Effect of acute hypercapnia on PTH-stimulated phosphaturia in dietary P_i-deprived rat

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GUNTUPALLI, JAYARAMA, BARBARA MATTHEWS, BRIAN CARLIN, AND EDMUND BOURKE. *Effect of acute hypercapnia on PTH-stimulated phosphaturia in dietary P_i-deprived rat.* Am. J. Physiol. 253 (Renal Fluid Electrolyte Physiol. 22): F34–F40, 1987.—The effects of respiratory acidosis on renal inorganic phosphate (P_i) handling are controversial. Clearance experiments, therefore, were performed in fasted, chronically parathyroidectomized (PTX), dietary P_i-deprived rats. The objectives were twofold: to study the effects of compensated and uncompensated hypercapnia per se on renal P_i excretion and to examine the interaction between acute hypercapnia, dietary P_i, and parathyroid hormone (PTH) on the renal handling of P_i. Acute hypercapnia increased the plasma P_i (Δ 2.82 ± 0.65 mg/dl, $P < 0.05$) without altering the glomerular filtration rate (GFR). The FE_{P_i} increased (Δ 7.26 ± 0.48%, $P < 0.001$) but the TR_{P_i}/GFR also increased. PTH (3 $U \cdot kg^{-1} \cdot h^{-1}$) superimposed on hypercapnia resulted in a plasma P_i comparable to hypercapnia alone. The FE_{P_i} (7.56 ± 0.78 vs. 24.43 ± 2.20%; $P < 0.001$) was higher and the TR_{P_i}/GFR (117 ± 4 vs. 80 ± 2 μg/min, $P < 0.01$) lower, in the former group. PTH infusion during normocapnia resulted in a lower FE_{P_i} (0.20 ± 0.10 vs. 24.43 ± 2.20%, $P < 0.001$) and a higher TR_{P_i}/GFR (106 ± 2 vs. 80 ± 2 μg/min, $P < 0.01$) compared with PTH infusion during hypercapnia. Urinary adenosine 3′,5′-cyclic monophosphate (cAMP) excretion was similar between the groups. During hypercapnia, when the extracellular acidemia was neutralized, the phosphaturic action of PTH persisted. These studies offer direct evidence that in chronically PTX, dietary P_i-deprived rats, the phosphaturic action of PTH is restored by hypercapnia per se. This effect appears to be independent of extracellular acidemia, changes in the plasma P_i and calcium, urinary pH and Na and cAMP excretion.

acute hypercapnia; phosphaturia of parathyroid hormone; respiratory acidosis

ALTHOUGH the effects exerted on the renal handling of inorganic phosphorus (P_i) by acute metabolic acidemia (1, 3, 9, 13), alkalemia (10, 11, 12, 20), and acute hypocapnia (6, 8) have been delineated, the effects of acute hypercapnia (4, 6, 19) remain controversial. In nonfasting rats fed a normal-P_i diet, hypercapnia was shown to be phosphaturic (19). However, hypercapnia was associated with a significant increase in the filtered load of bicarbonate (HCO_3) and urinary pH, factors known to be phosphaturic (10, 12). Also, in this study, failure to reverse the phosphaturic effect of hypercapnia by normalizing the plasma pH with HCO_3^- could be attributed to the phosphaturic effect of HCO_3^-. In contrast, in the thyroparathyroidectomized hamster, which appears to be naturally resistant to the phosphaturic action of PTH, hypercapnia failed to significantly increase P_i excretion in the present of PTH (7). Recently, it has been proposed that hypercapnia per se is not phosphaturic in acutely parathyroidectomized (PTX) dietary P_i-deprived rats (6). The possible restoration of the phosphaturic action of PTH by hypercapnia, however, was not examined. Hence, to resolve this issue, clearance experiments were performed in chronically PTX rats fed a low-P_i diet, which are resistant to the phosphaturic action of PTH (16), extracellular fluid volume expansion (14), and HCO_3^- (15). The objectives were twofold: to study the effect of uncompensated and compensated hypercapnia per se on the renal handling of P_i, independent of the variables known to affect renal P_i excretion, and to examine the interaction between acute hypercapnia, dietary phosphorus, and PTH on the renal handling of P_i.

METHODS

Seven groups of Sprague-Dawley rats weighing 200–250 g were studied. Animals were housed in individual metabolic cages on a time scale of 12 h of light and 12 h of darkness and fed Purina rat chow containing calcium 1.2 g% and phosphorus 0.89 g%, with free access to distilled water. Nine to ten days prior to the experiment, parathyroidectomy was performed with an electrocautery under direct microscopic visualization. The PTX was confirmed by demonstrating a serum calcium <7.0 mg/dl after an overnight fast. After confirmation of PTX and 60 h before the experiment a low-P_i diet (0.07 mg%) was instituted. Only animals demonstrating weight gain on this diet were used in the study.

All experiments were started after an overnight fast with free access to distilled water. The animals were anesthetized with intraperitoneal pentobarbital sodium. Core body temperature was maintained at 37°C on a heated table and monitored with a thermistor rectal probe. After tracheostomy, the animals were intubated with a polyethylene endotracheal tube (PE-240) and ventilated with a ventilator for small rodents (Harvard rodent ventilator, model 680, Lexington, MA). The in-

haled gas mixture contained 40% O_2-60% N_2, except during hypercapnia, as described below. The left carotid artery and the right jugular vein were cannulated with polyethylene catheters (PE-50) for arterial blood sampling and infusions, respectively. The urinary bladder was catheterized with PE-50 tubing through a small suprapubic incision for urine collection, taking care to minimize the dead space.

General Protocol

After preparation of the animals as described above, a base-line arterial blood gas (80 μl) was obtained. The tidal volume and the respiratory rate were adjusted to maintain the P_{CO_2} around 40 mmHg during the equilibration, control, and experimental phases, except where otherwise indicated. Steady-state arterial blood gases were confirmed by subsequent sampling (80 μl) at the beginning of the control and end of the experimental phases, respectively. Pilot studies with hourly sampling indicated stable acid-base parameters using the present protocol. The following general protocol, consisting of three phases was initiated: *1*) an equilibration period of 80 min, *2*) a control phase of 60 min consisting of three 20-min clearance periods, and *3*) an experimental phase of 180 min during which six clearance periods of 30 min each were observed. For each clearance hour 60 μl of arterial blood were drawn and immediately centrifuged. Only experiments without visible hemolysis were included. Thus, in the studies reported, none of the maneuvers employed induced a statistically significant change in the serum potassium concentration (results not shown). In all instances blood samples were replaced with an equal volume of normal saline. Immediately after venous cannulation, using a Harvard pump, the animals were extracellular fluid volume (ECFV)-expanded at the rate of 5 ml·100 $g^{-1}\cdot h^{-1}$ with a solution containing 80% isotonic saline (150 mM) and 20% isotonic sodium bicarbonate (150 mM). Preliminary experiments indicated a fall in plasma HCO_3^- in animals volume expanded with NaCl alone. Sufficient [*methoxy*-^{3}H]inulin was added to the infusate to maintain radioactivity five to six times above the background throughout the experiment.

Effect of ECFV expansion, PTH infusion, and time course of the experiment on P_i excretion (group 1) (n = 6). The general protocol was used throughout the experiment, except that during the experimental phase, freshly reconstituted parathyroid hormone 1—34 peptide (PTH) (Beckman Instruments, Palo Alto, CA) (6,000 IU activity·mg^{-1}) was added to the infusate to deliver 3 U·$kg^{-1}\cdot h^{-1}$.

Effect of acute hypercapnia on P_i excretion in PTX rats (group 2) (n = 6). The general protocol was followed up to the end of the control phase. At the beginning of the experimental phase, the inspired gas mixture was adjusted to contain 10% CO_2-40% O_2-50% N_2.

Effect of acute hypercapnia on the phosphaturic action of PTH (group 3) (n = 6). In animals similarly treated as in *group 2*, PTH was added to the infusate during the experimental phase to deliver 3 U·$kg^{-1}\cdot h^{-1}$.

Effect of increased filtered load of P_i on P_i excretion (group 4) (n = 5). At the beginning of the experimental phase sodium phosphate 20 mg·100 ml^{-1} (150 mM), (Na_2HPO_4:NaH_2PO_4, 4:1) replaced the $NaHCO_3$ in the infusate.

Role of increased filtered load of P_i on the phosphaturic action of PTH (group 5) (n = 5). The same protocol was used as in *group 4*, except that at the beginning of the experimental phase PTH was added to the infusate to deliver 3 U·$kg^{-1}\cdot h^{-1}$.

Effect of restoration of extracellular pH on P_i excretion during acute hypercapnia (group 6) (n = 6). This group was prepared and studied as in *group 2*, except that during the experimental phase isotonic sodium bicarbonate replaced the NaCl in the infusate.

Effect of hypercapnia per se on the phosphaturic action of PTH independent of systemic acidemia (group 7) (n = 6). This group was prepared and studied as in *group 6* except that during the experimental phase, in addition to isotonic bicarbonate, PTH was added to the infusate to deliver 3 U·$kg^{-1}\cdot h^{-1}$.

Analytical techniques. Urinary and arterial pH and gas tensions were measured on an ABL-2 (Radiometer-America). Plasma (P) HCO_3^- was calculated from the Henderson-Hasselbalch equation. P_i was measured by the method of Chen et al. (2). Sodium was measured on a flame photometer (Instrumentation Laboratory, 343, Lexington, MA). [*Methoxy*-^{3}H]inulin in plasma and urine was measured on a liquid scintillation counter (Packard-Tricarb 460 CD, Downers Grove, IL) and was used to calculate the glomeruler filtration rate (GFR). Calcium was measured on an atomic absorption spectrophotometer (Perkin-Elmer, model 313, Norwalk, CT). Ionized calcium was measured with an ion-selective electrode (Orion). Urinary adenosine 3′,5′-cyclic monophosphate (cAMP) was measured by radioimmunoassay (New England Nuclear, Boston, MA) modified after Steiner et al. (17). Clearance (C) of a substance was calculated by the standard formula (UV/P) and the fractional excretion (FE), by dividing C by the clearance of inulin and expressed as a percentage. Tubular reabsorption of P_i (TR_{P_i}/GFR) was calculated by using the formula [(GFR·plasma P_i) − $U_{P_i}V$] and expressed as micrograms per minute per milliliter per GFR. The symbol, Δ, refers to changes between the control and the third hour of the experimental phases. Results are expressed as means ± SE. Statistical analysis was done by the paired Student's *t* test for comparison within the groups and unpaired Student's *t* test where comparisons were made only once between groups. Where multiple comparisons were made between groups, one-way analysis of variance with repeated measures was used followed by the Student-Newman-Keuls test. Differences were considered significant at $P < 0.05$.

RESULTS

Effects of ECFV expansion, PTH, and time course of the experiment on P_i excretion (group 1). The GFR and arterial pH, P_{CO_2}, HCO_3^- were stable throughout the experiment (Tables 2 and 3). There was a small but significant increase in P_{P_i} (8.99 ± 0.53 vs. 10.69 ± 0.44 mg/dl, $P < 0.05$). As the filtered load increased the TR_{P_i}/GFR also increased (Table 1). Neither ECFV ex-

TABLE 1. *Effects of hypercapnia, PTH, and P_i infusion on the renal handling of phosphorus and sodium*

	Control			1st h			2nd h			3rd h		
	FE_{P_i}	FE_{Na}	R_{P_i}	FE_{P_i}	FE_{Na}	R_{P_i}	FE_{P_i}	FE_{Na}	R_{P_i}	FE_{P_i}	FE_{Na}	R_{P_i}
Normocapnia + PTH, *group 1*	0.12±0.03	6.30±0.75	89±2	0.08±0.02	7.01±0.82	96±4	0.17±0.07	7.47±0.97	102±3*	0.20±0.08	7.53±1.11	106±2*
Hypercapnia, *group 2*	0.32±0.11	6.61±0.68	92±2	2.62±1.02	7.67±0.77	109±4*	6.22±1.52*†	9.17±1.14	114±4*	7.56±0.78*	8.42±1.02	117±4*
P vs. *group 1*	NS	NS	NS	<0.05	NS	NS	<0.05	NS	<0.05	<0.05	NS	<0.05
Hypercapnia + PTH, *group 3*	0.26±0.19	7.01±0.19	97±2	10.11±2.73*‡	7.84±1.12	96±5	22.42±2.92*†‡	8.37±0.93	85±3*‡	24.43±2.20*‡	8.04±0.82	80±2*‡
P vs. *group 1*	NS	NS	NS	<0.05	NS	NS	<0.05	NS	<0.05	<0.05	NS	<0.05
Normocapnia + P_i, *group 4*	0.28±0.06	6.06±1.12	103±3	0.48±0.14	7.18±1.12	114±4*	0.84±0.28‡§	9.01±1.23	121±4*§	1.01±0.30‡§	7.41±0.63	129±3*§
P vs. *group 1*	NS	NS	<0.05	NS	NS	NS	NS	NS	<0.05	NS	NS	<0.05
Normocapnia + P_i + PTH, *group 5*	0.18±0.09	7.28±1.14	92±2	0.62±0.29§	8.19±0.33	117±2*§	1.94±0.64*§	7.93±0.60	121±3*§	3.92±0.31*‡§	7.56±0.30	122±2*§
P vs. *group 1*	NS	NS	NS	NS	NS	<0.05	<0.05	NS	<0.05	<0.05	NS	<0.05

Values are means ± SE. FE_{P_i}, fractional excretion of phosphorus %; FE_{Na}, fractional excretion of sodium %; R_{P_i}, tubular reabsorbtion of P_i per 1 ml GFR (TR_{P_i}/GFR) μg/min. * Significant difference from the control phase within the group, $P < 0.05$ (Student's *t* test). † Significant difference from the preceding experimental hour, $P < 0.05$ (Student's *t* test); ‡§ Significant difference compared with corresponding periods in *groups 2* and *3*, respectively, $P < 0.05$ (analysis of variance, Student-Newman-Keuls test). *P*, refers to comparison of *group 1* with *groups 2–5*, $P < 0.05$, (analysis of variance, Student-Newman-Keuls test).

pansion nor PTH infusion resulted in any significant increase in P_i excretion. The FE_{Na} were stable throughout the experiment (Table 1). Neither the total plasma calcium nor the ionized fraction (3.21 ± 0.13 vs. 3.14 ± 0.15 mg/dl, NS) increased significantly during the experiment.

Effect of acute hypercapnia on P_i excretion (group 2). The GFR remained stable throughout the experiment (Table 2). The plasma P_i increased significantly during hypercapnia (9.56 ± 0.49 vs. 12.48 ± 0.98 mg/dl, $P < 0.01$). The P_{P_i} (3rd experimental h) was higher than *group 1* (Table 2). Hypercapnia (control vs. experimental phase) resulted in a significant increase in FE_{P_i} (Table 1). As the plasma P_i increased, the TR_{P_i}/GFR also increased during hypercapnia. This value was also higher than that observed in *group 1* (Table 1). The FE_{Na} tended to increase compared with the control phase. Acute hypercapnia significantly decreased the arterial pH and increased both P_{CO_2} and HCO_3^- (Table 3). The urine pH did not change significantly during hypercapnia (6.65 ± 0.19 vs. 6.41 ± 0.15, NS), and the values obtained during the experimental phase were not different from the corresponding phase in *group 1* (Table 3). Both the total plasma calcium (7.71 ± 0.15 vs. 7.42 ± 0.17 mg/dl, NS) and the ionized calcium (3.07 ± 0.12 vs. 3.13 ± 0.10 mg/dl, NS) levels were stable throughout the experiment.

Permissive effect of acute hypercapnia on the phosphaturic action of PTH (group 3). The GFR was stable throughout the control and experimental phases (Table 2) but the P_{P_i} increased during hypercapnia (9.68 ± 0.25 vs. 11.57 ± 0.36 mg/dl, $P < 0.01$). The P_{P_i} (3rd experimental h) was similar to *groups 1* and *2* (Table 2). Hypercapnia with PTH was associated with a significant decrease in the TR_{P_i}/GFR within the group (Table 1). This value was also lower than that observed during the experimental phase (3rd h) of *group 1* (80 ± 2 vs. 106 ± 2 μg/min, $P < 0.05$) and *group 2* (117 ± 4 μg/min, $P < 0.05$). The changes in acid-base parameters were similar to *group 2* (Table 3). The plasma calcium (7.21 ± 1.10 vs. 7.51 ± 1.21 mg/dl, NS) and urinary Na excretion (Table 1) were stable throughout the experiment.

Effect of increased filtered load of P_i on P_i excretion (group 4). The GFR was stable throughout the experiment (Table 2). Plasma P_i rose during P_i infusion (10.34 ± 0.12 vs. 13.06 ± 0.78 mg/dl, $P < 0.02$). During the experimental phase this resulted in plasma levels of P_i comparable to those in the hypercapnic phase of animals in *group 2* (13.06 ± 0.78 vs. 12.48 ± 0.98 mg/dl, NS). The arterial pH, P_{CO_2}, and HCO_3^- remained within normal limits (Table 3). P_i infusion resulted in no significant changes in the FE_{P_i} (Table 1). The TR_{P_i}/GFR, however, increased significantly. The FE_{P_i} was significantly lower in *group 4* compared with *group 2* (3rd experimental h) (1.01 ± 0.30 vs. 7.56 ± 0.78%, $P < 0.05$), but the differences in TR_{P_i}/GFR did not reach statistical significance. The total plasma calcium (8.05 ± 0.17 vs. 7.63 ± 0.15 mg/dl, NS), urinary pH (Table 3), and Na excretion (Table 1) were stable throughout the experiment.

Effect of increased filtered load of P_i on the phosphaturic action of PTH (group 5). The GFR and plasma P_i were not significantly different from those observed in *group*

TABLE 2. *Effects of hypercapnia, PTH, and P_i infusion on plasma phosphorus and GFR*

	Control		1st h		2nd h		3rd h	
	P_{P_i}	GFR	P_{P_i}	GFR	P_{P_i}	GFR	P_{P_i}	GFR
Normocapnia + PTH, *group 1*	8.99±0.53	2.06±0.08	9.92±0.49	1.82±0.17	10.40±0.43*†	2.02±0.18	10.69±0.44*	2.02±0.16
Hypercapnia, *group 2*	9.56±0.49	2.00±0.13	11.17±0.48*	2.19±0.11	11.97±0.44*	2.24±0.10	12.48±0.98*	2.01±0.11
P vs. *group 1*	NS	NS	NS	NS	<0.05	NS	<0.05	NS
Hypercapnia + PTH, *group 3*	9.68±0.25	2.27±0.13	10.70±0.33*	1.97±0.06	11.59±0.33*†	2.20±0.07	11.57±0.36*	2.02±0.09
P vs. *group 1*	NS	NS	NS	NS	<0.05	NS	NS	NS
Normocapnia + P_i, *group 4*	10.34±0.12	2.13±0.12	11.55±0.88*	2.18±0.10	12.13±0.70*	2.14±0.10	13.06±0.78*	2.28±0.08
P vs. *group 1*	NS	NS	NS	NS	<0.05	NS	<0.05	NS
Normocapnia + P_i + PTH, *group 5*	9.29±0.17	2.07±0.13	11.84±0.23*	2.14±0.16	12.42±0.27*	2.15±0.16	12.82±0.19*	2.17±0.20
P vs. *group 1*	NS	NS	<0.05	NS	<0.05	NS	<0.05	NS

Values are means ± SE. P_{P_i}, plasma P_i, mg/dl; GFR, glomerular filtration rate, ml/min. * Significant difference from the control phase within the group, $P < 0.05$ (Student's *t* test). † Significant difference from the preceding experimental hour, $P < 0.05$ (Student's *t* test). *P*, comparison of *group 1* with *groups 2–5*, $P < 0.05$ (analysis of variance, Student-Newman-Keuls test).

TABLE 3. *Effects of uncompensated and compensated hypercapnia on systemic and urinary pH and plasma bicarbonate and P_{CO_2}*

	pH		*P*	P_{CO_2}, mmHg		*P*	HCO_3^-, mmol/l		*P*	UpH		*P*
	C	E		C	E		C	E		C	E	
Normocapnia + PTH, *group 1*	7.37±0.01	7.40±0.09	NS	41±2	42±1	NS	23±2	26±1	NS	6.32±0.10	6.47±0.14	NS
Hypercapnia, *group 2*	7.36±0.02	7.09±0.01	<0.001	41±5	91±1	<0.001	23±1	28±1	<0.05	6.51±0.16	6.41±0.15	NS
P vs. *group 1*	NS	<0.05		NS	<0.05		NS	NS		NS	NS	
Hypercapnia + PTH, *group 3*	7.38±0.01	7.10±0.02	<0.001	42±2	86±5	<0.001	22±2	26±2	NS	6.40±0.10	6.43±0.17	NS
P vs. *group 1*	NS	<0.05		NS	<0.05		NS	NS		NS	NS	
Normocapnia + P_i, *group 4*	7.36±0.02	7.37±0.01*†	NS	40±2	39±2*†	NS	23±1	24±1*	NS	6.53±0.10	6.46±0.11	NS
P vs. *group 1*	NS	NS		NS	NS		NS	NS		NS	NS	
Normocapnia + P_i + PTH, *group 5*	7.38±0.01	7.41±0.01*†	NS	40±3	39±2*†	NS	23±1	24±1	NS	6.26±0.17	6.48±0.56	NS
P vs. *group 1*	NS	NS		NS	NS		NS	NS		NS	NS	
Hypercapnia + HCO_3^-, *group 6*	7.36±0.02	7.32±0.01*†	NS	44±3	93±2	<0.001	23±2	42±2*†	<0.001	6.32±0.04	7.40±0.05*	<0.05
P vs. *group 1*	NS	<0.05		NS	<0.05		NS	<0.05		NS	<0.05	
Hypercapnia + HCO_3^- + PTH, *group 3*	7.36±0.01	7.34±0.02*†	NS	41±2	89±3	<0.001	22±1	44±2*†	<0.001	6.38±0.10	7.42±0.02†	<0.05
P vs. *group 1*	NS	NS		NS	<0.05		NS	<0.05		NS	<0.05	

Values are means ± SE. C, control phase; E, 3rd h of the experimental phase; *P* in boxhead, significance of difference compared with the control phase; pH, P_{CO_2}, and HCO_3^- refer to the arterial blood values; UpH, urinary pH. *† Significant difference compared with corresponding periods in *groups 2* and *3*, respectively, $P < 0.05$ (analysis of variance, Student-Newman-Keuls test). *P*, comparison of *group 1* with *groups 2–7*, $P < 0.05$ (analysis of variance, Student-Newman-Keuls test).

3 (Table 2). The acid-base parameters remain within the normal range (Table 3). However, the FE_P (3.92 ± 0.31 vs. 24.43 ± 2.20%, $P < 0.05$) was significantly lower in this group compared with the corresponding values in *group 3* (Table 1). Also, the TR_{P_i}/GFR in the experimental phase of this group (122 ± 2 vs. 80 ± 2 μg/min, $P < 0.05$) was higher than that observed in the corresponding phase of *group 3*. Again, the plasma calcium (7.87 ± 0.12 vs. 7.02 ± 0.18 mg/dl, NS), urinary pH (Table 3), and Na excretion (Table 1) were stable throughout the experiment.

Neutralization of the respiratory acidemia with $NaHCO_3$ (group 6). Again, the GFR was stable throughout the experiment but the P_{P_i} increased significantly during hypercapnia (9.88 ± 0.43 vs. 15.80 ± 1.11 mg/dl, $P < 0.001$) (Table 5). Isotonic $NaHCO_3$ infusion during hypercapnia prevented a decrease in systemic pH (7.36 ± 0.02 vs. 7.32 ± 0.01, NS) (Table 3). During compensated hypercapnia, the FE_{P_i} increased significantly compared with the control phase (Table 4). This increment (10.10 ± 1.37 vs. 7.56 ± 0.78%, NS) was not different from that observed in *group 2*. As the filtered load of P_i increased, the TR_{P_i}/GFR also increased significantly in this group (Table 4). During compensated hypercapnia, the urine pH and plasma HCO_3^- were significantly higher compared with the corresponding phases of all previous groups (Table 3).

Permissive effect of hypercapnia per se on the phosphaturic action of PTH independent of systemic acidemia (group 7). The GFR remained unchanged throughout the experiment (Table 5). Similar to *group 6*, the P_{P_i} increased (9.90 ± 0.30 vs. 11.20 ± 0.29 mg/dl, $P < 0.02$) significantly during hypercapnia. PTH and $NaHCO_3$ infusion during hypercapnia prevented any change in the

TABLE 4. *Effects of hypercapnia without systemic acidemia on the phosphaturic action of PTH*

	Control			1st h			2nd h			3rd h		
	FE_{P_i}	FE_{Na}	R_{P_i}	FE_{P_i}	FE_{Na}	R_{P_i}	FE_{P_i}	FE_{Na}	R_{P_i}	FE_{P_i}	FE_{Na}	R_{P_i}
Hypercapnia + HCO_3^-	0.21	6.53	97	2.42	6.92	121	8.53	7.02	130	10.01	7.37	141
group 6	±0.12	±1.07	±2	±1.20	±0.32	±2*	±1.99*†	±1.13	±2*	±1.37*	±1.32	±3*†
P	NS	NS	NS	<0.03	NS	<0.01	<0.01	NS	<0.001	<0.001	NS	<0.001
Hypercapnia + HCO_3^-	0.26	7.02	99	7.38*	7.58	87	15.37	8.53	86	26.51	8.43	82
+ PTH, *group 7*	±0.06	±0.64	±1	±1.01	±0.28	±2*	±1.12*†	±1.37	±2*	±1.21*†	±0.91	±2*

Values are means ± SE. FE_{P_i}, fractional excretion of phosphorus %; FE_{Na}, fractional excretion of sodium %; R_{P_i}, tubular reabsorption of P_i per 1 ml GFR (TR_{P_i}/GFR) μg/min. * Significantly different from the control phase within the group, $P < 0.05$ (Student's *t* test). † Significantly different from the preceding experimental hour, $P < 0.05$ (Student's *t* test). *P*, intergroup comparison between *groups 6* and *7*.

TABLE 5. *Effects of hypercapnia without systemic acidemia on plasma phosphorus and GFR*

	Control		1st h		2nd h		3rd h	
	P_{P_i}	GFR	P_{P_i}	GFR	P_{P_i}	GFR	P_{P_i}	GFR
Hypercapnia + HCO_3^-,	9.88	2.23	12.22	2.15	13.80	2.17	15.80	2.10
group 6	±0.43	±0.14	±0.20*	±0.30	±0.10*	±0.10	±1.11*†	±0.10
P	NS	NS	NS	NS	<0.05	NS	<0.05	NS
Hypercapnia + HCO_3^- + PTH,	9.90	2.21	9.99	2.17	11.07	2.13	11.20	2.15
group 7	±0.30	±0.12	±0.08	±0.10	±0.23*†	±0.14	±0.29*	±0.08

Values are means ± SE. P_{P_i}, plasma phosphorus, mg/dl; GFR, glomerular filtration rate, ml/min. * Significantly different from the control phase within the group, $P < 0.05$ (Student's *t* test). † Significantly different from the preceding experimental hour, $P < 0.05$ (Student's *t* test). *P*, intergroup comparison between *groups 6* and *7*.

extracellular pH (7.36 ± 0.01 vs. 7.34 ± 0.02, NS) but the arterial P_{CO_2} was similar to that observed in *groups 3* and *4* (Table 3). PTH infusion during compensated hypercapnia not only resulted in a significantly higher FE_{P_i} compared with *group 6*, but was also associated with a significant reduction in the TR_{P_i}/GFR within the group (Table 4), (Fig. 1) and compared with *group 6*. During the 3rd h of the experimental phase, neither the P_{P_i} (11.20 ± 0.29 vs. 11.57 ± 0.36 mg/dl, NS) nor the GFR (2.15 ± 0.08 vs. 2.02 ± 0.09 ml/min, NS) differed from values obtained during the corresponding phase of *group 3*. Furthermore, both the FE_{P_i} (26.51 ± 1.21 vs. 24.43 ± 2.20%, NS) and the TR_{P_i}/GFR (82 ± 2 vs. 80 ± 2 μg/min, NS) were similar between the 3rd experimental h of *groups 7* and *3*, respectively.

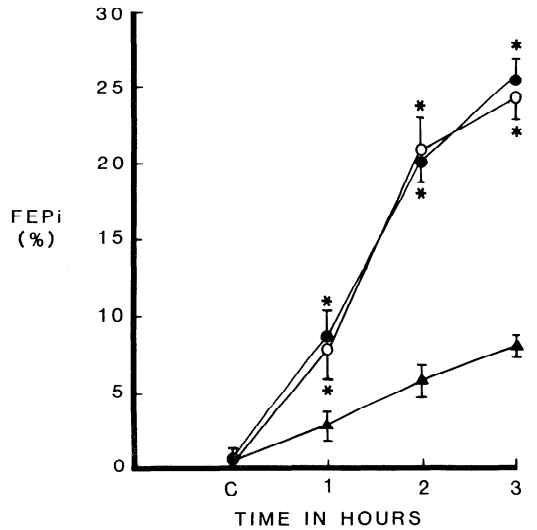

FIG. 1. Effects of compensated and uncompensated hypercapnia on phosphaturic action of parathyroid hormone (PTH). Values are means ± SE in each group. Compensated (*group 7, filled circles*) and uncompensated (*group 3, open circles*) hypercapnia with PTH, hypercapnia per se without PTH (*group 2, filled triangles*); C, control phase; 1–3, 1st, 2nd, and 3rd h of experimental phase; FE_{P_i}, fractional excretion of P_i. *Asterisk*, significantly different from the corresponding phase of hypercapnia per se.

cAMP excretion in response to hypercapnia, normocapnia + PTH, and hypercapnia + PTH with and without compensation of systemic pH. The increase in urinary cAMP (control vs. 3rd h of experimental phase) in *groups 1* (34 ± 5 vs. 81 ± 8 pmol·ml^{-1}·GFR, $P < 0.02$) and *3* (42 ± 10 vs. 96 ± 8 pmol·ml^{-1}·GFR, $P < 0.01$) was similar (Δ 47 ± 8 vs. Δ 54 ± 8 pmol·ml^{-1}·GFR, NS). Acute hypercapnia per se (*group 2*) was associated with a significant increase in cAMP excretion (22 ± 3 vs. 43 ± 4, Δ 21 ± 4 pmol·ml^{-1}·GFR, $P < 0.03$), which was nonetheless smaller than that observed in *group 3* (Δ 21 ± 4 vs. 54 ± 8 pmol·ml^{-1}·GFR, $P < 0.05$). The urinary cAMP response to PTH in *group 5* (48 ± 11 vs. 110 ± 8 pmol·ml^{-1}·GFR, $P < 0.01$) was similar to that seen in *group 3* (Δ 66 ± 8 vs. Δ 54 ± 8 pmol·ml^{-1}·GFR, NS) and *group 1*. The increase in cAMP excretion in *group 6* (35 ± 5 vs. 60 ± 4 pmol·ml^{-1}·GFR, $P < 0.02$) did not differ from that seen in *group 2* (Δ 25 ± 4 vs. Δ 21 ± 4 pmol·ml^{-1}·GFR, NS). Similarly, the increase observed in *group 7* (30 ± 5 vs. 88 ± 6 pmol·ml^{-1}·GFR, $P < 0.01$) did not differ from *group 3* (Δ 58 ± 6 vs. Δ 54 ± 8 pmol·ml^{-1}·GFR, NS) or *group 1*.

DISCUSSION

Earlier studies on the effects of hypercapnia on the renal handling of P_i have yielded conflicting results (4, 6, 19), which may reflect differences in the experimental design, species, or more importantly, not considering

certain factors known to influence the renal handling of P_i. In the present studies, chronically PTX dietary P_i-deprived rats were chosen to study the effects of acute hypercapnia, since earlier observations have demonstrated that this animal model is resistant to the phosphaturic actions of PTH (16), bicarbonaturia (15), changes in the filtered load of P_i (5), and ECFV expansion (14).

Infusion of PTH during normocapnia (*group 1*) resulted in a significant increase in the P_{P_i} during the course of the experiment, probably secondary to mobilization of P_i from the bone. This observation is consistent with the earlier studies of Steele et al. (16). Neither increased P_{P_i} nor PTH were phosphaturic in this group. Acute hypercapnia (*group 2*) was associated with an increase in the P_{P_i} and FE_{P_i}, compared with the control phase. This increased phosphaturia, however, was not associated with any reduction in the tubular reabsorption of P_i. In recent studies in acutely PTX dietary P_i-deprived rats, Haramati (6) demonstrated that the tubular capacity to reabsorb P_i was similar in hypercapnia and normocapnia, when comparable filtered loads were achieved by P_i infusion. The present results confirm, in part, these observations. Thus, in the present study, the increased filtered load of P_i is a major determinant of the phosphaturia observed during acute hypercapnia. The lack of such a phosphaturic response to a similar filtered load of P_i in normocapnia (*groups 1* and *4*), however, argues against changes in the filtered load of P_i as the sole determinant of the observed phosphaturia during hypercapnia. Certain differences in experimental design may account for the apparent differences in results and interpretation between the present study and those of Haramati (6). Thus, in contrast to the latter study, animals in the present investigation were fasted, a maneuver known to alter renal phosphate handling. More importantly, the duration of hypercapnia in the present study was more prolonged (180 vs. 60 min). Indeed, in the present study, phosphaturia over and above that attributable to an increased filtered load was not observed until the 3rd h of hypercapnia. In contrast to respiratory acidemia under these conditions, our previous studies with metabolic acidemia (3) resulted in a marked phosphaturia without an increase in either the plasma P_i concentration or the filtered load.

In thyroparathyroidectomized rats fed a normal-P_i diet, increased filtered load or excretion of HCO_3^- is associated with phosphaturia (20). This may have contributed to the increased FE_{P_i} observed by Webb et al. (19) in hypercapnic rats. Although the plasma HCO_3^- increased in the present hypercapnic groups, the values achieved in the experimental phase did not differ from the corresponding phase of the normocapnic groups (*groups 1* and *4*), nor were there any significant differences in the urinary pH between the groups or within the groups (Table 3). Furthermore, dietary P_i-deprived rats are resistant to the phosphaturic effects of increased filtered load or excretion rates of HCO_3^- (15).

In the present studies, during constant PTH infusion, hypercapnia (*group 3*) resulted in a restoration of the phosphaturic action of PTH as evidenced by a significant reduction in the tubular reabsorption of P_i compared with the control phase. The studies of Webb et al. (19) suggest a significantly greater phosphaturia in parathyroid intact rats compared with thyroparathyroidectomized animals, but tubular reabsorption of P_i was not measured nor was the effect of hypercapnia on plasma PTH concentration evaluated.

To study the mechanism of this permissive effect of hypercapnia on the phosphaturic action of PTH, urinary cAMP levels were studied. The increment in the urinary cAMP excretion in acutely hypercapnic rats infused with PTH (*group 3*) was greater than that observed in hypercapnia alone (*group 2*), but was similar to that observed in the normocapnic rats infused with a similar dose of PTH (*group 1*), although the P_i excretion is significantly lower in the latter group. Furthermore, in normocapnic rats infused with P_i and PTH (*group 5*), the increment in cAMP excretion was similar to *groups 1* and *3*. Nephrogenous cAMP generation was not directly measured in the present study. If the urinary cAMP excretion reflects changes in the renal generation of cAMP, an assumption not yet confirmed, the present data would suggest that the restoration of the phosphaturic action of PTH observed in the presence of hypercapnia, is mediated by events independent of cAMP generation.

It is possible that the increased filtered load of P_i observed during hypercapnia and PTH infusion may have altered the segmental P_i reabsorption along the length of the nephron leading to an increased P_i delivery to the PTH-sensitive nephronal segments, resulting in phosphaturia. The lack of a similar change in the tubular reabsorption of P_i in *group 5* in which comparable PTH infusion and filtered loads of P_i to *group 3* were achieved makes such a possibility unlikely. Although this observation (*group 5*) is in contrast to the recent findings of Haramati et al. (5), the higher dose of PTH employed in the latter study might explain this apparent difference. The reason for the lack of the expected fall in serum calcium following P_i infusion (*groups 4* and *5*) in this study is not readily apparent.

In the study previously alluded to (19), in PTH-intact rats, the phosphaturia of hypercapnia was sustained even when the extracellular acidemia was neutralized with $NaHCO_3$, implying that the observed phosphaturia was a consequence of hypercapnia per se, rather than acidemia. However, the potential role of HCO_3^- in the phosphaturia (12, 20) was not excluded. Since dietary P_i-deprived rats are resistant to the phosphaturic action of HCO_3^-, rats in *groups 6* and *7* were infused with HCO_3^- to normalize the decrease in extracellular pH. Neutralization of extracellular pH (*group 6*) did not prevent the rise in the plasma P_i induced by hypercapnia. As the plasma P_i increased, the tubular absorption of P_i also increased. Thus, the increase in FE_{P_i} during the hypercapnic phase in this group appears to be largely a function of the increased filtered load of P_i as in *group 2*. Two other factors could have contributed to the increased tubular reabsorption of P_i (TR_{P_i})/GFR observed in this group. As recently shown in the detailed study of Quamme (13) both an increase in proximal convoluted tubular pH and metabolic alkalosis induced by bicarbon-

ate infusion increased tubular phosphorus reabsorption in acutely PTX rats, provided their dietary phosphorus intake was not increased. The urinary alkalinization in the present *group 6* is presumptive evidence of a similar change in the proximal tubules and it is possible that the bicarbonate load could have induced an additional effect, although in contradistinction to the experiments of Quamme, frank alkalemia was avoided in the present investigation. As demonstrated in *group 7*, however, hypercapnia per se, even when unassociated with extracellular acidemia, fully restored the phosphaturic action of PTH. This is evidenced by an increase in FE_{P_i} and a concomitant reduction in the TR_{P_i} within the group. Also, the values obtained during hypercapnia in this group are similar to those observed in *group 3* (hypercapnia + PTH), (Fig. 1). Furthermore, this restoration of the phosphaturic action of PTH appears to be independent of changes in the filtered load of P_i, since similar filtered loads induced by the superimposition of P_i infusion on PTH infusion in normocapnic animals (*group 5*) were associated with an increase in the tubular reabsorption of P_i.

The mechanism of restoration of the phosphaturic action of PTH by hypercapnia in dietary P_i-deprived rats requires further investigation. It is possible that the intracellular acidosis induced by hypercapnia persisted despite extracellular neutralization (18). The persistent tubular responsiveness to PTH in compensated respiratory acidemia in these experiments points toward some factor in the intracellular environment. Alternatively, an effect of hypercapnia per se on the metabolic sequence involved in the tubular transport of P_i may be operative.

In summary, in chronically PTX dietary P_i-deprived rats, acute hypercapnia is phosphaturic predominantly secondary to an increase in the filtered load of P_i. In addition, hypercapnia restores the phosphaturic action of PTH, independent of the filtered loads of phosphorus and bicarbonate, plasma calcium, Na excretion, ECFV expansion, urinary pH, and cAMP excretion. Neutralization of the concomitant respiratory acidemia did not diminish the hypercapnia-induced tubular responsiveness to PTH.

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