Parathyroid Hormone Acutely Increases Polyphosphoinositides of the Rabbit Kidney Cortex by a Cycloheximide-sensitive Process

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ABSTRACT Parathyroid hormone (PTH) rapidly increases the concentrations of diphosphoinositide and triphosphoinositide in rabbit kidney cortex. Cycloheximide pretreatment abolishes these effects of PTH. These findings are similar to those reported for adrenocorticotropin and cyclic AMP action in the adrenal cortex, and suggest a common mechanism. Cycloheximide-sensitive effects of PTH, e.g., phosphaturia, may require polyphosphoinositides and/or other phospholipids.

INTRODUCTION

Parathyroid hormone (PTH)[1] regulates a variety of renal functions, including Ca^{++} reabsorption (1), 1α-hydroxylation of 25-hydroxycholecalciferol (2), amino acid transport (3), and phosphate (Pi) excretion (4). The underlying mechanism(s) in these processes is not fully understood. Increased cyclic (c)AMP generation (5) and cellular uptake of Ca^{++} (6) are stimulated by PTH, and these "second messengers" influence a variety of biochemical processes via protein phosphorylation (7, 8), subsequent enzyme activation or deactivation, and perhaps other mechanisms.

In the adrenal cortex, we have recently found that ACTH rapidly increases polyphosphoinositides (PPI) (9), and the latter, by virtue of their polyphosphorylated head groups, stimulate mitochondrial pregnenolone synthesis (10), which is rate-limiting in steroidogenesis (11). cAMP also increases adrenal PPI, and this increase is blocked by inhibitors of protein synthesis, e.g., puromycin and cycloheximide (CH) (12). Becuase of similarities between ACTH and PTH action in their respective target tissues (both hormones increase cAMP [5, 13], Ca^{++} uptake [6, 14], mitochondrial cytochrome P-450-mediated hydroxylations [2, 15], and uptake of amino acids [3, 16]), it seemed worthwhile to study effects of PTH on renal PPI. Our findings, herein reported, show that PTH, like ACTH, increases PPI in a target tissue by a CH-sensitive process.

METHODS

White male New Zealand rabbits, weighing 4–6 lb, were injected with physiological saline or 500 U of bovine PTH in saline (Lilly Chemical Products Inc., Templeton, Mass., and Sigma Chemical Co., St. Louis, Mo.) intramuscularly 15 min before killing by cervical dislocation and decapitation. (This dose of PTH was required to produce consistent effects on renal Pi and cAMP excretion—see below). Where indicated, rabbits were injected intraperitoneally with 100 mg of CH in saline 30 min before PTH.

For analysis of tissue phospholipids, kidneys were removed rapidly and chilled in ice-cold physiological saline. Portions of the cortex were obtained, weighed, and homogenized in water containing 250 mM sucrose and 50 mM Tris, pH 7.5. The homogenates were extracted with acidified chloroform-methanol as described by Hauser et al. (17). (Unlike the adrenal gland (9), acidification is required for more complete extraction of PPI from kidney cortex homogenates.) The washed

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[1] *Abbreviations used in this paper*: CH, cycloheximide; DPI, diphosphoinositide; PA, phosphatidic acid; Pi, phosphate; PI, phosphatidylinositol; PPI, polyphospoinositides; PTH, parathyroid hormone phosphate; TPI, triphosphoinositide.

TABLE I
Effects of PTH on DPI and TPI Levels in Rabbit Kidney Cortex

Treatment	Nanograms DPI-P		Nanograms TPI-P	
	Per 100 mg tissue*	Δ due to PTH*	Per 100 mg tissue*	Δ due to PTH*
Saline (control)	519±82(10)	—	119±24(10)	—
PTH in saline	814±71(10)	+296±26(10)	241±34(10)	+122±25(10)
P, PTH vs. control	<0.025‡	<0.001§	<0.01‡	<0.001§

In each experiment, one control and one PTH-treated rabbit were employed, and phospholipids were determined in parallel on three to five replicate portions of kidney cortex from each rabbit. Because of some variability in absolute *P* values obtained from one experiment to another, and because of less variability of the increments as a result of PTH treatment, *t* testing of the mean increments gave a greater degree of statistical significance than standard *t* testing of the absolute mean values.

* Mean values±SEM, with the number of experiments shown in parentheses.

‡ *P* determined by standard *t* testing.

§ *P* determined by *t* testing of the mean difference (increment) as a result of PTH treatment.

extracts (17) were dried under N_2 and subjected to thin layer chromatography in system B (successive unidimensional development with chloroform: methanol: 4.3 M NH_4OH [90:65:20, vol/vol/vol] followed by η-propranol: 4.3 M NH_4OH containing 10 mM cyclohexamediaminetetraacetic acid [65:35, vol/vol]), as described previously (9). Purified phospholipids were identified and digested, and phosphorus was measured as described previously (9).

In experiments where renal phosphaturic responses to PTH were evaluated, catheters were placed in the urinary bladders and stomachs of the rabbits. Water (50 ml/h) was given per stomach tube over the course of the next 5 h to ensure adequate urine flow. PTH (500 U) was administered, and urine was collected over 3 h and subjected to replicate Pi determinations (coefficient of variation ~4% by the method of Fiske and Subbarow (18). Where indicated, 100 mg CH was given 45 min before PTH (this treatment alone did not affect urine flow or basal Pi excretion).

RESULTS

The concentrations of the PPI, diphosphoinositide (DPI), and triphosphoinositide (TPI) in rabbit kidney cortex are shown in Table I. DPI levels were similar to those reported for rat kidney by Hauser et al. (19), whereas TPI levels were less.[2] PTH treatment for 15 min caused DPI and TPI to increase by 57 and 103%, respectively. In addition to increasing DPI and TPI, PTH also increased mean (±SE) levels of phosphatidic acid (PA) from 202±34 to 438±54 (14 determinations each, $P < 0.001$). Furthermore, PTH increased phosphatidylinositol (PI) to a degree comparable with that of DPI and TPI (Table II).

Because ACTH effects on steroidogenesis and adrenal PPI are blocked by CH (12), we tested effects of this inhibitor. Administration of CH 30 min before PTH completely inhibited effects of PTH on PI, DPI, and TPI (Table II). In addition, CH also blocked the phosphaturic effect of PTH (Table III), and in other studies,[3] CH inhibited PTH-induced increases in amino acid transport in renal membrane vesicle preparations.

DISCUSSION

The increase in renal PPI after PTH treatment is quite similar to that observed in the adrenal cortex after ACTH treatment. Both occur rapidly, are accompanied by increases in PA and PI, and are blocked by CH. This is of considerable interest because PTH and ACTH both increase cAMP in their target tissues, and because cAMP increases adrenal PPI (12). Thus, it seems possible that an increase in PPI may be a general control mechanism in cAMP-mediated hormone action.

It is of interest that the PTH-induced increase in PPI is associated with net increases in PA and PI. Lo et al. (20) observed rapid PTH-induced increases in [^{32}P]Pi incorporation into PA and PI, but this could be the result of a primary increase in PA, or PI breakdown and resynthesis of PA and PI. The presently observed net increase in PI argues against the latter possibility. Also, in the adrenal gland, ACTH (21) reportedly

[2] The reason for this difference is unknown, but may be the result of differences in extraction techniques or species. With nonacid extraction techniques described previously (9), the values obtained for DPI and TPI were slightly less than those obtained with the present acid extraction technique, but the ratio of DPI to TPI was not appreciably different. Moreover, the relative effect of PTH on DPI and TPI was virtually the same with the acid and nonacid extraction techniques. It may further be noted that the ratio of DPI to TPI observed presently in the kidney cortex is similar to that which we found in the ACTH-stimulated adrenal cortex (9). In attempts to employ the acid extraction technique of Hauser et al. (19), DPI and TPI recoveries were relatively poor as compared with the presently used acid and nonacid extraction techniques.

[3] P. Bidot. Manuscript in preparation.

TABLE II

Comparison of PTH and CH Effects on Inositides of the Kidney Cortex

Treatment	Phospholipid-P/100 mg tissue*			
	PI	DPI	TPI	PC + PE
	ng			
Saline (control)	5,431±138(5)	504±68(5)	268±36(5)	23,327±928(5)
PTH in saline	8,244±853(5)	848±78(5)	730±118(5)	22,475±1,002(5)
CH + PTH	6,580±287(5)	536±64(5)	346±54(5)	24,911±598(5)

* Mean values±SEM, with the number of determinations shown in parentheses. Phospholipids were determined from replicate (5) tissue samples that were extracted and chromatographed in parallel. The comparability of values of phosphatidylcholine (PC) plus phosphatidylethanolamine (PE) indicates the reproducibility of the analytic techniques and provides an internal standard for evaluation of other changes in phospholipids. Comparable effects of PTH and CH on PPI and PI were obtained in other experiments.

increases net levels of PI, and we have found[4] that: (*a*) ACTH and cAMP increase adrenal PA and PI[5] levels before DPI and TPI, (*b*) CH and puromycin block all effects of ACTH and cAMP on PA, PI, DPI, and TPI, and (*c*) ACTH-induced increases in PI kinase appear to be the result of increased PI availability. Needless to say, further studies of PTH and cAMP action on renal phospholipid metabolism will be required to determine whether the increase in PPI is entirely similar to that in the adrenal cortex.

It should be noted that a primary increase in PA after PTH (or ACTH) treatment has decidedly different implications than that which occurs with PI breakdown stimulated by α-adrenergic agents, acetylcholine, and a variety of neurotransmitters, hormones, and other substances not generally operative via cAMP (22–25). Increases in PI and PPI would accompany a primary increase in PA, whereas the opposite would occur with PI breakdown.

Although it is presently unclear why CH causes parallel inhibition of PTH effects on PPI and certain renal functions, phosphaturia, and amino acid transport, the following may be considered: (*a*) nonspecific effects of CH, (*b*) requirement for protein synthesis for concomitant, but otherwise, unrelated effects of PTH, or (*c*) a requirement for protein synthesis (? a labile protein) in PTH effects on PPI and involvement of PPI in certain PTH effects. From adrenal studies,

[4] Manuscript submitted for publication.

[5] We did not observe >10% increase in the PI area of chromatograms in our original studies (9), but in the solvent systems used, i.e., system A, PI was not clearly separated from phosphatidylserine and perhaps other phospholipids.

TABLE III

Effects of CH on PTH-induced Phosphaturia

Experiment	Treatment		Micrograms Pi excreted/min		
	Period I	Period II	Period I	Period II	Period II / Period I
1	None	None	77	83	1.08
	None	PTH	89	125	1.40
	CH	PTH	31	27	0.87
2	None	None	38	41	1.08
	None	PTH	102	208	2.04
	CH	PTH	66	17	0.26
3	None	None	93	109	1.17
	None	PTH	45	96	2.13
	CH	PTH	129	121	0.94
4	None	PTH	49	290	5.94
	CH	PTH	79	62	0.78

the first possibility seems unlikely because puromycin, another inhibitor of protein synthesis, inhibits stimulatory effects of ACTH and cAMP on inositide and PA metabolism.[6] The second explanation cannot be ruled out, but it is tempting to favor the third explanation because PPI may be involved in the stimulation of steroidogenesis by ACTH (12).

The effects of cAMP on renal inositides have been studied in vitro by Lo et al. (20) and Baricos et al. (25), and inhibition of [^{32}P]Pi incorporation has been observed. In adrenal sections in vitro, we have also observed that cAMP inhibits [^{32}P]Pi incorporation into PA, PI, DPI, TPI, and other phospholipids, while causing net increases (~100%) in these substances (unpublished observations). Obviously, labeling experiments may be misleading.

PPI have unique chemical properties, bind Ca^{++} avidly, are present in plasma and other cellular membranes, and turn over very rapidly (23, 24). Thus, these phospholipids may serve as ideal candidates for rapid modulation of membrane-associated cellular functions. Particularly in PTH action, it is not difficult to visualize a relationship between PPI and subsequent effects of PTH on Ca^{++} uptake (ACTH-induced Ca^{++} uptake in the adrenal is blocked by CH [14]) and subsequent PO_4 release (Ca^{++} activates enzymes that hydrolyze DPI and TPI [23, 24]). In addition, by analogy to ACTH and PPI effects on cholesterol hydroxylation by mitochondrial P-450 (10, 15), the increase in 1α-hydroxylation of 25-hydroxycholecalciferol after PTH treatment (2) may be the result of an increase in PPI. It will be of interest to test these possibilities in future studies.

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REFERENCES

1. Borle, A. B. 1968. Effects of purified parathyroid hormone on the calcium metabolism of monkey kidney cells. *Endocrinology.* **83:** 1316–1322.
2. Rasmussen, H., M. Wong, D. Bikle, and D. B. P. Goodman. 1972. Hormonal control of the renal conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. *J. Clin. Invest.* **51:** 2502–2504.
3. Weiss, I. W., K. Morgan, and J. M. Phang. 1972. Cyclic adenosine monophosphate-stimulated transport of amino acids in kidney cortex. *J. Biol. Chem.* **247:** 760–764.
4. Albright, F., and R. Ellsworth. 1929. Studies on physiology of parathyroid glands, calcium and phosphorus studies on case of idiopathic hypoparathyroidism. *J. Clin. Invest.* **7:** 183–201.
5. Chase, L. R., and G. D. Aurbach. 1967. Parathyroid function and the renal excretion of 3′,5′-adenylic acid. *Proc. Natl. Acad. Sci. U. S. A.* **58:** 518–525.
6. Rasmussen, H., and A. Tenenhouse. 1968. Cyclic adenosine monophosphate, Ca^{++}, and membranes. *Proc. Natl. Acad. Sci. U. S. A.* **59:** 1364–1370.
7. Krebs, E. G. 1972. Protein kinases. *Curr. Top. Cell. Regul.* **5:** 99–133.
8. Sieghart, W., J. Forn, and P. Greengard. 1979. Ca^{++} and cyclic AMP regulate phosphorylation of same two membrane-associated proteins specific to nerve tissue. *Proc. Natl. Acad. Sci. U. S. A.* **76**(5): 2475–2479.
9. Farese, R. V., A. M. Sabir, and S. L. Vandor. 1979. Adrenocorticotropin acutely increases adrenal polyphosphoinositides. *J. Biol. Chem.* **254:** 6842–6844.
10. Farese, R. V., and A. M. Sabir. 1979. Polyphosphorylated glycerolipids mimic adrenocorticotropin-induced stimulation of mitochondrial pregnenolone synthesis. *Biochim. Biophys. Acta.* **575:** 299–304.
11. Stone, D., and O. Hector. 1954. Studies on ACTH action in perfused bovine adrenals: site of action of ACTH in corticosteroid agenesis. *Arch. Biochem. Biophys.* **51:** 457–469.
12. Farese, R. V., A. M. Sabir, S. L. Vandor, and R. E. Larson. 1980. Are phosphoinositides the cycloheximide-sensitive mediator in the steroidogenic actions of adrenocorticotropin and adenosine-3′,5′-monophosphate? *J. Biol. Chem.* In press.
13. Grahame-Smith, D. G., R. W. Butcher, R. L. Ney, and E. W. Sutherland. 1967. Adenosine-3′,5′-monophosphate as the intracellular mediator of the action of adrenocorticotropin hormone on the adrenal cortex. *J. Biol. Chem.* **242:** 5535–5541.
14. Leier, D. J., and R. A. Jungmann. 1973. Adrenocorticotropic hormone and dibutyryl adenosine cyclic monophosphate-mediated Ca^{++} uptake by rat adrenal glands. *Biochim. Biophys. Acta.* **329:** 196–210.
15. Jefcoate, C. R., E. R. Simpson, and G. S. Boyd. 1974. Spectral properties of rat adrenal-mitochondrial cytochrome P-450. *Eur. J. Biochem.* **42:** 539–551.
16. Hechter, O., and I. D. K. Halkerston. 1964. On the action of mammalian hormones. *In* The Hormones. G. Pincus, K. V. Themann, and E. B. Astwood, editors. Academic Press, Inc., New York. **5:** 697–816.
17. Hauser, G., J. Eichberg, and F. Gonzalez-Sastre. 1971. Regional distribution of polyphosphoinositides in rat brain. *Biochim. Biophys. Acta.* **248:** 87–95.
18. Fiske, C. G., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66:** 375–400.
19. Hauser, G., and J. Eichberg. 1973. Improved conditions for the preservation and extraction of polyphosphoinositides. *Biochem. Biophys. Acta.* **326:** 201–209.
20. Lo, H., D. C. Lehotay, D. Katz, and G. S. Levey. 1976. Parathyroid hormone-mediated incorporation of ^{32}P-orthophosphate into phosphatidic acid and phosphatidylinositol in renal cortical slices. *Endocr. Res. Commun.* **3**(6): 377–385.
21. Laychock, S. G., J. C. Shen, E. L. Carmines, and R. P. Rubin. 1978. The effect of corticotropin on phospholipid metabolism in isolated adrenocortical cells. *Biochim. Biophys. Acta.* **528**(3): 355–363.
22. Hokin, L. E. 1969. Functional activity in glands and synaptic tissue and the turnover of phosphatidylinositol. *Ann. N. Y. Acad. Sci.* **165:** 695–709.
23. Hawthorne, J. N., and D. A. White. 1975. Myoinositol lipids. *Vitam. Horm.* **33:** 529–573.
24. Michell, R. H. 1975. Inositol phospholipids and cell surface receptor function. *Biochim. Biophys. Acta.* **415**(1): 81–147.
25. Baricos, W. H., M. W. Hurst, and C. G. Huggins. 1977. Effects of cyclic nucleotides and related agents on renal polyphosphoinositide metabolism. *In* Cyclitols and Phosphoinositides. W. W. Wells, and E. Eisenberg, Jr., editors. Academic Press, Inc., New York. 223–230.

[6] Manuscript in preparation.