken to ensure that the rats had not fasted.

On the day of the experiment, the rats were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg) (Promonta, Hamburg, FRG) and prepared for renal clearance experiments. The animals were placed on a thermoregulated table and body temperature was monitored with a rectal probe and maintained at 37°C with a servocontrolled heat lamp. A tracheostomy was performed to maintain an unobstructed airway, and the animals were allowed to breathe spontaneously. Polyethylene catheters were placed in the carotid artery for blood pressure measurement and blood sampling, in the jugular vein for infusing solutions, and in the bladder for collecting urine samples.

All rats were TPTX by heat cautery to remove the influence

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of endogenous PTH. After TPTX, a 2-h recovery period elapsed to achieve steady state conditions, during which time a 2% inulin solution was infused at a rate of 2% body wt/h for the duration of the experiment. At this point one of four protocols was performed.

Group I: control, Pi, Pi. The purpose of this protocol was to evaluate the effects of Pi infusion alone, within the same time period as the group given both Pi and PTH (group III). After the recovery period, a 30-min control clearance (C) was taken. Subsequently, phosphate was added to the inulin infusion at a concentration calculated to deliver 1 μmol/min·100 g for the duration of the experiment. The phosphate solution was a 4:1 mixture of dibasic:monobasic sodium Pi salts adjusted to pH 7.4. A single bolus of Pi (30 μmol/100 g) was administered at the onset of the Pi infusion. The infusion of phosphate proceeded for 20 min before sequential 30 min clearance periods were taken over the next 3 h. Blood samples were obtained at the midpoint of each clearance period. The results represented as experimental period 1 (Pi) are the mean of the clearances during the 1st h of Pi infusion, and the results represented as experimental period 2 (Pi) are the mean of the clearances obtained during the 3rd h of Pi infusion.

Group II: control, PTH, PTH. The purpose of this protocol was to evaluate the effects of PTH infusion alone over the same time period as the group given both PTH and Pi (group IV). After the 2-h recovery period, a 30-min control clearance (C) was obtained. Subsequently, PTH (synthetic, bovine 1-34 PTH, Peninsula Laboratories, Inc., Belmont, CA) was administered as a bolus injection (249 ng/100 g) and infused at 8.3 ng/min·100 g for the duration of the experiment. The infusion of PTH continued for 40 min before consecutive 30-min clearance periods were taken over the next 3 h. The results given for experimental period 1 (PTH) are the mean of the clearances obtained during the 1st h of PTH infusion, and the results given for experimental period 2 (PTH) are the mean of the clearances obtained during the 3rd h of PTH infusion.

Group III: control, Pi, Pi + PTH. The purpose of this protocol was to evaluate the renal effects of PTH given in the presence of ongoing Pi infusion. This protocol was similar to group I, except that PTH was administered concurrently with phosphate during the latter part of the experiment. Bolus and maintenance infusion doses for both Pi and PTH were calculated as described earlier for groups I and II. Data given for experimental period 1 (Pi) represents the mean of the clearances obtained during the infusion of phosphate alone, and the data given for experimental period 2 (Pi + PTH) represents the mean of the results obtained during the second hour of combined infusion.

Group IV: control, PTH, PTH + Pi. The purpose of this protocol was to evaluate the effects of Pi infusion given in the presence of ongoing PTH infusion. This protocol was similar to group II, except that Pi was administered with PTH during the latter part of the experiment. The data given for experimental period 1 (PTH) represents the mean of the clearances obtained during the infusion of PTH alone, and the data given for experimental period 2 (PTH + Pi) represents the mean of the results obtained during the 2nd h of combined infusion.

Analysis. Inulin concentrations in plasma and urine samples were measured by the anthrone method (9). The glomerular filtration rate was equated with the clearance of inulin. Phosphate concentrations in plasma and urine samples were measured by the phosphomolybdate method described by Chen *et al.* (10), and cAMP concentrations in urine were determined by radioimmunoassay (New England Nuclear, Boston, MA).

All values are expressed as means ± SE. Statistical comparisons were made with paired and unpaired Student's *t*-tests where appropriate.

RESULTS

Time control experiments: either Pi or PTH alone. The results obtained in the time control experiments from groups I and II are summarized in Table 1. The infusion of Pi alone in immature TPTX rats led to a predictable increase in the plasma Pi concentration. However, the fractional excretion of Pi rose to a maximum of only 5% during the second period of Pi infusion. In all groups (I–IV), mean arterial blood pressures declined over the course of the experiment. However, the GFR remained stable.

The results from rats given only PTH (group II) are also presented in Table 1. Plasma Pi concentration did not change significantly with infusion of the hormone. The low fractional excretion of phosphate during the control period reflected the adequacy TPTX. During PTH infusion, fractional phosphate excretion rose to a maximum of 5.7%, which is significantly attenuated to what has been previously obtained with this dose in older animals, *i.e.* fractional excretion of phosphate in the range of 20–30% (6).

Experiments in presence of both PTH and Pi. Table 2 contains the results obtained from group III rats, which received Pi infusion first followed by the combined administration of Pi and PTH. Once again, the infusion of Pi alone led to a significant rise in the plasma Pi concentration, with only a minimal increase in the fractional excretion of Pi. After the addition of PTH, the fractional excretion of Pi increased markedly from 3.7 to 21.8%. This occurred without a significant change in plasma Pi concentration.

In group IV, the reverse experiment was performed in which PTH was administered first, and then combined with Pi infusion. The results are given in Table 2. As seen in the time control experiments of group II, there was no significant change in the plasma Pi concentration during the administration of PTH alone and the rise in the fractional excretion of Pi was again attenuated. However, with the simultaneous infusion of both PTH and Pi, there was a dramatic increase in fractional excretion of phosphate from 3.6 to 27.7%.

To test whether the enhanced phosphaturic response to PTH was related to an augmentation in the signal transduction of the hormone at the level of the second messenger, determinations were made of the urinary excretion of cyclic AMP. The relationship between the renal excretion of cAMP and the fractional excretion of phosphate is shown in Figure 1. The values for urinary cAMP excretion during the control period or during infusion of phosphate alone were similar in all the groups. During infusion of PTH (group II and in experimental period 1 of group IV), there was a marked increase in urinary cAMP, but Pi excretion was minimal. During the combined infusion of PTH and Pi (groups III and IV), a significant increase in fractional Pi excretion was observed, but the urinary cAMP excretion rose to the same level as in group II. Accordingly, the lack of a phosphaturic response to PTH (when PTH was infused alone in groups II and IV) was not due to reduced generation of cAMP by PTH. By the same token, the onset of phosphaturia in the presence of both PTH and Pi was not due to an augmented cAMP response. Rather, it appears that the effects of PTH on Pi and cAMP excretion can be dissociated, and that the diminished phosphaturic response to PTH is not a consequence of an inability of the developing kidney to respond to the hormone.

DISCUSSION

The purpose of this study was to determine whether a phosphaturic response to PTH could be elicited in immature rats. The results of this study demonstrate that, whereas the infusion of Pi or PTH alone does not significantly alter Pi excretion in immature rats, the combination of Pi infusion and PTH, given in either order, results in a marked phosphaturia in these animals. Moreover, the combination of PTH and Pi infusion produced a phosphaturia that was much larger than the sum of the excretion of Pi induced by the administration of each stimuli alone. This points to a synergistic interaction between the effects of phosphate infusion and PTH in the immature kidney.

Table 1. *Effects of phosphate or PTH infusions alone on renal handling of phosphate in immature TPTX rats**

Period	PPi (mM)	FEPi (%)	FENa (%)	GFR (ml/min)	MAP (mmHg)
Group I					
C	2.97 ± 0.23	0.01 ± 0.01	0.20 ± 0.13	0.92 ± 0.09	97 ± 5
Pi	3.96 ± 0.25†	0.18 ± 0.06	0.34 ± 0.13	1.09 ± 0.09	94 ± 3
Pi	4.69 ± 0.28†	4.93 ± 1.90†	0.15 ± 0.04	0.95 ± 0.09	83 ± 3
Group II					
C	3.17 ± 0.15	0.12 ± 0.12	0.04 ± 0.02	0.80 ± 0.08	101 ± 4
PTH	3.09 ± 0.16	5.71 ± 1.75†	0.13 ± 0.02	0.93 ± 0.04	98 ± 3
PTH	3.09 ± 0.16	2.93 ± 1.36†	0.21 ± 0.11	0.97 ± 0.04	92 ± 3

* Values are means ± SE from six animals in group I and six animals in group II. Pi, phosphate; PPi, plasma phosphate concentration; FEPi, fractional excretion of phosphate; FENa, fractional excretion of sodium; MAP, mean arterial pressure.

† $p < 0.05$ compared to the control period.

Table 2. *Effects of combined infusion of phosphate and PTH on renal handling of phosphate in immature TPTX rats**

Period	PPi (mM)	FEPi (%)	FENa (%)	GFR (ml/min)	MAP (mmHg)
Group III					
C	2.98 ± 0.26	0.01 ± 0.01	0.12 ± 0.09	1.25 ± 0.18	95 ± 4
Pi	4.17 ± 0.35*	3.69 ± 2.70*	0.52 ± 0.20	1.30 ± 0.10	97 ± 2
Pi + PTH	4.20 ± 0.40*	21.75 ± 3.46*	0.47 ± 0.30	1.27 ± 0.08	91 ± 3
Group IV					
C	2.95 ± 0.24	0.04 ± 0.04	0.19 ± 0.08	1.12 ± 0.13	97 ± 4
PTH	2.88 ± 0.11	3.60 ± 1.20*	0.27 ± 0.07	1.14 ± 0.10	101 ± 2
PTH + Pi	3.30 ± 0.20	27.72 ± 3.31*	0.25 ± 0.11	1.12 ± 0.06	95 ± 5

* Values are means ± SE from seven animals in group III and seven animals in group IV. Pi, phosphate; PPi, plasma phosphate concentration; FEPi, fractional excretion of phosphate; FENa, fractional excretion of sodium; MAP, mean arterial pressure.

† $p < 0.05$ compared to the control period.

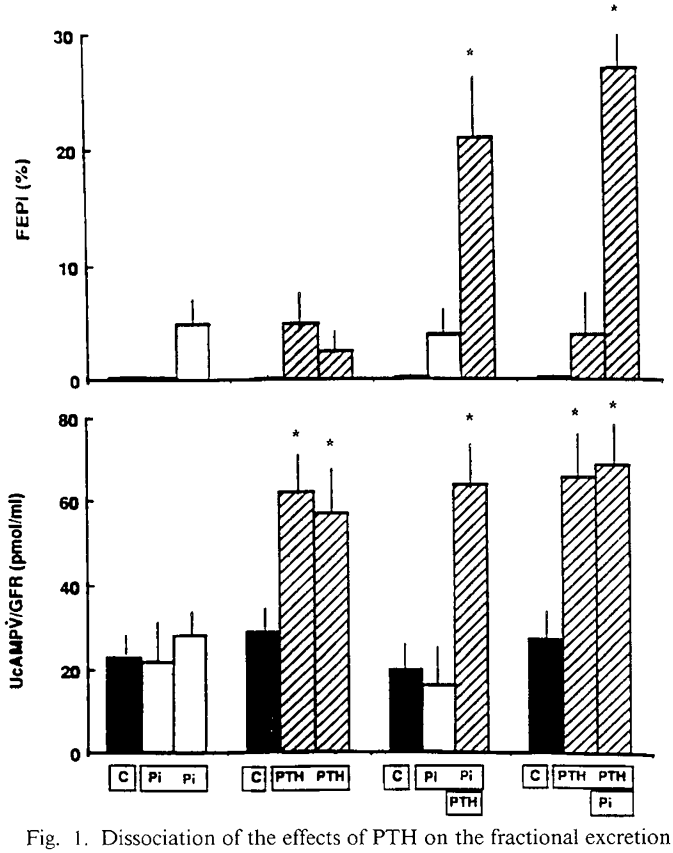

Fig. 1. Dissociation of the effects of PTH on the fractional excretion of phosphate (FEPi) and on the urinary excretion of cAMP (expressed as UcAMPV/ml of GFR) in immature, acutely TPTX rats. In every instance that PTH was administered (*hatched bars*), the urinary excretion of cAMP increased significantly (*lower panel*). However, an increase in FEPi (*upper panel*) occurred only during the combined infusion of phosphate (Pi) and PTH.

The mechanism for the attenuated phosphaturic response to PTH in the immature kidney is still not clear. The notion that the blunted phosphaturic response in the newborn is related to a decrease in receptor number or sensitivity appears unlikely, in view of the significant increase in urinary cAMP excretion during infusion of PTH. Indeed, urinary cAMP excretion rose to a similar extent in each instance that PTH was administered, whether in the presence or absence of Pi infusion. The fact that a phosphaturia occurred only when PTH was combined with Pi infusion again demonstrates that the phosphaturic and urinary cAMP effects of PTH can be dissociated. Thus, these findings concur with previous suggestions that the renal PTH-adenylate cyclase-cAMP axis is intact in developing animals (11–13). However, the possibility that a defect might be present in the hormonal signal transduction at a step beyond cAMP generation cannot be excluded.

Spitzer *et al.* (14) have proposed that low intracellular Pi concentrations may be an important driving force for the avid reabsorption of Pi seen in immature animals. It is conceivable that under those conditions a favorable concentration gradient would exist across the luminal membrane to facilitate the transport of Pi into the tubular cell, perhaps even in the presence of PTH. Consequently, the infusion of Pi might limit this enhanced reabsorption by raising the intracellular Pi concentration. In our study, however, the magnitude of the phosphaturia in the presence of both PTH and Pi was similar even though, when Pi was added to an ongoing PTH infusion, the extent of repletion of intracellular Pi stores was, presumably, not as large as in the reverse experiment.

Perhaps the results can best be accounted for by an alternative explanation related to the nephron sites of Pi reabsorption. Although direct measurements of the segmental handling of Pi in immature rats are not available, data obtained in other states of Pi conservation may be applicable in this regard. Both Pi deprivation (15, 16) and respiratory alkalosis (17) in adult rats are states characterized by an attenuated phosphaturic, but a normal cAMP, response to PTH. Results from several laboratories indicate that, although PTH still inhibits Pi reabsorption in

the proximal convoluted tubule in these states, a phosphaturia does not occur because of enhanced Pi reabsorption in the pars recta and distal nephron segments (18–22). In a similar manner, moderate Pi infusions to Pi-deprived adult rats, at rates which do not raise Pi excretion, also have been shown to decrease Pi uptake in proximal tubule brush border membrane vesicles (23), and increase the delivery of phosphate out of the proximal convoluted tubule (24). Because of the many similarities in the renal handling of phosphate between young, rapidly growing rats and Pi-deprived adult rats, it is reasonable to propose that nephron sites beyond the proximal tubule reabsorb significant amounts of Pi in the developing kidney. Accordingly, when PTH or Pi were administered separately, some inhibition of Pi reabsorption in the proximal tubule may have occurred. However, a phosphaturia did not ensue because the more distal sites could reabsorb the increased load of Pi. In contrast, when Pi and PTH were combined, the magnitude of the load delivered to the *pars recta* and distal tubule could have overwhelmed the Pi transport capacity of those segments, resulting in the increase in Pi excretion that was observed. This notion would also explain why a phosphaturia of similar magnitude occurred even when the order of administration of Pi and PTH was reversed.

Bonjour and Fleisch (25) have proposed that the tubular reabsorption of Pi is regulated by the balance between the demand of the animal for phosphate and the available dietary Pi supply. This thesis is clearly reflected in the parallels between the renal handling of Pi in immature rats fed normal Pi diet and Pi-deprived adult rats. Both the immature rats (that have an increased demand for Pi due to an accelerated rate of growth) and Pi-deprived adult rats (that have a reduced supply of phosphate) exhibit an elevated tubular capacity to reabsorb Pi (2, 15) and an apparent reduced sensitivity to PTH (6, 16); adaptations that serve to facilitate the renal conservation of Pi.

In summary, the results of this study indicate that a phosphaturic response to PTH can be elicited in immature rats by combined infusion of Pi and PTH, and that the phosphaturia is dissociated from the increase in urinary excretion of cAMP. The findings also suggest that the attenuated phosphaturic response to PTH seen in the neonate does not represent functional immaturity of the developing kidney, but rather may serve as an appropriate adaptation that contributes to Pi retention during growth and development.

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