Calcitonin decreases the renal tubular capacity for phosphate reabsorption

RUDOLFS K. ZALUPS AND FRANKLYN G. KNOX
Nephrology Research Laboratories, Department of Physiology and Biophysics, Mayo Clinic and Foundation, Rochester, Minnesota 55905

ZALUPS, RUDOLFS, K., AND FRANKLYN G. KNOX. *Calcitonin decreases the renal tubular capacity for phosphate reabsorption.* Am. J. Physiol. 245 (Renal Fluid Electrolyte Physiol. 14): F345–F348, 1983.—The effects of pharmacologic doses of synthetic salmon calcitonin on the renal tubular capacity of phosphate (P_i) transport were determined in the presence and absence of maximally phosphaturic doses of parathyroid hormone (PTH). Thyroparathyroidectomized rats were given graded infusions of P_i (1, 2, and 3 μmol/min) to prevent the hypophosphatemic effects of calcitonin and to determine the maximum transport of P_i for the kidney (Tm_{P_i}/GFR). The maximum transport of P_i for the rats treated with calcitonin was 2.46 ± 0.27 μmol/ml. This value was significantly less than that of 3.88 ± 0.32 μmol/ml ($P < 0.05$) for the control animals but was significantly greater than the maximum transport of P_i of 1.16 ± 0.05 μmol/ml ($P < 0.05$) for the rats treated with PTH. Furthermore, there was no significant difference between the maximum transport of P_i for the rats treated with PTH and that of 1.04 ± 0.05 μmol/ml for the rats treated with PTH plus calcitonin. We conclude that pharmacologic doses of calcitonin decrease the tubular capacity for P_i reabsorption of the kidney and that the effect is significantly smaller than that of maximally phosphaturic doses of PTH.

parathyroid hormone; thyroparathyroidectomy; maximum transport of P_i

CALCITONIN, WHICH IS A POTENT hypophosphatemic agent, has been reported to have a marked phosphaturic effect in the rat by some investigators (4, 5, 15) but not by others (11, 14, 17, 18). Part of the controversy is perhaps related to the difficulty in dissociating the hypophosphatemic from the direct effects of the hormone on the kidney. Oberleithner et al. (13) attempted to circumvent the systemic effects of calcitonin by dripping the hormone on superficial proximal tubules. They found that calcitonin was phosphaturic and, furthermore, that the phosphaturic effect of calcitonin was additive to the phosphaturic effect of parathyroid hormone (PTH). Milhaud and Moukhtar (12) and Anast et al. (1), on the other hand, reported that calcitonin and PTH did not have an additive effect on the excretion of phosphate (P_i) at the level of the whole kidney.

In the present study, graded infusions of phosphate were used to control for hypophosphatemia and to determine the effects of pharmacologic doses of calcitonin on the tubular capacity of P_i transport of the kidney in the presence and absence of PTH. Thus, it was possible to test whether calcitonin inhibits P_i transport and whether the inhibition of P_i transport produced by a maximal phosphaturic dose of PTH is enhanced by the presence of calcitonin.

MATERIALS AND METHODS

Clearance experiments were carried out on four groups of five male Sprague-Dawley rats (250–310 g) stabilized on a normal P_i diet (0.7% P_i content, Ralston Purina Co., St. Louis, MO). Both diet and water were given ad libitum.

On the morning of the experiments, the rats were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg body wt) (Byk-Gulden Konstanz, Hamburg, West Germany) and then placed on a heated table where a rectal probe was inserted to monitor body temperature. A midline incision was made through the skin on the ventral surface of the neck and thyroparathyroidectomy (TPTX) was performed using thermocautery. Subsequently, a tracheotomy and intubation of the trachea with PE-240 tubing were performed in order to maintain a clear airway and to allow the animals to breathe spontaneously. Catheters were then placed in jugular veins (PE-50) for infusions, in the carotid artery (PE-50) for monitoring blood pressure and the sampling of blood, and in the bladder (PE-100) for urine collections.

Following TPTX, a 2-h recovery period was allowed for the attainment of a steady state. Thirty minutes after the start of the recovery period, three separate infusions were started, each at a rate of 2 ml/h. One infusion was 6% inulin, the second was 0.9% saline, and the third was one of four treatment solutions, each of which designates one of the four protocols used in this study. The description of each solution is as follows. Solution 1 was 0.9% saline; solution 2 contained calcitonin (Calcimar, salmon calcitonin, Armour Pharmaceutical Co., Kankakee, IL) and was designed to deliver the hormone at 1 $U \cdot kg^{-1} \cdot min^{-1}$; solution 3 contained PTH (synthetic, 1–34, Beckman Instruments, Palo Alto, CA) and was designed to deliver a maximal phosphaturic dose of 1 $U \cdot kg^{-1} \cdot min^{-1}$; and solution 4 contained PTH plus calcitonin and was designed to deliver both hormones at a rate of 1 $U \cdot kg^{-1} \cdot min^{-1}$. After the recovery period, a 20-min control clearance period was taken. Then the saline infusion, used in all four groups, was replaced with successive P_i solutions formulated to deliver P_i at the rates of 1, 2, and 3 μmol/

min, in that order. The solutions were made from a 4:1 mixture of dibasic:monobasic sodium P_i salts (pH 7.4). The concentrations of the solutions were 30, 60, and 90 mM P_i, respectively. Each graded P_i infusion proceeded for 15 min prior to the start of a 20-min clearance period. At the midpoint of each clearance period a sample of blood was taken.

Inulin and P_i concentrations in the plasma and urine were determined by the anthrone (10) and Chen (7) methods, respectively. Calcium concentrations were determined by atomic absorption spectrometry, and sodium concentrations were measured by flame photometry.

All values are expressed as means ± SE. Statistical comparisons between means were first made by one-way analysis of variance followed by multiple comparison testing using Tukey's method.

RESULTS

During the control clearance period for the group given saline, the absolute reabsorption of P_i ($Reab_{P_i}$) was 6.93 ± 0.39 μmol/min, the fractional excretion of P_i (FE_{P_i}) was 1.1 ± 0.3%, and the absolute excretion of P_i ($U_{P_i}V$) was 0.08 ± 0.02 μmol/min (Table 1). These values were not significantly different from those of the group given calcitonin. The $Reab_{P_i}$ of 1.16 ± 0.05 μmol/min, FE_{P_i} of 31.6 ± 2.8%, and $U_{P_i}V$ of 1.72 ± 0.31 μmol/min for the group given PTH were, however, significantly different from the corresponding values for the group given calcitonin and the group given saline. Finally, there was no significant difference in $Reab_{P_i}$, FE_{P_i}, or $U_{P_i}V$ between the group given PTH and the group given PTH plus calcitonin.

Values for plasma P_i were plotted against normalized values of reabsorption of P_i per glomerular filtration rate ($Reab_{P_i}$/GFR) in order to construct curves indicating the maximum transport of P_i (Tm_{P_i}/GFR) for each of the four treatments used in this study (Fig. 1). The maximum transport of P_i for the group given saline was 3.88 ± 0.32 μmol/ml, which was significantly greater than that for the group given calcitonin (2.46 ± 0.27 μmol/ml). For the group given PTH, the maximum transport of P_i of 1.16 or 0.05 μmol/ml was significantly less than that for both the group given calcitonin and the group given saline but was not significantly different from the value of 1.04 ± 0.05 for the group given PTH plus calcitonin.

Plasma P_i in the group receiving saline was 2.52 ± 0.17 mM during the control clearance period. The values for plasma P_i for the groups given calcitonin, PTH, and PTH plus calcitonin were all lower than that for the group given saline, with the lowest value being 1.49 ± 0.04 mM, which was for the group given PTH plus

TABLE 1. *Effects of phosphate infusions on renal function in the presence of PTH and calcitonin*

Group	Amount of P_i Infused, μmol/min	$Reab_{P_i}$/GFR, μmol/ml	$Reab_{P_i}$, μmol/min	FE_{P_i}, %	$U_{P_i}V$, μmol/min	Plasma P_i, mM	GFR, ml/min	Plasma Ca, mM	FE_{Na}, %	BP, mmHg
Saline (n = 5)	0	2.49 ± 0.17	6.93 ± 0.39	1.1 ± 0.3	0.08 ± 0.02	2.52 ± 0.17	2.8 ± 0.3	1.76 ± 0.05	0.8 ± 0.1	143 ± 4
	1	3.09 ± 0.18	9.36 ± 0.78	1.8 ± 0.3	0.12 ± 0.03	3.13 ± 0.18	3.0 ± 0.2	1.64 ± 0.06	1.0 ± 0.3	139 ± 5
	2	3.74 ± 0.26	11.45 ± 0.78	4.3 ± 1.5	0.54 ± 0.20	3.91 ± 0.29	3.1 ± 0.2	1.47 ± 0.06	1.3 ± 0.4	135 ± 5
	3	3.88 ± 0.32	11.78 ± 1.47	16.7 ± 2.6	2.42 ± 0.50	4.65 ± 0.34	3.0 ± 0.2	1.31 ± 0.06	1.9 ± 0.5	130 ± 3
CT (n = 5)	0	1.81 ± 0.10	7.41 ± 0.63	2.7 ± 0.8	0.22 ± 0.06	1.86 ± 0.11	4.1 ± 0.2	1.68 ± 0.03	2.1 ± 0.5	148 ± 3
	1	2.15 ± 0.10	8.55 ± 0.60	6.8 ± 1.9	0.62 ± 0.15	2.31 ± 0.11	4.0 ± 0.2	1.54 ± 0.04	2.9 ± 0.4	144 ± 4
	2	2.32 ± 0.17	8.39 ± 0.77	14.2 ± 3.0	1.35 ± 0.25	2.70 ± 0.12	3.6 ± 0.2	1.42 ± 0.04	2.7 ± 0.4	138 ± 5
	3	2.46 ± 0.27	8.74 ± 1.30	25.1 ± 3.6	2.80 ± 0.33	3.25 ± 0.23	3.5 ± 0.2	1.31 ± 0.04	2.9 ± 0.4	131 ± 5
PTH (n = 5)	0	1.16 ± 0.05	3.64 ± 0.44	31.6 ± 2.8	1.72 ± 0.31	1.72 ± 0.12	3.1 ± 0.3	1.86 ± 0.09	0.9 ± 0.2	144 ± 3
	1	1.00 ± 0.06	3.01 ± 0.30	45.8 ± 3.3	2.53 ± 0.21	1.86 ± 0.11	3.0 ± 0.2	1.89 ± 0.17	1.6 ± 0.1	139 ± 4
	2	0.80 ± 0.05	2.21 ± 0.13	61.8 ± 3.6	3.65 ± 0.34	2.12 ± 0.10	2.8 ± 0.2	1.82 ± 0.21	2.6 ± 0.3	131 ± 5
	3	0.81 ± 0.08	2.40 ± 0.38	65.9 ± 3.2	4.52 ± 0.30	2.39 ± 0.06	2.9 ± 0.2	1.76 ± 0.19	2.4 ± 0.4	131 ± 6
PTH + CT (n = 5)	0	1.01 ± 0.06	3.30 ± 0.16	32.3 ± 3.3	1.58 ± 0.17	1.49 ± 0.04	3.3 ± 0.2	1.78 ± 0.02	2.8 ± 0.3	144 ± 2
	1	1.04 ± 0.05	3.65 ± 0.21	38.1 ± 1.5	2.23 ± 0.03	1.67 ± 0.06	3.5 ± 0.1	1.70 ± 0.00	3.5 ± 0.3	133 ± 2
	2	0.96 ± 0.15	3.41 ± 0.59	48.6 ± 6.5	3.17 ± 0.43	1.85 ± 0.10	3.5 ± 0.1	1.61 ± 0.02	3.4 ± 0.2	126 ± 2
	3	0.85 ± 0.03	2.79 ± 0.24	59.3 ± 1.5	4.07 ± 0.31	2.10 ± 0.04	3.3 ± 0.2	1.49 ± 0.01	3.8 ± 0.5	125 ± 2

Values are means ± SE; PTH, parathyroid hormone; CT, calcitonin; P_i, phosphate; $Reab_{P_i}$, reabsorbed phosphate; FE_{P_i}, fractional excretion of phosphate; $U_{P_i}V$, absolute excretion of phosphate; GFR, glomerular filtration rate; FE_{Na}, fractional excretion of sodium; BP, mean arterial blood pressure. Statistical comparisons of means from the control clearance periods:

	$Reab_{P_i}$/GFR	$Reab_{P_i}$	FE_{P_i}	$U_{P_i}V$	Plasma P_i	GFR	Plasma Ca	FE_{Na}	BP
CT vs. saline	*	NS	NS	NS	*	*	NS	*	NS
PTH vs. saline	*	*	*	*	*	NS	NS	NS	NS
CT vs. PTH	*	*	*	*	NS	NS	NS	*	NS
CT vs. PTH + CT	*	*	*	*	NS	NS	NS	NS	NS
PTH vs. PTH + CT	NS	NS	NS	NS	NS	NS	NS	*	NS
PTH + CT vs. saline	*	*	*	*	*	NS	NS	*	NS

Mean comparisons were made following one-way analysis of variance using Tukey's method. NS, no statistical difference; *, statistically different ($P < 0.05$). PTH + CT, group given parathyroid hormone plus calcitonin. PTH, group given parathyroid hormone. CT, group given calcitonin. Saline, group given saline.

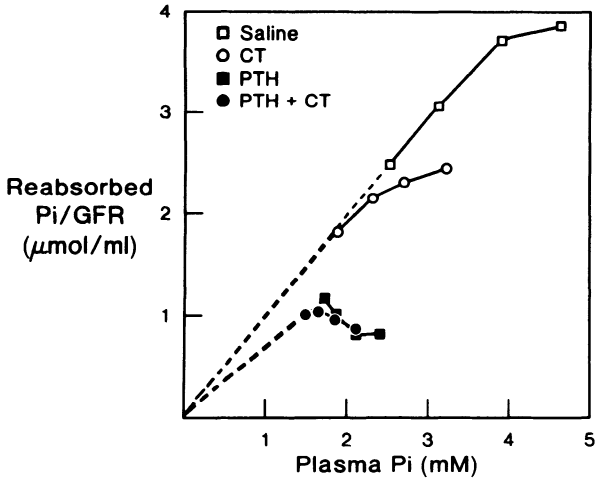

FIG. 1. Maximum transport of P_i ($Reab_{P_i}$/GFR) for calcitonin was significantly less than that for saline ($P < 0.05$) but was significantly greater than that for PTH ($P < 0.05$). There was no statistical difference between maximum transport of P_i for PTH and that for PTH + calcitonin.

calcitonin. The infusions of P_i resulted in increases in plasma P_i in all groups, with the greatest changes seen during the last infusion of P_i (3 μmol/min). The greatest change was seen in the group given saline (Δ = 2.13 mM), while the smallest change was detected in the group given PTH plus calcitonin (Δ = 0.61 mM).

At base line, plasma Ca was below 1.90 mM for all groups, indicating the efficacy of TPTX. With successive infusions of P_i, plasma Ca decreased in all groups.

During the experiments, blood pressure fell slightly in each group, particularly toward the end of the experiments. However, all values were well within the range for normal renal function (>100 mmHg).

Glomerular filtration rate (GFR) remained relatively constant throughout the experiments in all groups. In the group given calcitonin, GFR was slightly higher than in the other groups, but again was within the normal range.

Fractional excretion of Na (FE_{Na}) was greater in the group given calcitonin than in the group given PTH and the group given saline. The most pronounced natriuresis was found in the group given PTH plus calcitonin.

DISCUSSION

The hypophosphatemic effect of calcitonin is well documented (2, 19, 20, 21) and is consistent with the findings of the present study. Phosphate infusions were used to differentiate the hypophosphatemic effect from the direct effect of calcitonin on the renal handling of P_i and to determine the capacity for reabsorption of P_i (8, 9). The results of the present study show that with increasing rates of infusion of P_i, calcitonin causes a greater increase in both the absolute and fractional excretion of P_i compared with controls. In addition, when $Reab_{P_i}$/GFR is evaluated as a function of plasma P_i, calcitonin significantly decreased the capacity for tubular reabsorption of P_i (Fig. 1).

In the absence of phosphate infusions, calcitonin caused no significant change in FE_{P_i} when compared with controls. Carney and Thompson (6) and Quamme (18) have also reported that calcitonin causes only slight increases in FE_{P_i} in TPTX rats. There are, however, reports that calcitonin has a marked effect on FE_{P_i} (4, 5, 15). In studies by Poujeol et al. (17), both phosphaturic and hypophosphaturic responses were noted in the same study. The nature of the discrepancies in results between these studies is not clear, although in the present study it is apparent that when the fall in plasma P_i is corrected there is a definite inhibitory effect of calcitonin on the tubular transport of P_i.

As was expected, PTH caused a significant increase in the FE_{P_i} and $U_{P_i}V$, consistent with findings from other studies (4, 5). The inhibitory effect of PTH on P_i transport was also reflected by the low maximum transport of P_i, which was similar to that reported by Frick et al. (9).

The issue as to whether calcitonin has an additive effect to the phosphaturic effect of PTH was addressed recently by Oberleithner et al. (13). With the intent to avoid the systemic effects of calcitonin, they compared the effects of PTH and calcitonin applied topically to superficial proximal tubules microinfused with ^{33}P-labeled P_i in the presence of a systemic infusion of PTH. Their results showed that a greater amount of ^{33}P-labeled P_i was recovered in the urine with the application of calcitonin than with the application of PTH, and therefore they concluded that calcitonin had an additive phosphaturic effect to PTH. In the present study, however, there was no significant difference between the maximum transport of P_i for PTH and that for PTH plus calcitonin, which indicates that at the whole kidney level calcitonin does not have an additive effect to maximally phosphaturic doses of PTH on the inhibition of maximal rates of P_i transport. On the other hand, the phosphaturic effect of PTH was additive to calcitonin. This result does not rule out additivity of effects at physiologic levels of the two hormones.

It has been previously shown that calcitonin is natriuretic in the rat (3, 16). In the present study, a mild natriuresis was detected in rats treated with calcitonin, but the greatest natriuretic response was found in rats treated with PTH plus calcitonin. This suggests that PTH may enhance the natriuretic effect of calcitonin.

Based on the findings of the present study, we conclude that pharmacologic doses of both calcitonin and PTH decrease the tubular capacity for P_i reabsorption, with calcitonin having a less potent effect. Moreover, calcitonin does not have an additive effect to a maximal phosphaturic dose of PTH in decreasing the tubular transport of P_i for the kidney.

We appreciate the technical help of Marcine Onsgard, the secretarial help of June M. Hanke, and the advice and assistance of Theresa J. Berndt and Aviad Haramati.

This work was supported by National Institutes of Health Grant AM-19715.

Received 29 November 1982; accepted in final form 30 March 1983.

REFERENCES

1. ANAST, C., C. D. ARNAUD, H. RASMUSSEN, AND A. TENENHOUSE. Thyrocalcitonin and the response to parathyroid hormone. *J. Clin. Invest.* 46: 57–64, 1967.

2. ANDERSON, J. B., AND R. V. TALMAGE. The effect of calcium infusion and calcitonin on plasma phosphate in sham-operated and thyroparathyroidectomized dogs. *Endocrinology* 93: 1222–1226,

1973.
3. AURBACH, G. D., AND D. HEATH. Parathyroid hormone and calcitonin regulation of renal function. *Kidney Int.* 6: 331–345, 1974.
4. BERNDT, T. J., AND F. G. KNOX. Effects of parathyroid hormone and calcitonin on electrolyte excretion in the rabbit. *Kidney Int.* 17: 473–478, 1980.
5. BERNDT, T. J., J. D. PFEIFER, F. G. KNOX, S. A. KEMPSON, AND T. P. DOUSA. Nicotinamide restores phosphaturic effect of PTH and calcitonin in phosphate deprivation. *Am. J. Physiol.* 242 (*Renal Fluid Electrolyte Physiol.* 11): F447–F452, 1982.
6. CARNEY, S., AND L. THOMPSON. Acute effect of calcitonin on rat renal electrolyte transport. *Am. J. Physiol.* 240 (*Renal Fluid Electrolyte Physiol.* 9): F12–F16, 1981.
7. CHEN, P. S., T. V. TORIBARA, AND H. WARNER. Microdetermination of phosphorus. *Anal. Chem.* 28: 1756–1758, 1976.
8. FRICK, A., AND I. DURASIN. Maximal reabsorptive capacity for inorganic phosphate (TmP_i) in the absence of parathyroid hormone in the rat: decrease of the TmP_i during prolonged administration of phosphate and the role of calcium. *Pfluegers Arch.* 377: 9–14, 1978.
9. FRICK, A., I. DURASIN, AND M. NEUWEG. Phosphaturic response of hydrocortisone in the presence and the absence of parathyroid hormone. *Pfluegers Arch.* 392: 99–105, 1981.
10. FÜHR, J., J. KACZMARCZYK, AND C. D. KRÜTTGEN. Eine einfache colorimetrische Methode zur Inulinbestimmung für Nierenclearanceuntersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin. Wochenschr.* 33: 729–730, 1955.
11. GABBIANI, G., B. TUCHWEBER, G. COTE, AND LEFORT. Action of thyroxin and calcitonin on experimental soft tissue calcification. *Calcif. Tissue Res.* 2: 30–37, 1968.
12. MILHAUD, G., AND M. S. MOUKHTAR. Antagonistic and synergistic actions of thyrocalcitonin and parathyroid hormone on the levels of calcium and phosphate in the rat. *Nature London* 211: 1186–1187, 1966.
13. OBERLEITHNER, H., F. LANG, R. GREGER, AND H. SPORER. Additivity to the phosphaturic action of parathyrin and calcitonin in the rat kidney. *Adv. Exp. Med. Biol.* 128: 129–134, 1980.
14. PECHET, M. M., E. BABADILLA, E. L. CAROLL, AND R. H. HESSE. Regulation of bone reabsorption and formation. Influences of thyrocalcitonin, parathyroid hormone, neutral phosphate and vitamin D. *Am. J. Med.* 43: 696–710, 1967.
15. POPOVTZER, M. M., M. S. BLUM, AND R. S. FLIS. Evidence for interference of 25(OH)vitamin D_3 with phosphaturic action of calcitonin. *Am. J. Physiol.* 232 (*Endocrinol. Metab. Gastrointest. Physiol.* 1): E515–E521, 1977.
16. PORS NIELSEN, S., B. BUCHANAN-LEE, E. W. MATTHEWS, J. M. MOSELY, AND C. C. WILLIAMS. Acute effects of synthetic porcine calcitonins on the renal excretion of magnesium, inorganic phosphate, sodium and potassium. *J. Endocrinol.* 51: 455–464, 1971.
17. POUJEOL, P., C. TOUVAY, N. ROINEL, AND C. DE ROUFFIGNAC. Stimulation of renal magnesium reabsorption by calcitonin in the rat. *Am. J. Physiol.* 239 (*Renal Fluid Electrolyte Physiol.* 8): F524–F532, 1980.
18. QUAMME, G. A. Effect of calcitonin on calcium and magnesium transport in rat nephron. *Am. J. Physiol.* 238 (*Endocrinol. Metab.* 1): E573–E578, 1980.
19. ROYCROFT, J., AND R. V. TALMAGE. Changes due to age in the hypocalcemic and hypophosphatemic effects of salmon calcitonin in growing rats. *Proc. Soc. Exp. Biol. Med.* 144: 17–29, 1973.
20. SORENSEN, O. H., AND I. HINDBERG. The acute and prolonged effect of porcine calcitonin on urine electrolyte excretion in intact and parathyroidectomized rats. *Acta Endocrinol.* 70: 295–307, 1972.
21. TALMAGE, R. V., AND J. J. B. ANDERSON. The effect of calcitonin on ^{32}P disappearance from plasma in parathyroidectomized and nephrectomized rats. *Proc. Soc. Exp. Biol. Med.* 141: 982–985, 1972.