Renal handling of phosphate during acute respiratory acidosis and alkalosis in the rat

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HARAMATI, AVIAD, AND DAVID NIENHUIS. Renal handling of phosphate during acute respiratory acidosis and alkalosis in the rat. Am. J. Physiol. 247 (Renal Fluid Electrolyte Physiol. 16): F598–F601, 1984.—Clearance experiments were performed in acutely thyroparathyroidectomized rats to evaluate the renal handling of phosphate during respiratory acidosis (R ACID) and alkalosis (R ALK) in rats fed either a normal (0.7%) or low (0.07%) phosphate diet for 4 days. Different acid-base states were achieved by varying the mixture of carbon dioxide in the inspired air. Each group received graded infusions of phosphate to control for differences in plasma phosphate (Pp) and to determine the maximum transport capacity of phosphate reabsorption (TmP/GFR). In rats fed a normal phosphate diet, Pp, and the fractional excretion of phosphate (FEpi) were significantly greater in R ACID than in R ALK. However, there were no differences between R ACID and R ALK when FEpi was evaluated as a function of the Pp, and values for TmP/GFR during R ACID were not different from those during R ALK. In rats fed low phosphate diet, Pp, during R ACID was significantly greater than during R ALK, yet FEpi was less than 1% in all groups due to an adaptive increase in TmP/GFR. Further, the TmP/GFR was similar irrespective of the acid-base state. We conclude that acute respiratory acid-base changes do not alter the intrinsic capacity of the kidney to reabsorb phosphate.

phosphate transport capacity; carbon dioxide; normal phosphate diet; low phosphate diet

IT HAS BEEN KNOWN FOR MANY YEARS that renal phosphate excretion is influenced by the respiratory acid-base state (1, 2, 7, 13). However, only recently has the mechanism of these effects been examined in detail. Webb et al. (17) reported that the phosphaturia accompanying respiratory acidosis was not due to the concomitant hyperphosphatemia or to any of the other factors that may also change, such as parathyroid hormone, plasma bicarbonate, and extracellular acidosis. Rather, the authors concluded that Pco2 per se determines the phosphaturia observed during acute hypercapnia. A similar conclusion was reached by Hoppe et al. (11) in studies investigating the effects of respiratory alkalosis on renal phosphate excretion. In this case, the hypophosphaturia was attributed to decreased Pco2 per se rather than to the extracellular alkalosis or changes in the filtered phosphate load. Thus, these studies suggest that the alterations in phosphate excretion seen during respiratory acidosis and alkalosis are the result of a direct effect of changes in Pco2 on renal phosphate reabsorption.

There are, however, several points that remain unclear. For example, although the effects of Pco2 on phosphate excretion have been claimed to be independent of filtered load, a systematic study evaluating a range of plasma phosphate concentrations has not been performed. Such an analysis would correct for the different plasma phosphate concentrations present in respiratory acidosis and alkalosis, and, in addition, would answer the question of whether Pco2 affects the intrinsic capacity of the kidney to reabsorb phosphate. Furthermore, it is also not known whether respiratory acid-base disorders and, in particular, whether respiratory acidosis would alter phosphate excretion in phosphate deprived rats that avidly retain phosphate.

Accordingly, the present studies were performed with several objectives in mind. The first was to evaluate the renal handling of phosphate during respiratory acidosis and alkalosis in rats fed a normal phosphate diet and in those fed a low phosphate diet that display avid phosphate retention. In addition, we assessed the response of these groups to graded infusions of phosphate to control for changes in plasma phosphate induced by changes in the respiratory acid-base state and also to determine whether changes occur in the transport capacity of renal phosphate reabsorption.

METHODS

Experiments were performed on 33 Sprague-Dawley rats weighing 250–350 g. The animals were fed either a normal (0.7%) or low (0.07%) phosphate diet for 4 days prior to the experiment. The normal phosphate diet was prepared by supplementing the standard low phosphate diet (ICN Pharmaceuticals, Cleveland) with a mixture of sodium and potassium phosphate, ratio of monobasic to dibasic salts 1:4, to a final content of 0.7%. For rats fed a low phosphate diet, the sodium and potassium content in the diet was supplemented with sodium chloride and potassium chloride. All animals were given food and water ad libitum and care was taken to ensure that fasting had not occurred.

On the day of the experiment, the rats were anesthetized with an intraperitoneal injection of Inactin (100–120 mg/kg) and prepared for clearance experiments. The animals were placed on a heated table and body temperature was monitored with a rectal probe. Catheters were placed in jugular veins for infusions, in the carotid artery
for blood pressure measurement and blood sampling, and in the bladder for urine collection.

All rats underwent acute thyroparathyroidectomy (TPTX) and were verified as previously described (8, 9).

Following TPTX, a 2-h recovery period was allowed for attainment of a steady state, during which a 4% inulin solution was infused at 1.2 ml/h for the duration of the experiment. During the second hour of recovery, positive-pressure ventilation was applied. Initially, the animals were ventilated at 45 breaths/min at a tidal volume set according to the nomogram of Kleinman and Radford (12). After verification of normal blood acid-base values, the respiratory rate was increased to 92 breaths/min for all animals. Different acid-base states were achieved by varying the mixture of carbon dioxide and oxygen in the inspired air.

*Experiments in rats fed a normal phosphate diet.* The rats were divided into the following groups. Group 1: hypocapnic (n = 6), Group 2: normocapnic (n = 5), Group 3: hypercapnic (n = 6). Following the recovery period, which coincided with at least 30 min of acute acid-base change, a 30-min clearance period was taken during which saline was infused at 2 ml/h. In subsequent periods, the saline was replaced with phosphate-containing solutions calculated to deliver phosphate at progressively increasing rates of 1, 2, and 3 μmol/min. A 4:1 mixture of dibasic:monobasic sodium phosphate salts was used to attain concentrations of 30, 60, and 90 mM phosphate. All solutions were adjusted to pH 7.4, 300–310 mosmol/kg H2O, and infused at 2 ml/h. Phosphate infusions proceeded for 15 min before a 30-min clearance period was started. Blood samples were obtained at the midpoint of each clearance period.

*Experiments in rats fed a low phosphate diet.* These experiments were done with the following groups. Group 4: hypocapnic (n = 6), Group 5: normocapnic (n = 4), Group 6: hypercapnic (n = 6). The experimental protocol in these groups was identical to the one described above for rats fed a normal phosphate diet with the exception that additional solutions containing higher concentrations of phosphate were infused. Since the transport capacity of phosphate reabsorption is known to be elevated in phosphate-deprived rats (9, 15), solutions of 180 and 270 mM (which delivered phosphate at 6 and 9 μmol/min, respectively) were also infused to ensure that the maximum rate of phosphate reabsorption was obtained.

Inulin concentrations in plasma and urine were measured by the anthrone method (6). Phosphate was measured using the method of Chen et al. (4) and calcium was determined by atomic absorption spectrometry. Sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory).

All values are expressed as means ± SE. Statistical comparisons between groups were made with analysis of variance followed by Scheffe tests. Comparisons within groups were made with paired t tests.

**RESULTS**

*Experiments in rats fed a normal phosphate diet.* The results obtained in rats fed a normal phosphate diet are given in Table 1. The different acid-base states, with respiratory alkalosis present in the hypocapnic group (pH = 7.59 ± 0.01, PCO2 = 21.3 ± 1.1 mmHg), and respiratory acidosis present in the hypercapnic group (pH = 7.10 ± 0.02, PCO2 = 85.3 ± 4.2 mmHg). The normocapnic group served as controls (pH = 7.34 ± 0.01, PCO2 = 47.8 ± 1.8 mmHg). The respective acid-base states were maintained throughout the experiments.

During the control period, in which no phosphate was infused, plasma phosphate in the hypocapnic group (2.64 ± 0.09 mM) was significantly lower than in either normocapnic (3.85 ± 0.16 mM) or hypercapnic (4.29 ± 0.12 mM), P < 0.05. Fractional phosphate excretion was also significantly decreased in the hypocapnic group (2.3 ± 0.9%) compared with the hypercapnic group (11.0 ± 2.3%), P < 0.05. There were no differences in glomerular filtration rate or mean arterial blood pressure.

To compare phosphate excretion between the groups at similar plasma phosphate concentrations, after the control period each group was infused with increasing amounts of phosphate. The infusions of phosphate resulted in significant increases in plasma phosphate, fil-

<table>
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<th>Group</th>
<th>pH</th>
<th>PCO2 (mmHg)</th>
<th>PM (mM)</th>
<th>Fp, (μmol/min)</th>
<th>Rpg (μmol/min)</th>
<th>Rpg/GFR</th>
<th>Fp2, %</th>
<th>Rg,GFR</th>
<th>BP (mmHg)</th>
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Values are means ± SE; Fp, phosphate; Fp2, filtered phosphate load; Rpg, fractional phosphate reabsorption; GFR, glomerular filtration rate; BP, mean arterial blood pressure. Statistical comparisons are discussed in the text.
tered phosphate, and fractional excretion of phosphate in all groups (Table 1). The relationship between the fractional excretion of phosphate as a function of the plasma phosphate concentration is depicted in Fig. 1. Fractional phosphate excretion was lowest in the hypocapnic group and initially did not increase with the small rise in plasma phosphate. However, with further increases in plasma phosphate, fractional phosphate excretion increased markedly. Moreover, at equivalent plasma phosphate concentrations, there were no differences in fractional phosphate excretion between the groups. Accordingly, the relationship between fractional phosphate excretion and plasma phosphate appears to be similar in the three groups irrespective of the large differences in $\text{PCO}_2$.

The influence of differences in $\text{PCO}_2$ on the maximum transport capacity of phosphate reabsorption is shown in Fig. 2, where absolute phosphate reabsorption is plotted as a function of the filtered phosphate load normalized by the glomerular filtration rate. The peak point in each curve is taken as the transport maximum ($M_{\text{p}}/\text{GFR}$). As is evident, the phosphate transport maximum for the hypocapnic group (3.55 ± 0.30 μmol/ml) was not significantly different from that obtained for the normocapnic group (3.60 ± 0.20 μmol/ml) or for the hypercapnic group (3.83 ± 0.18 μmol/ml). Thus, differences in $\text{PCO}_2$ and pH that occur during respiratory acid-base disorders do not alter the intrinsic capacity of the kidney to reabsorb phosphate in rats fed a normal phosphate diet.

*Experiments in rats fed a low phosphate diet.* The results of experiments in rats fed a low phosphate diet are given in Table 2. The extent of acid-base changes in these groups was similar to that obtained in the rats fed normal phosphate diet, with respiratory alkalosis present in the hypocapnic group ($\text{pH} = 7.57 ± 0.02$, $\text{PCO}_2 = 26.4 ± 2.2$ mmHg) and respiratory acidosis present in the hypercapnic group ($\text{pH} = 7.06 ± 0.02$, $\text{PCO}_2 = 89.6 ± 3.6$ mmHg). Normal acid-base values were attained in the normocapnic group ($\text{pH} = 7.41 ± 0.04$, $\text{PCO}_2 = 40.2 ± 3.5$ mmHg).

Plasma phosphate during the control period was significantly higher in the hypercapnic group (3.88 ± 0.17 mM) than in either the normocapnic (2.07 ± 0.36 mM) or hypocapnic group (1.89 ± 0.26 mM), $P < 0.05$. Thus, changes in $\text{PCO}_2$ can alter the plasma phosphate concentration and, consequently, the filtered phosphate load in phosphate-deprived rats. However, in contrast to what was found in animals fed a normal phosphate diet, baseline fractional phosphate excretion did not differ but rather remained less than 1% in all groups. Mean arterial pressure during the control period did not vary among the groups. In all groups, blood pressure tended to decline during the course of the experiment, yet remained greater than 100 mmHg in every instance. Glomerular filtration

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**Fig. 1.** Fractional phosphate excretion (FE$_{Pi}$) plotted as a function of plasma phosphate concentration for each of the 3 groups fed normal or low phosphate diet. Units of $\text{PCO}_2$ are in mmHg.

**Fig. 2.** Relationship between phosphate reabsorption and filtered phosphate load. Both phosphate reabsorption and filtered load are normalized by GFR. Dashes connect lines to origin. Units of $\text{PCO}_2$ are in mmHg.
Plasma phosphate exceeded 5 mM, fractional phosphate excretion in phosphate-deprived rats, as in rats on a normal phosphate diet, was not altered by respira-
tory acid-base changes. In addition, as shown in Fig. 2, the higher maximum transport capacity of phosphate reabsorption present in rats fed a low phosphate diet was also unaffected by large differences in \( P_{\text{co}_2} \) and pH, values for \( \text{Tm}_{\text{p}}/\text{GFR} \) being 5.05 ± 0.03 in hypocapnia, 5.25 ± 0.50 in normocapnia, and 5.23 ± 0.21 \( \mu \text{mol/ml} \) in the hypocapnic group.

### DISCUSSION

The findings of the present experiments indicate that although both plasma phosphate and the fractional excretion of phosphate are higher during respiratory acidosis and lower during respiratory alkalosis, in animals fed a normal phosphate diet the differences in phosphate excretion disappear when fractional phosphate excretion is evaluated as a function of the plasma phosphate concentration (Fig. 1). Furthermore, in spite of large differences in \( P_{\text{co}_2} \) and pH between the groups, the maximum transport capacity of phosphate reabsorption was not significantly different (Fig. 2). Qualitatively similar results were obtained in phosphate-deprived rats (Figs. 1 and 2). Accordingly, these studies suggest that the differences in phosphate excretion following respiratory acid-base changes are largely the result of concomitant changes in plasma phosphate and do not involve alterations in the intrinsic capacity of the kidney to reabsorb phosphate.

Our results regarding the changes in plasma phosphate and the fractional excretion of phosphate during different respiratory acid-base states are consistent with previous reports (11, 17). However, the demonstration that the differences in fractional phosphate excretion are related to the associated changes in the plasma phosphate appears to differ with earlier studies. Indeed, other investigators have suggested that \( P_{\text{co}_2} \) per se exerts an effect on the kidney to alter phosphate reabsorption. Hoppe et al. (11) concluded that the hypophosphaturia present in respiratory alkalosis was independent of the plasma phosphate concentration since normalization of the filtered load did not correct the blunted excretion of phosphate. Similarly, Webb et al. (17) concluded that the phosphaturia produced during respiratory acidosis was independent of the increase in plasma phosphate or filtered phosphate load, although these investigators admitted that elevation of either may contribute to the phosphaturia. The fundamental difference between the present study and the previous ones mentioned is that both respiratory acidosis and alkalosis were evaluated over a wider range of plasma phosphate concentrations. Therefore, it was possible in the present investigation to examine the relationship between the fractional excretion of phosphate and plasma phosphate concentration for each acid base state. As shown in Fig. 1, in the range where fractional phosphate excretion varied with plasma phosphate, the relationship was similar in the three groups. It is of note, however, that when plasma phosphate was less than 3.9 mM in the hypocapnic group, fractional phosphate excretion did not increase in response to a small increase in plasma phosphate. In the study of Hoppe et al. (11), a blunted phosphaturia was noted in the presence of normal filtered load when plasma phosphate was in the range 2.3–2.7 mM, a range that we also found does not significantly influence phosphate excretion. Thus, greater levels of phosphate were present in respiratory alkalosis.
necessary to further increase plasma phosphate and thereby uncover the dependence of phosphate excretion on the plasma phosphate concentration. Furthermore, the lack of change in the phosphate transport capacity (Fig. 2) in spite of large differences in PCO₂ argues against a direct effect of PCO₂ on renal phosphate reabsorption.

The mechanism of the changes in plasma phosphate that occur during respiratory acidosis and alkalosis is not clear. The increase in plasma phosphate during acidosis is thought by some investigators to result from the mobilization of intracellular phosphorus stores, perhaps from bone (14). With regard to alkalosis, the suggestion has been made that increased muscle glycolysis may account for the accompanying hypophosphatemia (3, 14). Indeed, Hoppe et al. (11) reported elevated tissue phosphate content following hyperventilation in muscle and liver, but, interestingly, not in kidney. However, what is evident from the present studies is that the mechanism(s) involved in altering plasma phosphate with fluctuations in PCO₂ are not dependent on an adequate dietary supply of phosphate in the short term, since the changes in plasma phosphate still occurred in phosphate-deprived rats.

Although the response of plasma phosphate to respiratory acid-base changes was qualitatively similar regardless of the dietary regimen, differences were apparent in the excretion of phosphate. In rats fed a low phosphate diet, fractional phosphate excretion was initially very low and rose only at high plasma phosphate concentrations (greater than 5 mM). As shown in Fig. 1, the curve depicting the relationship between fractional excretion of phosphate and plasma phosphate concentration in phosphate-deprived rats was shifted to the right of the normal group. The resistance of phosphate-deprived rats to the phosphaturic effects of phosphate infusions has been reported previously by our laboratory and others (10, 15) and is a consequence of an increase in the transport capacity of phosphate reabsorption (Fig. 2). Thus, despite similar increases in plasma phosphate in phosphate-depleted and phosphate-replete rats, the adaptive increase in the phosphate transport capacity limited the excretion of phosphate in rats fed a low phosphate diet. In regard to the effect of respiratory acid-base changes, not only were there no differences in the transport maximum of phosphate reabsorption among the 3 groups, but once the reabsorptive capacity was exceeded, the precipitous increase in fractional phosphate excretion was similar regardless of the acid-base state.

The observations obtained during conditions of maximal phosphate transport do not preclude subtle effects of PCO₂ on phosphate reabsorption in the absence of phosphate infusion. Furthermore, the finding that changes in PCO₂ did not change the maximal transport capacity of phosphate reabsorption for the entire kidney does not rule out the possibility that PCO₂ may have different effects at various nephron sites. Indeed, several studies have reported that changes in PCO₂ may alter phosphate reabsorption in the proximal tubule (h, 16, 18).

In summary, our results indicate that although respiratory acid-base changes alter plasma phosphate concentration and phosphate excretion in rats fed a normal phosphate diet, changes in plasma phosphate concentration but not in phosphate excretion occur in phosphate-deprived rats because of the adaptive increase in the phosphate transport capacity. Furthermore, the infusion of phosphate revealed that the magnitude of phosphate excretion was related to the concomitant changes in plasma phosphate. Finally, under either dietary regimen, acute respiratory acid-base changes did not alter the intrinsic capacity of the kidney to reabsorb phosphate.

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