Effect of Prostaglandin E_1 on Certain Renal Actions of Parathyroid Hormone

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ABSTRACT Parathyroid hormone increased basal adenyl cyclase activity and that increase was inhibited by prostaglandin E_1 (PGE_1). Tissue cyclic 3′,5′-adenosine monophosphate (cyclic AMP) concentrations were increased by parathyroid hormone and that increase was likewise inhibited by PGE_1. Both parathyroid hormone and dibutyryl cyclic AMP increased ^{32}P incorporation into renal cortical phospholipids. PGE_1 diminished the effect of parathyroid hormone but not dibutyryl cyclic AMP to influence that parameter. PGE_1 likewise modulated the effect of parathyroid hormone but not dibutyryl cyclic AMP to decrease fractional phosphate reabsorption by the renal tubule. It is suggested that PGE_1 inhibits the effect of parathyroid hormone by decreasing its effect on adenyl cyclase. Such interaction may be important in modulating the intracellular action of parathyroid hormone on kidney cortex.

INTRODUCTION

Prostaglandins inhibit several hormonal actions mediated by cyclic 3′,5′-adenosine monophosphate (cyclic AMP)[1] (1–6) by interfering with the generation of that cyclic nucleotide (1–8). In addition, local release of prostaglandins in response to hormonal stimulation has been described in various tissues (9–11). In adipose tissue, catecholamine stimulation in vitro has been reported to result in release of a sufficient amount of prostaglandins to inhibit the cyclic AMP-mediated lipolytic action of the catecholamines (11). These observations and the known ubiquitous distribution of prostaglandins (1) led to the proposal that they might participate in an important local physiologic regulating mechanism functioning to modulate hormonal actions mediated by the adenyl cyclase-cyclic AMP system (5, 7, 11).

Subsequent data indicated, however, that in certain tissues, prostaglandins themselves enhanced cyclic AMP generation (12), mimicked the biologic action of effector hormone (13), or appeared to have no significant interaction with the effector hormone (14). Further, the responses observed varied with different prostaglandins. These results indicated that the effects of prostaglandins were dependent upon the particular prostaglandin, tissue, and hormonal action examined.

Several renal responses to the prostaglandins have been described (4, 6, 15, 16). These include the observations that prostaglandin E_1 (PGE_1) inhibits the actions of vasopressin to (*a*) increase water permeability in isolated collecting ducts of rabbits (4); (*b*) increase renal medullary cyclic AMP levels (6); and (*c*) induce antidiuresis (16). Additionally, PGE_1 itself increases cyclic AMP concentration in the outer medulla (6). Considerable data has accumulated to suggest that the physiologic actions of parathyroid hormone (PTH) on the renal cortex, like those of vasopressin on the renal medulla (17), are mediated by PTH activation of the adenyl cyclase-cyclic AMP system (18–24). In the present study we investigated the interaction between PTH and PGE_1 on several renal cortical responses to PTH. The parameters examined were the effect PGE_1 on (*a*) PTH stimulation of renal cortical adenyl cyclase activity and tissue cyclic AMP concentrations; (*b*) PTH and dibutyryl cyclic AMP (DBC) augmentation of renal cor-

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This work was presented in part at the 43rd Annual Meeting of the Central Society for Clinical Research, 6 November 1970, Chicago, Ill.

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Received for publication 3 January 1972 and in revised form 12 April 1972.

[1] *Abbreviations used in this paper:* CIN, inulin clearance; cyclic AMP, cyclic 3′,5′-adenosine monophosphate; DBC, dibutyryl cyclic AMP; PGE_1, prostaglandin E_1; PTH, parathyroid hormone; TRP, tubular reabsorption of phosphate.

tical incorporation of ^{32}P into phospholipids; and (*c*) PTH and DBC potentiation of renal phosphate excretion. The data demonstrate that PGE_1 inhibits, completely or partially, PTH stimulation of each of these parameters but does not antagonize the actions of DBC. The results are consistent with the hypothesis that PGE_1 by interfering with activation of PTH-sensitive adenyl cyclase may serve as a modulator of certain renal responses to PTH.

METHODS

Adenyl cyclase activity and cyclic AMP concentration were studied in the renal cortical tissue of male Sprague-Dawley rats, weighing 180–240 g, and sacrificed by decapitation. Adenyl cyclase activity was determined using the method of Marcus and Aurbach (25) with modification. The homogenates of renal cortex were centrifuged at 2000 *g* in 0.25 M sucrose, 0.05 M tris buffer, pH 7.4, and 10% (v/v) dimethyl sulfoxide, for 10 min at 4°C. The particulates were resuspended in 0.05 M tris buffer. The study hormones, parathyroid extract (Eli Lilly and Company, Indianapolis, Ind.) and/or prostaglandin E_1 (generous gift of Dr. J. Pike, The Upjohn Company, Kalamazoo, Mich.) at the concentrations as indicated in the results, were mixed with an appropriate amount of adenyl cyclase preparation, ^{14}C-labeled adenosine triphosphate (ATP), and ATP regeneration system with pyruvic kinase (25). The mixtures were incubated for 15 min at 37°C, and then the reaction was terminated by boiling for 3 min. Cyclic AMP-^{14}C was then extracted and assayed as described by Krishna, Weiss, and Brodie (26).

For determination of cyclic AMP concentration, renal cortex was sliced to a thickness of less than 0.5 mm and a weight of 40–70 mg. The slices were incubated in Krebs-Ringer bicarbonate buffer (KRBB), pH 7.4, containing 10 mM theophylline, 200 mg bovine albumin/100 ml, 50 mg glucose/100 ml, and the study hormones as indicated in the results. The slices were incubated at 37°C. After 15 min incubation, the slices were homogenized in 5% trichloroacetic acid and centrifuged at 700 *g*. Cyclic AMP was then extracted and assayed using the method (27) described previously with modification.

The incorporation of radioactive inorganic ^{32}P was studied in renal cortical tissue from mongrel dogs. Under sodium pentobarbital anesthesia, a left nephrectomy was rapidly performed. Renal cortical slices weighing 50–100 mg were incubated in 25-ml Erlenmeyer flasks containing 2 ml of KRBB, 2 mg glucose, 20 μCi of ^{32}P ($Na_2H^{32}PO_4$ from New England Nuclear, Boston, Mass.), and test substances under an atmosphere of 95% O_2 and 5% CO_2. In each experiment all conditions were examined in cortical slices obtained from a single kidney. Substances tested and their final concentrations were PTH 3 U/ml, DBC (sodium N^6, O_2-Dibutyryladenosine-3′-5′-cyclic monophosphate, Calbiochem, San Diego, Calif.) 500 μg/ml, and PGE_1 10^{-6} M alone or in combination with PTH or DBC. Flasks were incubated in a metabolic shaker for 2 hr at 37°C. The reaction was stopped by homogenizing slices in 10% trichloroacetic acid. The phospholipid fraction was isolated and assayed for ^{32}P incorporation according to methods previously described (28).

Each of the above in vitro parameters was determined in triplicate for every experimental condition tested. Mean values from the triplicate determinations were compared using Student's *t* test for paired values to analyze statistical significance. *P* values were then obtained from standard tables.

Phosphate excretion was studied. Eight mongrel dogs weighing 10–20 kg were anesthetized with sodium pentobarbital (30 mg/kg) intravenously and maintained with 25-mg doses as necessary. Through abdominal and flank incisions, both ureters and the left renal artery were catheterized. Isotonic sodium chloride infusions were begun into the left renal artery (0.1 ml/min) and the right jugular vein (1.7 ml/min). An inulin prime (50 mg/kg) was administered into the left jugular vein and a sustaining infusion of 25 mg/ml per min was then initiated. After a 30 min stabilization period, three 15-min control urines were collected separately from each ureter (collection period I), blood was sampled at the midpoint of each 15 min urine collection from a femoral vein. An infusion of PGE_1 in 0.85% sodium chloride, 1.0 μg/0.1 ml per min, was then begun into the left renal artery and three additional 15-min urine collections and blood samples were obtained (period II). An infusion of PTH, 0.1 U/kg per min, was subsequently started via the right jugular vein at the same infusion rate as the saline control. Urines and blood samples were collected as described above (period III).

In six additional dogs a similar experimental protocol was employed to evaluate possible DBC and PGE_1 interaction. In this case, DBC, 20 μg/kg per min, was infused for 45 min into the right jugular vein.

Blood and urine samples were analyzed for inulin (29) and phosphorus (30). Mean values for inulin clearance (CIN) and per cent tubular reabsorption of phosphate (per cent TRP) were calculated separately for each kidney over each total 45 min control and experimental collection period. Mean values from the right kidney were statistically compared with those from the corresponding collection period on the left. In addition the various collection periods in the same kidney were compared with each other using *t* test for paired values.

Different animal models were utilized in these studies for technical reasons. A larger number of slices from each kidney was necessary for the ^{32}P incorporation into phospholipid experiments. Accordingly, the dog was chosen over the rat. Likewise, the design for evaluating PTH-PGE_1 interaction in vivo required a large animal. However, in a small series ($n = 4$) PTH increased cyclic AMP concentration from 5.2±SEM 1.3 to 14.9±0.9 in slices of dog renal cortex. The addition of PGE_1 10^{-6}M to the PTH 10 U/ml decreased the value to 6.2±0.1. These results indicate that the use of two models introduces no particular problem in interpretation of the results.

RESULTS

Adenyl cyclase activity. As shown in Fig. 1, preliminary investigation indicated that a dose-response relationship existed between PTH concentration and adenyl cyclase activity. PTH 1 U/ml was found to be a submaximal dose. In further studies (Table I), mean control adenyl cyclase activity of the renal cortex was 28± SE 2.0 pmoles cyclic AMP/mg protein per 15 min. PTH, 1 U/ml, increased the mean level to 42±5, $P < 0.002$. PGE_1 10^{-4}M alone had no effect on renal cortical adenyl cyclase activity, mean 31±3 (Table I). However, when this dose of PGE_1 was combined with PTH, 1 U/ml,

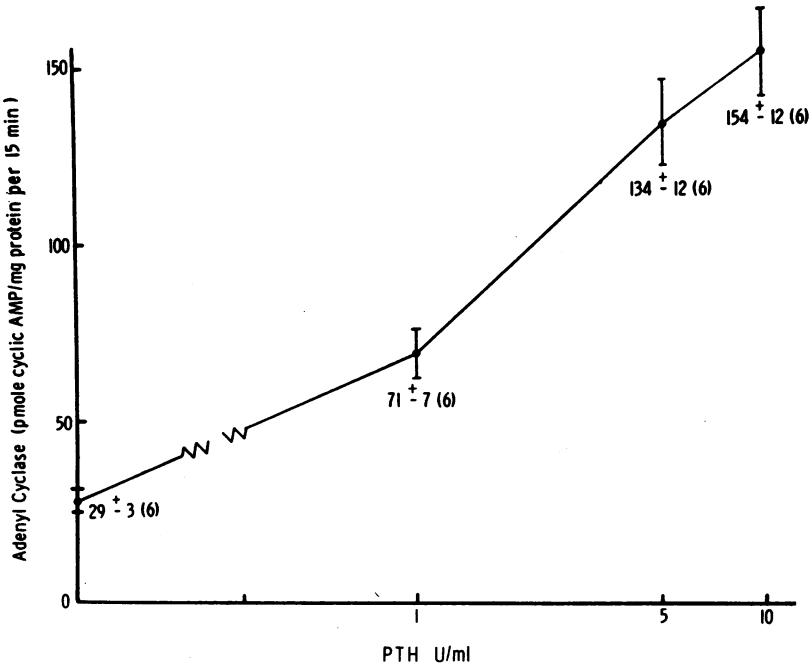

FIGURE 1 Response of renal cortical adenyl cyclase of the rat to parathyroid extract (PTE). A significant increase over basal adenyl cyclase, expressed as pmoles of cyclic AMP/mg protein per 15 min, occurred in response to 1 U/ml of PTE. PTE, 10 U/ml, did not increase adenyl cyclase significantly over the activity observed with 5 U/ml of PTE. Values for adenyl cyclase shown are mean ±SE. Numbers in parentheses give the size of each experimental population.

mean adenyl cyclase activity was 29±2, a significant reduction from the value observed with PTH alone (42±5), $P < 0.02$. PGE_1, 10^{-6}M, had no effect on PTH-induced increases in adenyl cyclase activity.

Cyclic AMP concentration. PTH, 10 U/ml, was found to be the lowest concentration to increase cyclic AMP concentration of rat renal cortical slices. This concentration of PTH increased cyclic AMP levels from a mean control value of 4.1±SE 0.6 nmoles cyclic AMP/g of wet tissue to 7.6±0.7, $P < 0.01$ (Table II). PGE_1 10^{-6}M alone had no effect on cyclic AMP concentration, mean 4.4±0.7. However, when PTH, 10 U/ml, was combined with PGE_1 10^{-6}M cyclic AMP concentration, 4.9±0.5 was significantly lower than the level observed with PTH alone (7.6±0.7), $P < 0.01$. PGE_1 10^{-7}M had no effect on PTH-induced increases in cyclic AMP concentration.

^{32}P incorporation into phospholipids. PTH, 3 U/ml, significantly increased ^{32}P incorporation into phospholipids by canine renal cortical slices (Table III), mean increase from basal values 100.3±SEMD 18.1 cpm/mg wet tissue per 120 min, $P < 0.0005$. ^{32}P incorporation was also significantly increased by DBC, 500 mg/ml, mean

TABLE I

Interaction between PGE_1 and PTH on Adenyl Cyclase Activity in Rat Renal Cortex

Hormone	Adenyl cyclase activity	
	pmoles cyclic AMP/mg protein per 15 min	
Control	28±2*	(19)
PGE_1 10^{-4} M	31±3	(12)
PTH 1 U/ml	42±5	(24)
PTH 1 U/ml + PGE_1 10^{-4} M	29±2	(14)

* Values shown are mean ±SE. Figures in parentheses represent the size of each experimental population.

TABLE II

Interaction between PGE_1 and PTH on Cyclic AMP Concentration in Rat Renal Cortex

Hormone	Cyclic AMP concentration	
	nmoles/g wet tissue per 15 min	
Control	4.1±0.6*	(16)
PGE_1 10^{-6} M	4.4±0.7	(17)
PTH 10 U/ml	7.6±0.7	(16)
PTH 10 U/ml + PGE_1 10^{-6} M	4.9±0.5	(17)

* Values shown are mean ±SE. Figures in parentheses represent the size of each experimental population.

increase 69.4±15.4 with $P < 0.005$. Preliminary studies (not shown) indicated these to be the lowest doses of PTH and DBC effective in increasing ^{32}P incorporation significantly. PGE_1 10^{-6}M did not change ^{32}P incorporation, mean difference from basal incorporation + 3.8±8.7. However, when PGE_1 10^{-6}M was combined with PTH, 3 U/ml, ^{32}P incorporation was significantly decreased from that produced by this dose of PTH alone, mean difference, (PTH + PGE_1) − PTH, −80.0±15.9 with $P < 0.02$. This inhibition of PTH action by PGE_1 was, however, only partial since the combination of PGE_1 and PTH still resulted in a statistically significant increase in ^{32}P incorporation over the basal level, mean increase over basal 21.3±8.6, $P < 0.01$. No significant difference in ^{32}P incorporation was observed between that produced by the combination of DBC and PGE_1 and that seen with DBC alone, mean difference −2.4±8.8.

Tubular reabsorption of phosphate. After control (period I) observations, Infusion of PGE_1, (period II) 1 μg/min, into the left renal artery resulted in a decrease in per cent TRP by the left kidney, mean difference (period II-I) −7.3%±SEMD 3.0 $P < 0.05$. The difference in TRP between periods II and I was not significant for the right kidney, indicating the unilateral effect of PGE_1. Systemic infusion of PTH (period III) 0.1 U/kg per min, reduced per cent TRP in both kidneys, mean difference (period III-II) −15.2%±3.2 on the right and −11.0 ± 3.2 on the left with $P < 0.005$ (Fig. 2, Table IV). Comparison of the magnitude of the reduction in per cent TRP in each kidney in response to PTH revealed the mean decrease on the left (PGE_1 infused) kidney to be less than that on the right. Mean difference (right-left kidney) in reduction of per cent TRP in response to PTH was −4.0%±1.1. Although small, this difference in responsiveness was statistically significant, $P < 0.025$. No significant changes in mean CIN were observed in either kidney during the collection periods.

A phosphaturic action of intravenously infused DBC, 20 μg/kg per min, was also observed (Fig. 3, Table IV). This action of DBC has been previously described (23,

TABLE III

^{32}P Incorporation into Phospholipids by Canine Renal Cortical Slices

Condition	Difference in incorporation	
	cpm/mg tissue per 120 min	*P value*
PTH	100.3±18.1	<0.0005
DBC	69.4±15.4	<0.005
PGE_1	3.8±8.7	NS
(PTH + PGE_1)	21.3±8.6	<0.02 (compared with PTH alone)
(DBC + PGE_1)	67.0±13.9	NS (compared with DBC alone)

Values represent the mean difference ±SEMD between basal value and the experimental condition as listed. The basal value was 202.8±23.6. In each experiment, all conditions listed were assessed in three cortical slices obtained from a single kidney. Mean values of these three determinations for a given condition were compared with any other condition statistically by paired analysis using *t* test. Abbreviations: PTH, parathyroid hormone 3 U/ml; DBC, dibutyryl cyclic AMP 500 mg/ml; PGE_1, prostaglandin E_1 10^{-6} M; cpm, counts per minute of ^{32}P in phospholipid fraction; NS, not significant.

TABLE IV

Effect of PGE_1 on Renal Responses to PTH and DBC

		I		II		III		(III-II)		
		R	L	R	L	R	L	R	L	R-L
PTH										
CIN	$\bar{X}$	40.6	41.0	37.7	41.5	34.7	43.3	−2.9	−1.7	−0.8
	SEM	1.9	6.5	1.6	4.3	3.8	6.2	3.8	2.6	5.1
TRP	$\bar{X}$	85.6	88.1	82.2	80.8	65.4	68.9	−16.1‡	−12.0‡	−4.1*
	SEM	4.1	3.4	2.3	2.1	2.7	3.2	3.9	4.2	1.1
DBC										
CIN	$\bar{X}$	37.2	38.9	36.0	36.5	36.5	38.3	0.6	1.8	1.2
	SEM	6.0	9.5	4.4	7.4	3.3	5.8	1.3	1.3	0.9
TRP	$\bar{X}$	89.8	90.7	79.8	80.6	69.5	68.0	−10.2‡	−12.6‡	2.4
	SEM	6.1	4.5	6.8	5.8	3.4	3.1	2.1	1.9	1.5

Abbreviations: CIN, inulin clearance; TRP, per cent tubular phosphate reabsorption; R, right kidney; L, left kidney.

Period I is with saline into left renal artery; II PGE_1 1 μg per min into left renal artery; III PGE_1 into left renal artery and either PTH of DBC given systematically.

* $P < 0.025$.

‡ $P < 0.005$.

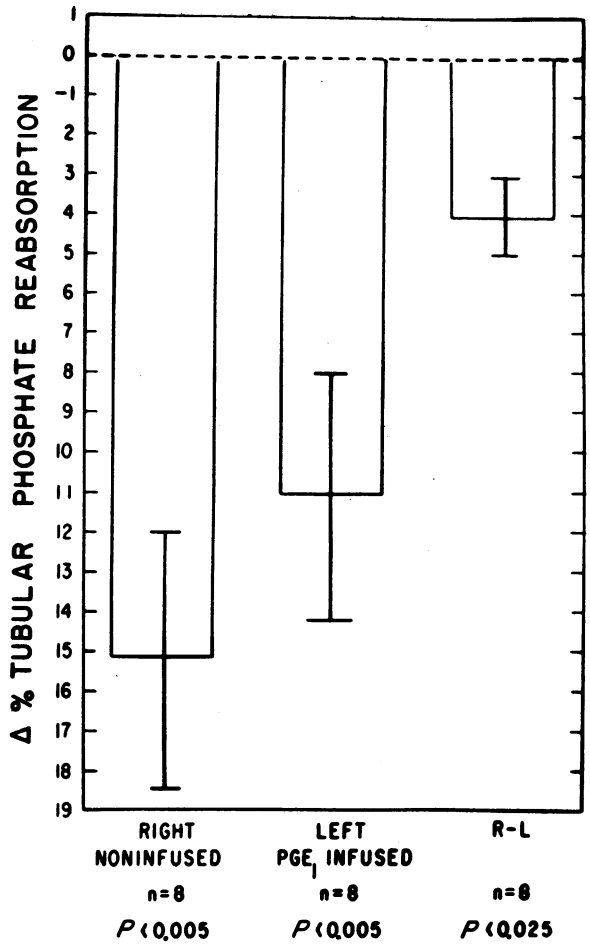

FIGURE 2 The changes in fractional reabsorption of phosphate after intravenous infusion of PTH are depicted. A significant decrease in fractional phosphate reabsorption was measured in both the PGE_1 infused and the noninfused kidney. Presented are the mean decrease and the SEM. The decrease, however, was consistently greater in the noninfused than in the infused kidney. The mean difference and the SEMD between the two sides are represented on the right.

24). Mean differences in per cent TRP in response to DBC (period III-II) were $-10.2\% \pm 2.1$ right and $-12.6\% \pm 1.9$ left, $P < 0.025$. However, no difference in the magniutde of the decrease in per cent TRP was observed between the right and left (PGE_1 infused) kidney. Mean difference (left-right kidney) in responsiveness was -2.4 ± 1.5, $P > 0.1$. Again no significant changes in CIN were noted.

DISCUSSION

The results indicate that PGE_1 inhibits the effect of submaximal doses of PTH to activate adenyl cyclase and increase intracellular cyclic AMP concentration in rat renal cortical tissue in vitro. The doses of PGE_1 effective in antagonizing these actions of PTH did not alone influence renal cortical adenyl cyclase activity or cyclic AMP concentration. In view of the differences in the assay systems, little biologic significance can probably be attached to the different concentrations of PTH and PGE_1 employed in the study of these two parameters. These in vitro systems also required relatively high concentrations of PGE_1, 10^{-4}M in the homogenates, and 10^{-6}M in the slices, to demonstrate an effect. PTH activation of renal cortical adenyl cyclase-cyclic AMP system is well documented (20, 21), with hormone-sensitive adenyl cyclase found predominantely in the plasma membrane fraction (20). In view of the present results, it is possible that PGE_1 at these concentrations might interfere with PTH activation of the adenyl cyclase-cyclic

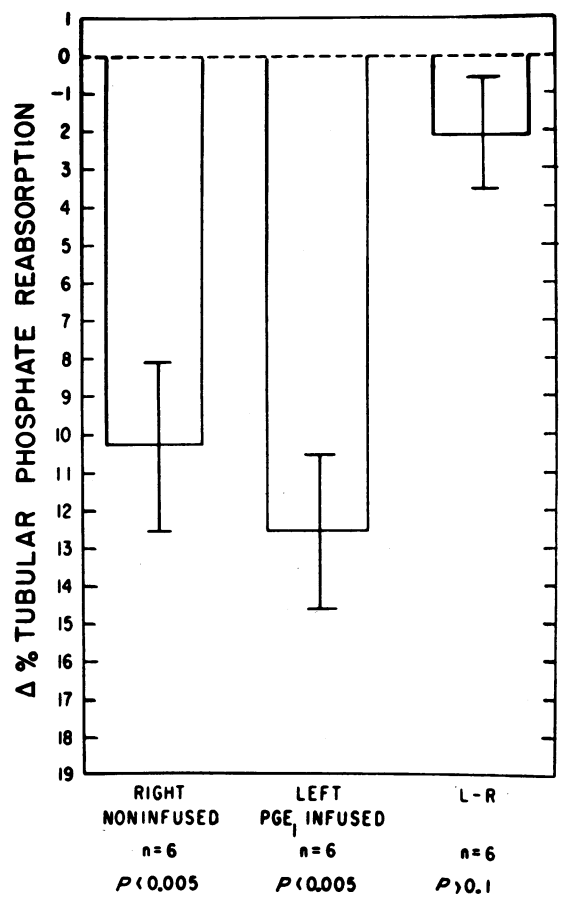

FIGURE 3 The changes in fractional phosphate reabsorption after infusion of DBC are depicted. A significant decrease in fractional phosphate reabsorption was measured in both the PGE_1-infused and the noninfused kidney. Presented are the mean decrease and the SEM. There was no consistent difference in the decrease between the infused and the noninfused kidneys. The mean difference and the SEMD between the two sides are represented on the right.

AMP system by competing with the hormone for specific membrane receptor sites at the surfaces of the cell. A similar mechanism has been proposed for PGE_1 inhibition of the action of vasopressin to increase water permeability (3, 4, 6) and that of catecholamines to stimulate lipolysis in adipose tissue (7).

The influence of PGE_1 on other renal cortical responses to PTH was also compatible with this hypothesis. It was demonstrated in vitro that PTH potentiated inorganic ^{32}P incorporation into phospholipids in canine renal cortical slices. Enhanced incorporation of ^{32}P into phospholipid in response to PTH had previously been reported using an in vivo model in which PTH was given intravenously to rats (31). In the present study, DBC also potentiated ^{32}P incorporation into phospholipids in vitro. Cyclic AMP mediation of this PTH action is thus suggested. PGE_1 10^{-6} M, which alone had no significant effect on ^{32}P incorporation, inhibited PTH but not DBC stimulation of this parameter. These results imply, therefore, that the antagonistic effect of PGE_1 on this action of PTH occurs at some point before hormonal stimulation of cyclic AMP generation and are consistent with the previously observed PGE_1 interference with adenyl cyclase activation.

The physiologic significance of PTH potentiation of ^{32}P incorporation into phospholipids is unclear. PTH stimulation of this parameter in intestine and bone as well as kidney suggest it may be a rather generalized response of PTH-sensitive tissues (32, 33). In addition, increased ^{32}P incorporation into phospholipids has been observed in other target tissues in response to other hormones (34–36).

Cyclic AMP is also thought to mediate the phosphaturic action of PTH (23, 24). Therefore, a possible interaction between PTH and PGE_1 was sought in vivo.

In order to compare the renal effects of PTH alone and PGE_1 and PTH together, experiments were designed to limit the effect of PGE_1 to one (the left) kidney. The design was tested in each experiment by comparing renal function in both kidneys when only the diluent was infused (period I) into the left kidney with the infusion of PGE_1 (period II). Increases in urine flow and in phosphate excretion occurred in period II only in the left kidney, confirming the unilateral effect of PGE_1. The systemic infusion of either PTH or DBC (period III) then permitted the evaluation of the effect of each on both a kidney with a documented PGE_1 effect and on one without that effect. For these reasons, period I was compared with period II to document the unilateral effect of PGE_1 and period III with period II to evaluate a possible interaction between PGE_1 and either DBC or PTH.

In the kidney selectively perfused with PGE_1, a small but statistically significant decrease in responsiveness to PTH was observed relative to the opposite control kidney as reflected by a decrease in the magnitude of PTH-induced inhibition of tubular phosphate reabsorption in the PGE_1-perfused kidney. An inhibitory effect of PGE_1 on the phosphaturic action of DBC was not seen. Therefore, qualitatively the interaction between PGE_1, PTH, and DBC in vivo appeared to be similar to that demonstrated in vitro. Since increase in glomerular filtration rate have previously been reported in response to the commercial PTH extract employed in this study (37), per cent TRP was employed as an index of tubular phosphate transport to negate the influence of this variable on phosphate excretion. Significant changes in mean CIN during different collection periods of a given experiment were not, however, noted in the present study, perhaps due to the lower total dose and slower rat of administration of PTH employed (37).

The effect of PGE_1 on PTH inhibition of tubular phosphate reabsorption was small. The physiologic importance of this interaction, therefore, remains uncertain. It is of interest in this regard that the magnitude of the phosphaturic response to PTH was reduced in the PGE_1-infused kidney despite the fact that PGE_1 infusion alone appeared to decrease per cent TRP. The explanation for this apparent inhibitory effect of PGE_1 on renal tubular phosphate reabsorption is unclear. A correlation between decreased proximal tubule sodium and phosphate reabsorption has recently been reported (24). The phosphaturic action of PGE_1 observed here could possibly be related to its natriuretic action (15). An independent effect of PGE_1 to decrease proximal tubular sodium (and phosphate) reabsorption might thus diminish its net inhibitory action on PTH-induced phosphaturia. Such a mechanism is speculative but deserves further study since it could explain the small in vivo effect of PGE_1 on PTH phosphaturic action compared with the more apparent in vitro interaction.

In summary, the data indicate that PGE_1 is able to modify several renal responses to PTH. This effect of PGE_1 would appear to depend upon its interference with PTH activation of sensitive renal cortical adenyl cyclase, inhibiting hormone-induced cyclic AMP generation and cyclic AMP-mediated hormonal actions. PGE_1 and perhaps other prostaglandins might, therefore, participate in an important local negative feedback system modulating the renal actions of PTH. Such a homeostatic mechanism would probably require local release of prostaglandins by the renal cortex in response to PTH stimulation in amounts sufficient to inhibit PTH action (10). Such local release of prostaglandins in the renal cortex has not been demonstrated. For this and other reasons discussed, the physiologic importance of the observed interaction between PGE_1 and PTH remains speculative.

REFERENCES

1. Bergström, S. 1967. Prostaglandins: members of a new hormonal system. *Science (Wash. D. C.).* **157**: 382.
2. Steinberg, D., M. Vaughan, P. J. Nestel, O. Strand, and S. Bergstrom. 1964. Effects of the prostaglandins on hormone-induced mobilization of free fatty acids. *J. Clin. Invest.* **43**: 1533.
3. Orloff, J., J. S. Handler, and S. Bergstrom. 1965. Effect of prostaglandin (PGE_1) on the permeability response of toad bladder to vasopressin, theophylline and adenosine 3',5'-monophosphate. *Nature (Lond.).* **205**: 397.
4. Grantham, J. J., and J. Orloff. 1968. Effect of prostaglandin E_1 on the permeability response of the isolated collecting tubule to vasopressin, adenosine 3',5'-monophosphate, and theophylline. *J. Clin. Invest.* **47**: 1154.
5. Horton, E. W. 1969. Hypotheses on physiologic roles of prostaglandins. *Physiol. Rev.* **49**: 122.
6. Beck, N. P., T. Kaneko, U. Zor, J. B. Field, and B. B. Davis. 1971. Effects of vasopressin and prostaglandin E_1 on the adenyl cyclase-cyclic 3',5'-adenosine monophosphate system of the renal medulla of the rat. *J. Clin. Invest.* **50**: 2461.
7. Butcher, R. W., J. E. Pike, and E. W. Sutherland. 1967. The effect of prostaglandin E_1 on adenosine 3',5' monophosphate levels in adipose tissue. Prostaglandins, Proceedings of the 2nd Nobel Symposium. S. Bergstrom and B. Samuelsson, editors. Almqvist & Wiksell, Publishers, Stockholm. 133.
8. Ramwell, P. W., and J. E. Shaw. 1968. Prostaglandin inhibition of gastric secretion. *J. Physiol. (Lond.).* **195**: 34P.
9. Ramwell, P. W., and J. E. Shaw. 1967. Prostaglandin release from tissues by drug, nerve, and hormone stimulation. Prostaglandins, Proceedings of the 2nd Nobel Symposium. S. Bergstrom and B. Samuelsson, editors. Almqvist & Wiksell, Publishers, Stockholm. 283.
10. Shaw, J. E., and P. W. Ramwell. 1967. Prostaglandin release from the adrenal gland. Prostaglandins, Proceedings of the 2nd Nobel Symposium. S. Bergstrom and B. Samuelsson, editors. Almqvist & Wiksell, Publishers, Stockholm. 293.
11. Shaw, J. E., and P. W. Ramwell. 1968. Release of prostaglandin from rat epididymal fat pad on nervous and hormonal stimulation. *J. Biol. Chem.* **243**: 1498.
12. Butcher, R. W., and C. E. Baird. 1968. Effects of prostaglandins on adenosine 3',5'-monophosphate levels in fat and other tissues. *J. Biol. Chem.* **243**: 1713.
13. Flack, J. D., R. Jessup, and P. W. Ramwell. 1969. Prostaglandin stimulation of rat corticosteroidogenesis. *Science (Wash. D. C.).* **163**: 691.
14. Bedwane, J. R., and E. W. Horton. 1968. The effects of prostaglandins E_1 and E_2 on ovarian steroidogenesis. *Life Sci.* **7**: 389.
15. Lee, J. B. 1969. Hypertension, natriuresis and the renal prostaglandins. *Ann. Intern. Med.* **70**: 1033.
16. Johnston, H. H., J. P. Herzog, and D. P. Lauler. 1967. Effect of prostaglandin E_1 on renal hemodynamics, sodium and water excretion. *Am. J. Physiol.* **213**: 939.
17. Orloff, J., and J. Handler. 1967. The role of adenosine 3',5'-phosphate in the action of antidiuretic hormone. *Am. J. Med.* **42**: 757.
18. Wells, H., and W. Lloyd. 1967. Effects of theophylline on the serum calcium of rats after parathyroidectomy and administration of parathyroid hormone. *Endocrinology.* **81**: 139.
19. 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.
20. Chase, L. R., and G. D. Aurbach. 1968. Renal adenyl cyclase: anatomically separate sites for parathyroid hormone and vasopressin. *Science (Wash., D. C.).* **159**: 545.
21. Melson, G. L., L. R. Chase, and G. D. Aurbach. 1970. Parathyroid hormone-sensitive adenyl cyclase in isolated renal tubules. *Endocrinology.* **86**: 511.
22. Nagata, N., and H. Rasmussen. 1968. Parathyroid hormone and renal cell metabolism. *Biochemistry.* **7**: 3728.
23. Rasmussen, H., M. Pechet, and D. Fast. 1968. Effect of dibutyryl cyclic adenosine 3',5'-monophosphate, theophylline, and other nucleotides upon calcium and phosphate metabolism. *J. Clin. Invest.* **47**: 1843.
24. Agus, Z. A., J. B. Puschett, D. Senesky, and M. Goldberg. 1971. Mode of action of parathyroid hormone and cyclic adenosine 3',5'-monophosphate on renal tubular phosphate reabsorption in the dog. *J. Clin. Invest.* **50**: 617.
25. Marcus, R., and G. D. Aurbach. 1969. Bioassay of parathyroid hormone *in vitro* with a stable preparation of adenyl cyclase from rat kidney. *Endocrinology.* **85**: 801.
26. Krishna, G., B. Weiss, and B. B. Brodie. 1968. A simple, sensitive method for the assay of adenyl cyclase. *J. Pharmacol. Exp. Ther.* **163**: 379.
27. Kaneko, T., and J. B. Field. 1969. A method for determination of 3',5'-cyclic adenosine monophosphate based on adenosine triphosphate formations. *J. Lab. Clin. Med.* **74**: 682.
28. Oka, H., and J. B. Field. 1966. Effects of ions on TSH stimulation of P^{32} incorporation into phospholipid thyroid slice. *Am. J. Physiol.* **211**: 1357.
29. Führ, J., J. Kaczmarczyk, and C.-D. Krüttgen. 1955. Eine einfache colorimetrische methode zur inulin-bestimmung für Nieren-Clearance-Untersuchungen bei Stoffwechselgesunden und Diabetrickern. *Klin. Worchenschr.* **33**: 729.
30. Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorous. *J. Biol. Chem.* **66**: 375.
31. Egawa, J., and W. F. Neuman. 1964. Effect of parathyroid extract on the metabolism of radioactive phosphate in kidney. *Endocrinology.* **74**: 90.
32. Borle, A. B., H. T. Keutmann, and W. F. Neuman. 1963. Role of parathyroid hormone in phosphate transport across rat duodenum. *Am. J. Physiol.* **204**: 705.
33. Egawa, J., and W. F. Neuman. 1963. Effects of parathyroid hormone on phosphate turnover in bone and kidney. *Endocrinology.* **72**: 370.
34. Morton, M. E., and J. R. Schwartz. 1953. The stimulation *in vitro* of phospholipid synthesis in thyroid tissue by thyrotropic hormone. *Science (Wash., D. C.).* **117**: 103.
35. Hokin, L. E., and M. R. Hokin. 1962. Changes in phospholipid metabolism on stimulation of protein secretion in pancreas slices. *J. Histochem. Cytochem.* **13**: 113.
36. Pastan, I. 1966. The effect of dibutyryl cyclic 3',5'-AMP on the thyroid. *Biochem. Biophys. Res. Commun.* **25**: 14.
37. Hiatt, H. H., and D. D. Thompson. 1957. The effects of parathyroid extract on renal function in man. *J. Clin. Invest.* **36**: 557.