Evidence for Two Separate Adenyl Cyclase Systems Responding Independently to Parathyroid Hormone and β-Adrenergic Agents in the Renal Cortex of the Rat[1] (37266)

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(Introduced by Alvin Sellers)

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It has been shown that parathyroid hormone (PTH) stimulates the activity of adenyl cyclase of the renal cortex and increases the concentration of cyclic 3′,5′-adenosine monophosphate (cAMP) in renal cortical tubules (1, 2) and in the urine (3). Cyclic AMP and its dibutyryl derivative mimic the renal action of PTH (4) and it has been suggested that cAMP is an intracellular mediator of PTH action on the kidney (1–4).

The biological effects of catecholamines are probably also mediated by changes in cAMP concentration in the target tissues (5). Isoproterenol and norepinephrine stimulate adenyl cyclase activity in homogenates of renal cortex (1) and increase cAMP concentration in renal cortical slices (6). These actions are mediated through the β-adrenergic properties of these agents since the β-adrenergic blocker, propranolol, abolishes their effects (1, 6).

Melson, Chase, and Aurbach found that the effect of PTH on adenyl cyclase activity of the renal cortex is not abolished by phentolamine or propranolol (1); these data suggest that PTH acts through receptors which are different from those influenced by the catecholamines. It is not as yet known whether distinct receptors are attached to the same enzyme or if there are two discrete adenyl cyclase systems with separate regulatory and catalytic units responsive independently to PTH and catecholamines. The present study was undertaken to evaluate this question.

Methods. Assay of adenyl cyclase activity in homogenates of renal cortex. Wistar male rats, weighing 180–200 g, were killed by decapitation. The kidneys were rapidly removed and the cortex was separated by sharp dissection and washed with 0.25 *M* sucrose and 0.1 m*M* ethylene glycol-bis(β-amino ethyl ether)-*N*, *N*′-tetraacetic acid (EGTA). The renal cortex was then homogenized by hand in a glass homogenizer containing 50 m*M* Tris–HCl (pH 7.5), 1 m*M* EGTA, 5 m*M* $MgCl_2$, and 10 m*M* theophylline. Adenyl cyclase activity was assayed by the method of Marcus and Aurbach (7) with slight modification. The incubation media contained 40 m*M* Tris–HCl (pH 7.5), 4 m*M* $MgCl_2$, 0.05% bovine serum albumin, 15 m*M* KCl, 0.4 m*M* EGTA, 8 m*M* theophylline, and 1.5 m*M* ATP-8-^{14}C (sp act 20–40 μCi/μmole); 10 m*M* creatine phosphate and 0.5 mg/ml creatine phosphokinase, 75 units/mg (Sigma Chemical Co.), were used as ATP regenerating system. Homogenate of renal cortex containing approximately 0.8–1.1 mg protein is placed in the incubation media. The effects of PTH, catecholamines, adrenergic blocking agents or sodium flouride were evaluated by adding these agents to the incubation mixture to a total volume of 60 μl. Incubation was carried out at 37° in a metabolic shaker, and the reaction was terminated at 10 min by adding 100 μl of carrier solution containing ATP, 4×10^{-2}

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M, and cyclic AMP, 1.25×10^{-3} M, in 10 mM Tris–HCl (pH 7.5). Reaction mixtures were immediately placed in boiling water bath for 3 min; then 0.4 ml of deionized water was added and the mixture was centrifuged for 10 min at 1000g. Cyclic AMP in the supernate was separated by 0.5×3.5 cm column of Dowex 50W-X8, and the cyclic AMP fraction was treated twice with 0.2 ml each of 5% $ZnSO_4 \cdot 7H_2O$ and 0.3 N $Ba(OH)_2$ as described by Krishna, Weiss and Brodie (8). One milliliter portion of the resultant supernate was added to 10 ml Aquasol (New England Nuclear) and the radioactivity was measured in liquid scintillation spectrometer. The absorption at 260 nm of an aliquot of the supernatant fluid was determined in order to calculate the recovery of cyclic AMP. Appropriate reaction blanks were prepared. Results are expressed as net cyclic AMP formed per milligram protein in excess of the blank reaction. Protein concentration was determined by the method of Lowry *et al.* (9).

Results. Effects of PTH and catecholamines on adenyl cyclase activity. The effects of PTH, catecholamines with and without adrenergic blocking agents, and a combination of PTH and isoproterenol on adenyl cyclase activity of renal cortical homogenates are shown in Table I. The dose–response relationship between these agents and the enzyme activity (Figs. 1 and 2) were evaluated in an effort to find the doses that exert the maximum effects.

As previously reported (1), PTH, isoproterenol, and norepinephrine increased the activity of adenyl cyclase. Propranolol abolished the effects of isoproterenol and norepinephrine but not those of PTH on the enzyme activity. Phentolamine did not interfere with the stimulation of the enzyme by these agonists. Significant activation of adenyl cyclase was observed with PTH at a concentration of 1 μg/ml and the maximum effect occurred with 100 μg/ml. A dose–response relationship between isoproterenol or norepinephrine and adenyl cyclase activity

TABLE I. Effects of Parathyroid Hormone, Catecholamines and Adrenergic Blocking Agents on Adenyl Cyclase Activity of Rat Renal Cortex.[a]

Expt and addition	Adenyl cyclase activity (pmoles cyclic AMP formed/mg protein/10 min)
I. None	19.6 ± 1.6 (6)
Isoproterenol, 10^{-5} M	33.0 ± 1.0 (4)
Norepinephrine, 10^{-5} M	31.2 ± 1.2 (6)
Propranolol, 10^{-4} M	18.8 ± 1.0 (3)
Phentolamine, 10^{-4} M	18.2 ± 1.1 (3)
Isoproterenol + propranolol	20.2 ± 1.2 (3)
Norepinephrine + propranolol	19.1 ± 1.1 (3)
Isoproterenol + phentolamine	33.1 ± 1.0 (3)
Norepinephrine + phentolamine	31.6 ± 1.2 (6)
NaF, 10 mM	128.0 ± 5.0 (3)
II. None	19.4 ± 1.0 (6)
Parathyroid hormone, 10 μg/ml	37.1 ± 1.1 (6)
Parathyroid hormone	
+ propranolol, 10^{-4} M	38.5 ± 1.6 (6)
+ phentolamine, 10^{-4} M	38.5 ± 1.0 (6)
NaF, 10 mM	130.0 ± 5.0 (3)
III. None	18.3 ± 0.9 (3)
Parathyroid hormone, 100 μg/ml	74.1 ± 1.5 (5)
Isoproterenol, 5×10^{-4} M	31.6 ± 1.2 (5)
Parathyroid hormone + isoproterenol	86.4 ± 1.0 (5)
NaF, 10 mM	135.0 ± 4.0 (3)

[a] Results are the mean ± standard error. The number of determinations is in parentheses.

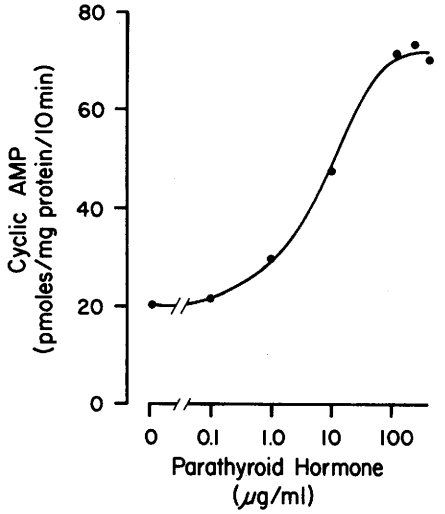

FIG. 1. A dose–response relationship between adenyl cyclase activity and parathyroid hormone concentrations. Each point represents a mean of triplicate determinations.

was also obtained. At equimolar concentrations, both isoproterenol and norepinephrine produced a similar degree of enzyme stimulation (Fig. 2). Maximal effects were observed with concentrations of 10^{-5} *M* for both isoproterenol or norepinephrine.

The combination of maximum doses of both PTH and isoproterenol resulted in an additive effect on the activity of adenyl cyclase (Table I). The amounts of cAMP formed above the basal levels were 55.8 pmoles/mg protein/10 min with PTH and 13.3 with isoproterenol. In the presence of both agonists the difference from basal levels was 68.1 pmoles/mg protein/10 min, a value approximately equal to the sum of cAMP produced by each agent alone. In Table II the details of five additional experiments are shown and in every study the effects of both agonists were additive.

Discussion. The results of the present study confirmed the previous observations that PTH and catecholamines stimulate adenyl cyclase activity of the renal cortex (1). The data also show that the receptors which respond to PTH or catecholamines are different since the beta blocking agent, propranolol, abolished the effects of isoproterenol and norepinephrine but not those of PTH.

Our data demonstrated that there is a dose–response relationship between isoproterenol or norepinephrine and adenyl cyclase activity. Melson, Chase and Aurbach (1) stated that they were unable to find a dose–response relationship, although the details of the data were not reported. The reasons for the discrepancy between our results and those of Melson, Chase and Aurbach are not clear, but may be related to the difference in the preparation of the cortical tissue for the enzyme assay between the two studies.

The finding of the present study that the effects of a combination of maximal doses of PTH and isoproterenol on adenyl cyclase activity are additive is consistent with the presence of two discrete adenyl cyclase systems in the renal cortex, and that these enzymes respond independently to the specific agonists. These two adenyl cyclase systems are probably located mainly in the cells of the renal tubule.

Available data indicate that PTH decreases the tubular reabsorption of phosphate, calcium and sodium in the proximal tubule (10, 11) and enhances calcium reabsorption in the distal nephron (11, 12). Parathyroid hormone augments the urinary excretion of cAMP and cAMP or its dibutyryl derivative mimics the renal action of PTH (3, 4). These observations strongly suggest that the renal action of PTH is mediated by adenyl cyclase-cyclic AMP system and that adenyl cyclase with receptors to PTH is present in

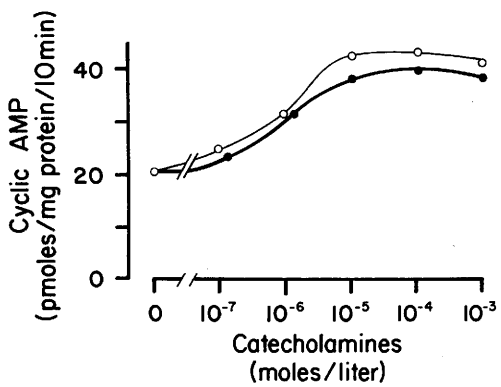

FIG. 2. A dose–response relationship between adenyl cyclase activity and isoproterenol (○—) or norepinephrine (●—). Each point represents a mean of triplicate determinations.

TABLE II. Additive Effects of Isoproterenol (ISO) and Parathyroid Hormone (PTH) on Adenyl Cyclase Activity.[a]

Expts	ISO	PTH	ISO + PTH	$\frac{\text{ISO alone + PTH alone}}{\text{(ISO + PTH) together}} \times 100$
1	13.3	55.8	68.1	101
2	12.9	50.7	61.5	103
3	12.3	41.4	53.1	101
4	9.5	41.6	56.4	91
5	14.5	42.4	60.7	94
6	11.2	47.7	54.4	108

[a] Values represent increment in cAMP formed over control (pmoles/mg protein/10 min). Values are the mean of 3–5 determinations.

the renal tubular cells. Isoproterenol has been shown to decrease proximal tubular reabsorption of sodium and this effect could also be produced by cAMP or its dibutyryl derivative (13, 14) suggesting that adenyl cyclase with beta adrenergic receptors is also located in the proximal tubular cell. In addition, catecholamines as well as cAMP stimulate renin synthesis in renal cortical tissue *in vitro* suggesting the presence of adenyl cyclase with beta adrenergic receptors in the juxtaglomerular apparatus as well (15).

The observation in the present study that the activation of adenyl cyclase with maximal doses of PTH was 3–4 times greater than that produced by maximal doses of isoproterenol may indicate that a smaller number of enzymatic units possess beta adrenergic receptors. It is also possible that these two separate enzymes are present in different cells and that the cell population containing the adenyl cyclase with beta adrenergic receptors is significantly smaller than the population having the enzymes with PTH receptors.

Summary. Parathyroid hormone (PTH), isoproterenol and norepinephrine stimulated adenyl cyclase activity of rat renal cortex. The effects of the latter two were abolished by propranolol but not by phentolamine. The effects of PTH were not influenced by these adrenergic blocking agents. Stimulation of the enzyme activity by a maximal dose of PTH was 3–4 times greater than that by a maximal dose of isoproterenol and the effects of both agonists were additive. These results indicate that in rat renal cortex there are two discrete adenyl cyclase systems responding independently to PTH and catecholamines, and the receptor for the latter agents being a beta adrenergic receptor.

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