Effects of Parathyroid Hormone and Prostaglandin E₁ *in vitro* on Release of Cyclic AMP from Kidney Cortical Tissue

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Synopsis

The interaction of parathyroid hormone (PTH) and prostaglandin E₁ (PGE₁) on cyclic AMP metabolism in the rat renal cortical tissue was examined *in vitro*, placing an emphasis on the process of release of the nucleotide from the tissue. PTH enhanced the release of cyclic AMP from cells even in the presence of 10 μ M cyclic AMP added to the incubation medium. This effect of PTH to accelerate cyclic AMP release was suppressed by simultaneous addition of PGE₁. Although PTH and PGE₁ showed an additive effect in stimulating the adenylate cyclase activity of the purified renal plasma membrane, in the experiment in which slices were employed the sum of cyclic AMP in the tissue and medium under simultaneous stimulation tended to be less than that under the stimulation by PTH alone. However, the increase by PTH of specific activity of cyclic AMP produced in slices prelabeled with [¹⁴C]adenine was further enhanced by the simultaneous addition of PGE₁, suggesting that PGE₁, by suppressing the release of cyclic AMP, keeps more cyclic AMP intracellularly and offers phosphodiesterase more substrate. It seemed that the release of cyclic AMP from the kidney cortical tissue could proceed against a concentration gradient and was regulated somehow by PTH and PGE₁. It was noted also that the change in the release process influenced the intracellular metabolism of cyclic AMP, indicating the importance of taking such an extracellular release mechanism into consideration in the study on the cellular metabolism of cyclic AMP.

The effect of parathyroid hormone (PTH) to increase the cyclic AMP concentration in the kidney and urine is well known (Chase and Aurbach, 1967; Nagata and Rasmussen, 1968). The level of cyclic AMP in the rat renal tissue maximally stimulated by PTH is 500 to 700% of the control and the increase of urinary excretion of cyclic AMP far exceeds this response (Kurokawa *et al.*, 1974). This marked increase in the urinary excretion of cyclic AMP suggests the presence of some mechanism related to cyclic AMP release rather than a simple reflection of

change in the cyclic AMP content in the tissue. So, in the study of the cellular metabolism of cyclic AMP, especially in the renal tissue, it seems necessary to discuss the release of cyclic AMP out of cells along with the rate of production and decomposition.

This study shows a stimulation by PTH of the cyclic AMP release from rat kidney slices and an antagonistic effect of prostaglandin E₁ (PGE₁) on this process.

Materials and Methods

Wistar male rats weighing 250 to 300 g were used. PTH (Wilson 340 U/mg) and porcine calcitonin (Armour 60 U/mg—a gift from Yamanouchi Pharmaceutical Co.) were dissolved in saline. PGE₁ was supplied from Ono Pharmaceutical Co. in two forms, G501 (lyophilized powder) and G511 (cyclodextrin conjugate). G501 was dissolved in 10 mM phosphate buffer pH 7.4 and water soluble G511 was dissolved in saline. Cyclodextrin conjugate (G511) was used in this series of experiments, unless otherwise indicated.

Kidney cortical slices (100 to 120 mg) prepared by a tissue slicer were incubated in 1.5 ml of Krebs-Ringer bicarbonate buffer containing 10 mM glucose in a 30 ml Erlenmeyer flask under a gas phase of 95% O₂–5% CO₂. Unless otherwise indicated, the incubation medium contained 2 to 10 mM theophylline. To examine the change in a cyclic AMP content in tissue and medium, the slices were preincubated for 5 to 30 min at 37°C and the test reagents were added. At the end of incubation, the slices were rapidly homogenized in 7.5% trichloroacetic acid in a glass homogenizer and the medium was acidified by adding 200 μ l of 50% trichloroacetic acid. The extraction and estimation of cyclic AMP were reported previously (Kurokawa *et al.*, 1974).

Newly synthesized cyclic AMP was estimated by measuring the radioactivity incorporated into cyclic AMP from [¹⁴C]ATP essentially according to the method reported by Shimizu *et al.* (Shimizu *et al.*, 1969). Kidney cortical slices (100 to 120 mg) preincubated for 30 min in the presence of 2 μ Ci of [¹⁴C]adenine were transferred to the incubation flask containing the test reagent and incubated further for 5 min. The neutralized trichloroacetic acid extract of the tissue or medium was charged onto a Dowex 50-X8 column (0.6 $\times$ 8 cm) along with 100,000 cpm of [³H]cyclic AMP and eluted with water. An aliquot of 1-ml fractions was counted for [³H]cyclic AMP. A fraction (1 ml) containing the highest activity of [³H]cyclic AMP was lyophilized, dissolved in 0.2 ml of 50% ethanol containing 5 mg/ml of cyclic AMP and purified further on silica gel thin layer chromatography (solvent system; isobutyric acid : water : NH₄OH = 64 : 33 : 3). Cyclic AMP visualized under UV lamp was extracted by sonication into 1 ml of water and a portion of it was counted for ¹⁴C and ³H in scintillation fluid (70% toluene, 30% Triton-X 100, 0.4 g 2,5-diphenyl oxazole and 0.01 g 1,4-bis [2-(5-phenyl oxazolyl)] benzene in 100 ml) by Aloca LSC 507 scintillation counter. Recovery of [¹⁴C] cyclic AMP was calculated from the amount of ³H found.

Purified renal plasma membranes were prepared by sucrose gradient centrifugation and its adenylate

cyclase activity was measured as previously reported (Kimura *et al.*, 1976). The phosphodiesterase activity of the tissue homogenate was assayed according to the method of Thompson and Appleman (Thompson and Appleman, 1971). ATP in the trichloroacetic acid extract of the kidney slices was measured enzymatically as described by Williamson using an Eppendorf fluorophotometer (Williamson and Corkey, 1969). Protein was determined by the method of Lowry *et al.* (Lowry *et al.*, 1951). Each experiment was run in triplicate and the significance of differences was evaluated by Student's *t* test.

Results

Effect of PTH on extracellular release of cyclic AMP

Table 1 shows the effect of PTH or PGE₁ on cyclic AMP accumulation. The effect of PGE₁ to increase cyclic AMP was dose-related and appeared to continue longer than that by PTH. Markedly increased levels of cyclic AMP were also found in the medium by incubating slices with PTH.

It was examined whether the release of cyclic AMP from the tissue was modified by the presence of added cyclic AMP in the medium as shown in Table 2. The release of cyclic AMP was estimated by measuring labeled cyclic AMP released from the prelabeled tissue. When incubated for 30 min with 4 μ M [¹⁴C] adenine, slices incorporated into ATP about 12.6% of the adenine, and about 1.8% of this was found as cyclic AMP. The incubation of this prelabeled tissue for 5 min in the presence of 10 U/ml of PTH resulted in a marked increase in radioactivity incorporated into cyclic AMP in the tissue as well as in the medium. The addition of 1 to 10 μ M of cyclic AMP into the medium during this test incubation did not change the PTH effect on cyclic AMP either in the tissue or in the medium.

To examine if the release process of cyclic AMP itself is modified by PTH, the dose of PTH was lowered and the distribution of cyclic AMP between the tissue and

Table 1. Effects of PTH and PGE₁ on cyclic AMP metabolism of kidney cortical tissue.

Treatment	Cyclic AMP (nmol/g wet tissue)		
	Tissue	Medium	30 min
	5 min	30 min	
Saline	2.80±0.35	1.94±0.18	0.34±0.03
PTH 10 U/ml	6.22±0.17**	2.72±0.04**	3.47±0.69**
20 U/ml	5.55±0.39**	3.20±0.16**	3.04±0.28**
PGE ₁ 0.1 µg/ml	2.14±0.07	1.88±0.02	0.39±0.04
1 µg/ml	3.47±0.19	2.26±0.23	0.53±0.04**
5 µg/ml	3.63±0.21*	3.59±0.08**	0.59±0.03**
10 µg/ml	4.93±0.29**	4.97±0.61**	0.83±0.14**

PTH or PGE₁ was added at the end of 5 min of preincubation. The medium contained theophylline at 2mM. Each mean±SEM is of three incubations.

* p<0.05, ** p<0.01 in comparing PGE₁ or PTH effects with saline treatment.

Table 2. Effect of extracellular cyclic AMP on the release of [¹⁴C]cyclic AMP from PTH-stimulated kidney cortical tissue.

Treatment	[¹⁴ C]cyclic AMP cpm × 10 ⁻³ /g wet tissue	
	Tissue	Medium
Saline	3.29 ± 0.35	1.71 ± 0.06
plus 10 ⁻⁵ M cyclic AMP	1.48 ± 0.20*	2.20 ± 0.16
PTH 10 U/ml	114.07 ± 11.68	61.63 ± 1.06
plus 10 ⁻⁶ M cyclic AMP	119.27 ± 5.53	59.74 ± 3.51
plus 10 ⁻⁵ M cyclic AMP	98.70 ± 2.22	50.71 ± 3.53

Kidney cortical tissue was preincubated for 30 min with [¹⁴C]adenine, transferred to a new incubation flask containing test solution, and incubated further for 5 min. [¹⁴C]cyclic AMP was extracted and purified as described in Methods. Each mean±SEM is of three incubations. * p<0.02

the medium was compared to that in the presence of PGE₁ or calcitonin (Fig. 1). As to the increase of cyclic AMP in the tissue, the effect of 0.5 U/ml of PTH was nearly equivalent to the effect of 0.5 U/ml of calcitonin or 10 µg/ml of PGE₁. However, there was a clear difference in the amount of cyclic AMP released out of the tissue into the medium, PTH inducing a greater release of cyclic AMP than the other stimulants. Such an action of PTH is further demonstrated by examining the effect of 0.1 U/ml of PTH. This dose of PTH, although the increase of tissue cyclic AMP was only a minimum, caused a similar or

Fig. 1. Effects of PTH, PGE₁ or calcitonin on cyclic AMP levels in the tissue and the medium. Slices were incubated in the medium containing 10mM theophylline. Saline or hormone was added after 5 min of preincubation and test incubation was carried out further for 1 or 5 min. To terminate the reaction, slices were homogenized in 7.5% trichloroacetic acid and media were acidified by adding trichloroacetic acid. Each mean±SEM is of 3 incubations. PGE₁ 10 µg/ml, calcitonin (CT) 500mU/ml. ○ Saline control, ●—Cyclic AMP in the tissue, ●-----Cyclic AMP in the medium.

even greater release of cyclic AMP as compared to that induced by 0.5 U/ml of calcitonin or 10 µg/ml of PGE₁.

Effect of PGE₁ on PTH induced change in cyclic AMP metabolism

Another possibility raised was that PGE₁ kept more cyclic AMP intracellularly because the appearance of cyclic AMP out of cells was extremely low when the slices

were stimulated by PGE₁ (Table 1 and Fig. 1). So the effect of simultaneous addition of PGE₁ and PTH was examined (Fig. 2). The level of cyclic AMP in the tissue at the end of 5 min of incubation with 10 U/ml of PTH plus 10 μ g/ml of PGE₁ was the same as that obtained by incubation with PTH alone. However, the release of cyclic AMP stimulated by PTH was decreased by this simultaneous addition of PGE₁. Both the cyclodextrin conjugate (G511) of PGE₁ and lyophilized powder (G501) inhibited the appearance of cyclic AMP in the medium to the same extent. It was confirmed that the binding assay of cyclic AMP itself was not affected by the presence of PGE₁ in the assay mixture. Levels of ATP in the kidney slices were not changed either by PTH or PGE₁ (control; 1.36 ± 0.09 μ mol/g wet tissue, PTH 10 U/ml; 1.25 ± 0.08 , PGE₁ 10 μ g/ml; 1.40 ± 0.05) probably excluding a change in an ATP-dependent transport process of cyclic AMP. It is noticed also that the sum of cyclic AMP in the tissue and medium is not enhanced but decreased by the simultaneous addition of PTH and PGE₁.

Beck *et al.* (Beck *et al.*, 1972) studied the effect of PGE₁ on the rat renal tissue and showed that an antagonistic action of PGE₁ on PTH induced an increase in adenylate cyclase activity. The results shown in Table 3, however, indicated that PGE₁ and PTH had an additive effect on renal cortical adenylate cyclase in accord with the finding by Chase on the rabbit kidney (Chase, 1975). In Table 4, it is shown that neither a preincubation of the slices with PGE₁ nor a direct addition of PGE₁ into the assay mixture affected phosphodiesterase activity.

Effects of PTH and/or PGE₁ on the metabolism of [¹⁴C]cyclic AMP

Table 5 shows an effect of incubation of the prelabeled tissue with PTH, PGE₁ or PTH plus PGE₁ on [¹⁴C]cyclic AMP

Fig. 2. Effect of PGE₁ on PTH-induced change in cyclic AMP metabolism. PTH (10 U/ml), PGE₁ (10 μ g/ml) or PTH plus PGE₁ was added at the end of 5 min of preincubation and the test incubation was continued for 5 min. Incubation media contained theophylline at 10 mM. PGE₁ in two forms, cyclodextrin conjugate (G511) and lyophilized powder (G501) dissolved in 10 mM phosphate buffer, was used. Each mean $\pm$ SEM is of three incubations. **p* < 0.01; significantly different from the value obtained by incubating PTH alone.

Table 3. Effect of PGE₁ on adenylate cyclase activity of purified renal cortical plasma membrane.

Addition	Cyclic AMP formed (pmol/min/mg protein)
None	16.3 ± 3.8
PGE ₁ 2 μ M	30.7 ± 4.5
10 μ M	41.0 ± 5.1
PTH 10 U/ml	263.8 ± 14.2
PGE ₁ 10 μ M plus PTH 10 U/ml	$325.5 \pm 13.2^*$

About 20 μ g protein of renal cortical plasma membranes purified by sucrose gradient centrifugation was incubated for 5 min. The reaction was terminated by trichloroacetic acid and the cyclic AMP purified through a neutral alumina column was measured by Gilman's binding method. Each mean $\pm$ SEM is of three incubations. **p* < 0.05; significantly different from the value obtained with PTH alone.

Table 4. Effect of PGE₁ on phosphodiesterase activity.

Exp I Enzyme activity measured in the presence of PGE ₁			
Substrate concentration		Cyclic AMP hydrolyzed (nmol/min/mg protein)	
		Basal	PGE ₁ 7.5 µg/ml
Cyclic AMP	5 × 10 ⁻⁶ M	0.74 ± 0.03	0.71 ± 0.02
	5 × 10 ⁻⁴ M	6.74 ± 0.98	6.60 ± 0.82
Exp II Enzyme activity in the homogenate prepared from the slices incubated with PGE ₁			
Substrate concentration		Cyclic AMP hydrolyzed (nmol/min/mg protein)	
		Basal	PGE ₁ 10 µg/ml
Cyclic AMP	5 × 10 ⁻⁶ M	0.41 ± 0.02	0.42 ± 0.03
	5 × 10 ⁻⁴ M	3.60 ± 0.12	3.91 ± 0.18

Renal cortical tissue homogenized in 0.25 M sucrose and 1 mM EDTA (pH 7.4) was sonicated for 2 min in an ultrasonic bath and was subjected to an enzyme assay. Enzyme preparation of 100 µg of protein was added to the assay mixture of 200 µl. In Exp II, the homogenate of the kidney cortical slices which were incubated for 5 min with PGE₁ was used as enzyme preparation. Each mean ± SEM is of three incubations.

accumulation in the presence of 10 mM theophylline. The specific activity of cyclic AMP was calculated from the level of cyclic AMP estimated by Gilman's assay in the experiment conducted under the same conditions except that it was not prelabeled with [¹⁴C]adenine. The specific activity of cyclic AMP in the medium was higher than that of intracellular cyclic AMP, indicating a preferential release of newly synthesized cyclic AMP. The amount of [¹⁴C]cyclic AMP in the slices stimulated by PTH or PGE₁ was about 20 or 5 times as much as the control, respectively. The marked increase of [¹⁴C]cyclic AMP in the medium induced by PTH was suppressed by the simultaneous addition of PGE₁. When the sum of [¹⁴C]cyclic AMP accumulation in the tissue and medium was taken into account, PGE₁ again suppressed the PTH effect. The PTH-induced increase of specific activity of cyclic AMP, however, seemed to be enhanced further by PGE₁.

Table 5. Effects of PTH, PGE₁ or PTH plus PGE₁ on kidney slices prelabeled with [¹⁴C]adenine.

	[¹⁴ C]cyclic AMP cpm × 10 ⁻³ /g	Cyclic AMP nmol/g	Specific activity cpm/nmol × 10 ⁻³
Tissue			
Saline	3.16 ± 0.87	1.83 ± 0.16	1.73
PTH 10 U/ml	65.37 ± 0.59	10.90 ± 0.45	6.00
PGE ₁ 10 µg/ml	15.06 ± 1.24	4.18 ± 0.11	3.60
PTH 10 U/ml + PGE ₁ 10 µg/ml	70.35 ± 1.90	10.50 ± 0.96	6.70
Medium			
Saline	1.39 ± 0.32	0.25 ± 0.02	5.58
PTH	50.02 ± 0.24	5.58 ± 0.29	8.96
PGE ₁	1.95 ± 0.19	0.40 ± 0.03	4.88
PTH + PGE ₁	33.60 ± 0.41**	2.86 ± 0.20**	11.75
Total			
Saline	4.55 ± 0.68	2.06 ± 0.18	2.19
PTH	115.39 ± 0.83	16.48 ± 0.82	7.00
PGE ₁	17.01 ± 1.73	4.58 ± 0.10	3.71
PTH + PGE ₁	103.95 ± 2.82	13.36 ± 1.39	7.78

Kidney slices preincubated for 30 min with [¹⁴C]adenine were transferred into new incubation flasks and incubated for 5 min in the presence or absence of hormone(s). [¹⁴C]cyclic AMP was extracted and purified as described in Methods. The levels of cyclic AMP were estimated by Gilman's assay on the samples obtained by an experiment, in which slices were preincubated for 30 min in the absence of adenine and incubated for 5 min in the new flasks. Each mean ± SEM is of three incubations.

** p < 0.01; significantly different from the value obtained by incubating with PTH alone.

Fig. 3 shows the sum of cyclic AMP in the tissue and medium obtained in an experiment in which the incubation was carried out in the absence of a phosphodiesterase inhibitor, theophylline. The increased cyclic AMP level caused by PTH was clearly suppressed by PGE_1 , but the effect of PTH to increase the specific activity of cyclic AMP was enhanced. Thus the effect of PGE_1 to modify PTH action was shown more clearly in the absence of theophylline than in its presence.

Fig. 3. Modification by PGE_1 of PTH induced changes in cyclic AMP metabolism in the absence of theophylline. Except for the absence of theophylline, the experimental procedure was the same as that shown in Table 5. The values shown are the sum of [^{14}C]cyclic AMP or cyclic AMP (estimated by Gilman's assay) in the tissue and the medium. The amount of [^{14}C]cyclic AMP in the tissue at the end of 30 min of pre-incubation has been subtracted from the value found at the end of test incubation. Each mean $\pm$ SEM is of 3 incubations. PTH 10 U/ml, PGE_1 10 $\mu\text{g/ml}$.

Discussion

Cyclic AMP is released out of the kidney cortical tissue even in the presence of high concentration (1–10 μM) of cyclic AMP added to the incubation medium, and PTH accelerates this process. As the concentration of intracellular cyclic AMP stimulated by PTH is calculated to be approximately

10 μM (Tables 1, 5 and Fig. 2), it seems that there is a process of cyclic AMP release which can proceed against a concentration gradient. The fact that the accumulation of [^{14}C]cyclic AMP in the control slices is significantly decreased by the addition of 10^{-5} M cyclic AMP needs further clarification.

The increase of cyclic AMP release in the presence of PTH cannot be explained simply by an elevation of the tissue level of the nucleotide, as a comparable elevation of tissue cyclic AMP induced by calcitonin or PGE_1 caused much less release of cyclic AMP into the medium (Fig. 1). It is possible, therefore, that the release of cyclic AMP from the cell is somehow regulated, and that some unknown action of PTH on the cell membrane facilitates this process. The fact that PGE_1 possesses an antagonistic action on this PTH effect supports the possible presence of such a regulated process. In the study on some tumor cells (Doore *et al.*, 1975) and bacteria (Saier, 1975), it has been reported that cyclic AMP is transported out of the cell by an energy dependent process. The inhibitory action of PGE_1 on PTH-induced cyclic AMP release may not be due to an alteration in energy metabolism since no change in the cellular ATP level was detected by PGE_1 .

An influence of the change in the release process on the intracellular cyclic AMP metabolism is suggested by the fact that PGE_1 cannot enhance the PTH effect but rather antagonize it when the increase in the sum of intra- and extracellular cyclic AMP is taken into consideration (Table 5, Figs. 2 and 3). The results obtained in these experiments on tissue slices are not consistent with the observation that the effect of PGE_1 on the adenylate cyclase activity of the purified renal plasma membrane is additive to that of PTH (Table 3) and that the phosphodiesterase activity of broken cell preparation is not affected by PGE_1 (Table 4). The experiment examining

the metabolism of radioactive cyclic AMP appears to have clarified the mechanism underlying this apparent discrepancy between the results obtained with broken cell preparation and tissue slices. Although the increase produced by PTH of the sum of intra- and extracellular [¹⁴C]cyclic AMP shows tendency to be suppressed by PGE₁, PGE₁ seems to enhance the PTH-induced increase in the specific activity of cyclic AMP (Table 5 and Fig. 3). This observation cannot be explained by a lowered production rate of cyclic AMP but suggests an increased rate of decomposition as a cause of the apparent antagonistic action of PGE₁. It seems that the prevention of cyclic AMP release by PGE₁ keeps cyclic AMP within the cell and offers phosphodiesterase more substrate, resulting in an increase in the portion of cyclic AMP decomposed intracellularly by the enzyme. This notion is supported by the finding that the apparent antagonistic effect of PGE₁ is enhanced by omission of the phosphodiesterase inhibitor (Fig. 3). Another possibility, however, that PGE₁ stimulates phosphodiesterase *in situ* directly or indirectly cannot be excluded in the present study. It is possible that PGE₁ acting on the intact cell induces an increased intracellular calcium concentration (Villani *et al.*, 1974) resulting in the stimulation of phosphodiesterase through the mechanism as shown by Kakiuchi *et al.* (1975).

The physiological significance of the

PTH-stimulated release of cyclic AMP and the probable antagonistic action of PGE₁ on this process is unknown. These facts, however, indicate the need to consider such an extracellular release mechanism in the study on the cellular metabolism of cyclic AMP.

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