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Renal Response to Phosphaturic Agents in Acutely and Chronically Parathyroidectomized Rats (39708)

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Introduction. Extracellular volume expansion is associated with a decreased tubular reabsorption of phosphorus in both intact and chronically parathyroidectomized animals but not in acutely parathyroidectomized animals (1-6). The reason for this discrepancy is not entirely apparent, even though it was the subject of numerous investigations (4, 5). The present study was undertaken to extend the previous studies by examining the effects of several phosphaturic agents including diuretics in chronically and acutely parathyroidectomized rats with varying loads of filtered phosphorus. The diuretics were acetazolamide and chlorothiazide. Acetazolamide acts primarily on the proximal tubule to decrease fluid reabsorption in association with carbonic anhydraze inhibition and suppression of proximal bicarbonate reabsorption. Acetazolamide has the capacity to produce phosphaturia in both intact and chronically parathyroidectomized animals (7). Chlorothiazide suppresses sodium reabsorption, primarily in the cortical diluting segment of the distal nephron. Its effect on phosphate reabsorption is markedly smaller than that of acetazolamide.

Methods. White female Sprague-Dawley rats (180-300 g) fed Purina pellet chow diet with tap water *ad libitum* were studied.

Experimental procedures and groups. All rats were parathyroidectomized (PTX) by cautery either 2 hr (PTX − 2 hr) or 2 days (PTX − 2 days) prior to the start of clearance studies. These consisted of three to four 20-min control urine collection periods followed by five to six periods of the same length during which the drug was administered. Control rats and rats receiving the drug were always run concurrently. The surgical procedures and the chemical analyses were described previously (8-10).

The following groups were studied.

Group 1: administration of chlorothiazide (1.5 mg/100 g/hr iv); (a) PTX, 2 hr; (b) PTX, 2 days; and (c) PTX, 2 hr plus infusion of isotonic Na_2HPO_4 (6 mg/100 g/hr).

Group 2: infusion with 0.9% NaCl (0.1 ml/100 g/min); (a), (b), and (c) as in Group 1.

Group 3: infusion of acetazolamide (1.0 mg/100 g/hr); (a) and (b) as in Group 1.

Group 4: infusion of calcitonin (0.5 U/kg/hr).

Results. All results are expressed as mean ± SE. The data presented in all tables were obtained by adding all 20-min urine collections belonging to the same experimental phases and calculating their arithmetic averages. The serum concentrations of phosphorus and calcium were calculated in the same fashion.

Chlorothiazide did not alter CP/CIN in acutely PTX rats but it effected a significant increase in CP/CIN in chronically PTX rats (Table I). Serum concentration and filtered loads of phosphorus, before and during the infusion of chlorothiazide in group 1b were higher than the corresponding values in group 1a, but the glomerular filtration rates did not differ significantly (Table II).

The administration of chlorothiazide to acutely PTX rats receiving iv phosphorus effected a significant increase in CP/CIN (Table I). There was no difference between the experimental and the control groups during the infusion of phosphorus but an increment in CP/CIN was noticed during the infusion of chlorothiazide resulting in a discrepancy of 0.071 between the groups. Serum concentrations and filtered loads of phosphorus in the control and the experimental groups did not differ significantly.

The effect of volume expansion with 0.9% NaCl in acutely and chronically PTX rats is shown in Table III. In group 2b volume expansion was associated with an in-

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TABLE I. COMPARISON OF THE AVERAGE FRACTIONAL EXCRETION RATES OF PHOSPHORUS IN THE EXPERIMENTAL AND THE CORRESPONDING CONTROL ANIMALS, IN DIFFERENT GROUPS, DURING BASELINE AND EXPERIMENTAL PERIODS.

Group	N	CP/GFR	
		Baseline clearances	Experimental clearances
1a. EXP[a] Acute PTX + TZ[b]	6	0.010 ±0.002	0.012 ±0.002
CNT[c] Acute PTX	6	0.008 ±0.002	0.011 ±0.003
p[d]		NS	NS
1b. EXP Chronic PTX + TZ	6	0.038 ±0.005	0.118 ±0.025
CNT Chronic PTX	6	0.030 ±0.009	0.038 ±0.001
p		NS	<0.01
1c. EXP Acute PTX + P[e] + TZ	6	0.126 ±0.018	0.354 ±0.013
CNT Acute PTX + P	6	0.118 ±0.014	0.238 ±0.011
p		NS	<0.001
2c. EXP Acute PTX + P + VE[f]	6	0.105 ±0.034	0.346 ±0.002
CNT Acute PTX + P	6	0.100 ±0.023	0.216 ±0.016
p		NS	<0.001
3a. EXP Acute PTX + ACTZ[g]	6	0.002 ±0.001	0.159 ±0.010
CNT Acute PTX	6	0.002 ±0.001	0.003 ±0.001
p		NS	<0.001
4a. EXP Acute PTX + CT[h]	6	0.0010 ±0.0002	0.044 ±0.018
CNT Acute PTX	6	0.0010 ±0.0004	0.002 ±0.001
p		NS	<0.01

[a] Experimental subgroup.
[b] Chlorothiazide was given during the experimental periods.
[c] Control subgroup.
[d] Compares the experimental with the control subgroups.
[e] Intravenous phosphorus was given during the baseline and experimental clearances in both subgroups.
[f] Extracellular volume expansion with saline was performed during experimental clearances.
[g] Acetazolamide was given during the experimental clearances.
[h] Calcitonin was given during the experimental clearances.

crease in urinary excretion of phosphorus and CP/CIN whereas in group 2a these variables did not change significantly. Similar results were reported in previous studies (4, 6). Glomerular filtration rates (CIN) and fractional excretion of sodium (CNa/CIN) in group 2a did not differ significantly from their respective values in group 2b, but serum concentrations and filtered loads of phosphorus in group 2b were higher than the corresponding values in group 2a (Table III).

The effect of volume expansion on renal handling of phosphorus in acutely PTX phosphate-loaded rats is shown in Table I. There was no difference in average CP/CIN between the experimental and the control animals during the infusion of phosphate. The addition of iv saline to the phosphate infusion in the experimental group produced an increment in CP/CIN, leading to a discrepancy between the experimental and the control group of 0.13. There was no significant difference in serum phosphorus concentrations, glomerular filtration rates, and filtered loads of phosphorus between the control and the experimental animals.

The effect of acetazolamide on renal handling of phosphorus in acutely PTX rats (group 3) is shown in Table I. There was no difference in the average CP/CIN between the experimental and control animals during the baseline collections. During the administration of acetazolamide to the experimental rats, average CP/CIN increased from 0.002 to 0.159, but in the control group CP/CIN did not change. In chronically PTX rats there was no difference in average CP/CIN between the experimental and control animals during the baseline collections. During the administration of acetazolamide to the experimental rats, average CP/CIN increased from 0.034 ± 0.003 to 0.19 ± 0.31 ($p < 0.01$), but in the control group CP/CIN did not change. Serum phosphorus concentrations, glomerular filtration rates, and filtered loads of phosphorus in the control and in the experimental animals did not differ significantly throughout the experiment, both in groups 3a and 3b.

The effect of calcitonin on renal handling of phosphorus in acutely PTX animals is shown in Table I. During the baseline, Cp/CIN in the experimental and the control animals did not differ significantly. During the infusion of calcitonin in the experimental rats CP/CIN increased from 0.001 to 0.044, but remained unchanged in the control group.

TABLE II. COMPARISON OF THE AVERAGE SERUM CONCENTRATIONS OF PHOSPHORUS, INULIN CLEARANCES, AND FILTERED LOADS OF PHOSPHORUS, BEFORE AND DURING CHLOROTHIAZIDE INFUSION IN ACUTELY PTX RATS WITH THE CORRESPONDING VALUES IN CHRONICALLY PTX RATS.

	Sp[a] (mg/100 ml)		GFR (ml/min)		Fp[b] (μg/min)	
Group	B[c]	TZ[d]	B	TZ	B	TZ
1a. Acutely PTX rats (N = 6)						
Mean	5.4	5.6	1.17	1.21	63	68
± SE	0.2	0.2	0.06	0.08	4	5
1b. Chronically PTX rats (N = 6)						
Mean	10.0	10.0	1.23	1.15	123	115
± SE	0.4	0.4	0.08	0.06	7	7
p[e]	<0.001	<0.001	NS	NS	<0.001	<0.001

[a] Serum phosphorus.
[b] Filtered load of phosphorus.
[c] Baseline clearances.
[d] Chlorothiazide was given during the experimental periods.
[e] Comparison of the acutely with the chronically PTX rats.

TABLE III. THE EFFECT OF EXTRACELLULAR VOLUME EXPANSION WITH NORMAL SALINE ON RENAL HANDLING OF PHOSPHORUS IN ACUTELY AND CHRONICALLY PTX RATS.

	GFR (ml/min)		Sp (mg/100 ml)		Fp (μg/min)		UpV[a] (μg/min)		CP/GFR		CNa/GFR	
Group	B[b]	VE[c]	B	VE	B	VE	B	VE	B	VE	B	VE
2a. Acutely PTX rats (N = 4)												
Mean	1.05	1.20	5.5	5.7	58	65	0.10	0.13	0.001	0.002	0.003	0.064
± SE	0.06	0.07	0.2	0.1	5	7	0.02	0.02	0.000	0.000	0.000	0.011
2b. Chronically PTX rats (N = 4)												
Mean	1.04	1.22	10.8	11.2	112	136	3.93	23.10	0.036	0.166	0.006	0.065
± SE	0.05	0.06	0.7	0.9	6	8	1.40	5.65	0.012	0.032	0.002	0.014
p[d]	NS	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	NS

[a] Urinary excretion of phosphorus.
[b] Baseline clearances.
[c] Extracellular volume expansion was performed during the experimental clearances.
[d] Compares the acutely with the chronically PTX rats.

In chronically PTX animals during the baseline, CP/CIN in the experimental and the control animals did not differ significantly. During the infusion of calcitonin in the experimental rats CP/CIN increased from 0.040 ± 0.002 to 0.142 ± 0.031 ($p < 0.01$), but remained unchanged in the control group.

In all groups of rats in all experiments serum calcium concentrations were less than 6.5 mg/100 ml and did not change significantly during the course of the experiments.

Discussion. The present study demonstrated phosphaturic effects of iv calcitonin and acetazolamide in acutely PTX rats. The effect of calcitonin like that of PTH is mediated through the activation of the adenylate cyclase/cyclic AMP system in renal cortex (11, 12). Therefore, similarly to PTH, calcitonin is an effective phosphaturic agent in acutely PTX rats. There is no adequate explanation, however, for the similar effect of acetazolamide.

Both chlorothiazide and iv saline failed to enhance urinary excretion of phosphorus in acutely PTX rats but induced phosphaturia in chronically PTX rats and in acutely PTX rats undergoing iv loading with phosphate. The response to intravenous saline in acutely PTX rats after phosphate loading in this study confirmed previous results in dogs (5).

The failure of chlorothiazide and saline to increase urinary excretion of phosphorus in acutely PTX animals is not-well understood, but two possible mechanisms are worth comment. First, because the bulk of filtered phosphorus in this situation is presumably

reabsorbed by PTH-responsive mechanism, and very little if any is reabsorbed by other mechanisms, suppression of PTH-independent systems for phosphorus reabsorption by chlorothiazide or saline loading cannot be an effective means of increasing urinary excretion of phosphorus. Second, although iv chlorothiazide and saline may suppress phosphorus reabsorption in acutely PTX rats it is eventually reabsorbed completely by the high capacity PTH-responsive system. The demonstration of phosphaturia with chlorothiazide and saline in phosphate-loaded acutely PTX rats implies that, as the reabsorptive capacity for phosphorus is deliberately exceeded, a greater proportion of filtered phosphorus is probably absorbed by PTH-independent mechanisms, that are readily suppressible by saline and chlorothiazide. The fact that the filtered loads of phosphorus in chronically PTX rats were higher than those in acutely PTX rats may at least partly account for the presence of the phosphaturic response to chlorothiazide and saline in chronically PTX rats, as opposed to its absence in acutely PTX animals.

Summary. Acutely PTX rats responded with phosphaturia to both iv calcitonin and acetazolamide but failed to respond to iv chlorothiazide or saline loading. Chronically PTX rats, and iv phosphate-loaded acutely PTX rats responded with phosphaturia to iv chlorothiazide and saline loading. These findings suggests that different agents may exert their phosphaturic effects by acting at different reabsorptive systems for phosphorus in the kidney.

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