Altered Activity of the Nucleotide Regulatory Site in the Parathyroid Hormone-Sensitive Adenylate Cyclase from the Renal Cortex of a Patient with Pseudohypoparathyroidism

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ABSTRACT A series of clinical studies suggest that the primary defect underlying pseudohypoparathyroidism is an abnormality of the parathyroid hormone-receptor-adenylate cyclase complex of the renal cortical cell plasma membrane. In the present study we compared parathyroid hormone-stimulated adenylate cyclase activity in membrane preparations from the renal cortex of three controls and a patient with pseudohypoparathyroidism. In the pseudohypoparathyroid preparation the $K_m$ for ATP was significantly greater and parathyroid hormone elicited markedly diminished adenylate cyclase activity at a subsaturating concentration of ATP. In contrast, the dose-response effect of enzyme activity to parathyroid hormone was the same in the control preparations, and that of the pseudohypoparathyroidism kidney, at a saturating concentration of ATP. The apparent alteration in enzyme kinetics, however, was normalized upon addition of guanosine 5'-triphosphate to the reaction mixtures. These results indicate that the defect in the parathyroid hormone-receptor-adenylate cyclase complex of the renal cell membranes, in our patient with pseudohypoparathyroidism, is an abnormal nucleotide receptor site of decreased activity. Such a defect may result in partial uncoupling of the parathyroid hormone receptor and adenylate cyclase, rendering the organ refractory to hormonal stimulation.

INTRODUCTION Pseudohypoparathyroidism (PsH) is a genetic disease characterized by target organ resistance to the action of parathyroid hormone (PTH). The failure of exogenously administered PTH to normally increase urinary or renal vein cyclic AMP (1), in patients with this disorder, suggests that the primary defect underlying this disease state is in the PTH-receptor-adenylate cyclase complex of the renal cortical cell plasma membrane. Nevertheless, Marcus et al. (2) have demonstrated that renal membrane adenylate cyclase activity was stimulated by PTH in a postmortem kidney preparation from a patient with presumed PsH. In these studies, however, no control preparations were available for comparison, and enzyme kinetics were not investigated. Moreover, a series of recent studies (3) indicates that the adenylate cyclase enzyme system is complex, containing at least three sites through which ligands interact and affect enzyme activity: the hormone receptor site, the nucleotide regulatory site (which reacts preferentially with guanosine 5'-triphosphate (GTP) or high concentrations of ATP, and the catalytic site (which reacts with Mg ATP$^{2-}$, the productive form of substrate). The abnormality in PsH, therefore, may be a result of (a) a deleted or abnormal PTH receptor, (b) absence of the adenylate cyclase enzyme, or (c) a defect in the enzyme system at the nucleotide or catalytic regulatory sites. In the present study we compared PTH-stimulated adenylate cyclase

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1Abbreviations used in this paper: GTP, guanosine 5'-triphosphate; PsH, pseudohypoparathyroidism; PTH, parathyroid hormone.
activity in renal cortical membranes prepared from kidney biopsies of three control patients and a patient with PsH. On the basis of these investigations we propose that the biochemical abnormality in our patient with PsH is the presence of an abnormal nucleotide regulatory site in the renal adenylate cyclase system.

METHODS

Open kidney biopsies from the patient with PsH and three patients with unrelated disorders (controls) were obtained under halothane anesthesia. The three controls required surgery for resection of a localized renal cell carcinoma, repair of a lacerated renal artery, and drainage of a renal abscess, respectively. The biopsies were obtained from normal kidney tissue. The patient with PsH required reimplantation of an ectopically located right ureter and the biopsy was obtained during this operation. In all instances portions of the biopsies were evaluated for pathology, and normal histology was observed. All procedures were approved by the Human Investigations Committee and performed with informed consent.

The kidney biopsies (1–1.5 g) were immediately placed in chilled 0.25 M sucrose, and the renal cortex was dissected. A particulate enzyme fraction was prepared from the cortical tissue of each kidney according to the methods of Marcus and associates (2, 4). Adenylate cyclase activity was measured in these preparations by a modification of the methods of Krishna et al. (5). Assays were performed in a volume of 50 μl containing Tris-HCl buffer, 30 mM (pH 7.4); MgCl₂, 10 mM; cyclic AMP, 0.1 mM; ATP, 1.5 mM (unless otherwise noted); [α-32P]ATP, 1–2 × 10⁵ cpm; phosphoenol pyruvate kinase, 40 μg/ml; and myokinase, 20 μg/ml. After incubation for 10 min at 37°C, the reaction was stopped by adding 1 ml of a solution containing cyclic [3H]AMP (15,000 cpm/ml), 100 μg ATP, and 50 μg cyclic AMP. The cyclic [3H]AMP formed was isolated by sequential chromatography as described by Salomon et al. (6). All results were corrected for product recovery (50–70%) on the basis of cyclic [3H]AMP and the results expressed after appropriate calculation of substrate specific activity, as picomoles of cyclic AMP formed per milligram of protein per 10 min. For all membrane preparations the adenylate cyclase reactions were linear over the course of the 10-min incubation (at ATP concentrations of 1.5 and 0.1 mM) and enzyme activity directly proportional to protein concentration (7) over the range (25–100 μg) used in the assays. Moreover, incubation of membrane preparations from the control kidneys and the PsH kidney for 10 min, in the presence of a reaction mixture containing 0.075 mM and 0.10 mM ATP, resulted in the preservation of 37.4±7.2 and 55.2±6.3% and 62.4±2.8 and 66.3±3.2% of the ATP, respectively (8). Thus, the expressed ATPase activity in the membrane preparations was equal.

PTH-stimulated adenylate cyclase activity was defined as the adenylate cyclase enzyme activity obtained when bovine PTH (1–84) was added to the incubation mixture. Data on control enzyme activity is expressed as the mean±SEM of two determinations on each of the three control preparations. The data on the PsH enzyme activity is the mean±SEM of three determinations. In individual experiments, involving either a control or PsH preparation, each data point is the mean of triplicate measurements.

Serum calcium was determined by atomic absorption spectrophotometry (normal 8.7–10.3 mg/dl). Serum creatinine was determined on the Multichannel Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.), and serum phosphorus by the colorimetric method of Dryer et al. (9) (normal 2.5–4.5 mg/dl). Urine specimens were stored at −20°C before analysis of phosphorus (10), creatinine (11), and cyclic AMP (12). The tubular maximum of phosphate reabsorption normalized to glomerular filtration was calculated by the method of Bijvoet (13) as previously described (14). Serum PTH was measured at the Mayo Medical Laboratories, Rochester, Minn., using a carboxy terminal specific assay employing GP,M antibody (15) (normal <40 μl eq/ml).

Materials. [α-32P]ATP and cyclic [3H]AMP were obtained from New England Nuclear Corp., Boston, Mass.; ATP (99–100%), cyclic AMP, phosphoenol pyruvate kinase, and myokinase were purchased from Sigma Chemical Co., St. Louis, Mo.; highly purified bovine PTH (1–84), 1,442 U/mg, was obtained from Inolex Corp. Park Forest South, Ill.; bovine parathyroid extract for parenteral use (in humans) of proved activity was provided by Eli, Lilly & Co., Indianapolis, Ind.

Case description. The patient was a 16-yr-old female who presented with the characteristic physical and chemical abnormalities of PsH. Multiple subcutaneous calcifications were noted over the distal phalanges bilaterally as well as over the heel surfaces. The metacarpals were uniformly short. Examination of the nails, hair, head, eyes, ears, nose, throat, chest, heart, and abdomen was normal and the neurologic examination gave normal results.

The serum calcium concentration was 7.8±0.2 mg/dl (20 determinations) and the serum phosphorus 5.1±0.3 (25 determinations). Serum PTH, on two occasions, was elevated at 90 and 130 μl eq/ml. The diagnosis of PsH was confirmed by demonstrating renal refractoriness to PTH: intravenous infusion of parathyroid extract of proven activity (200 U) on three occasions resulted in a maximum urinary cyclic AMP excretion of 3.9±1.5 μmol/g creatinine (normal 20–500) and a maximum decrease in tubular maximum of phosphate reabsorption normalized to glomerular filtration of 0.70±0.1 mg/dl (normal 0.95±0.33) (16).

RESULTS

In initial studies adenylate cyclase activity was readily detectable, at a saturating concentration of ATP (1.5 mM), in the three control preparations and that from the PsH kidney. The basal enzyme activity in these experiments averaged 186.2±30.1 pmol cyclic AMP formed/mg of protein per 10 min in the controls, whereas that in the PsH preparation was 144.3±32.4. Furthermore, PTH exhibited a dose-response effect on enzyme activity and the maximum activity, achieved upon stimulation with 50 μg/ml PTH, was 322.6±18.0 in the controls and 279.4±12.4 in the PsH preparation. These values, in both the basal and hormone-stimulated state, are not significantly different. Moreover, the characteristics of the dose response of enzyme activity to PTH were identical in the controls and PsH preparations. As illustrated in the representative experiment shown in Fig. 1, PTH stimulation resulted in a significant increase of adenylate cyclase activity at a concentration of 10 μg/ml, and the response was a linear function of the hormone concentration from 10–20 μg/ml in the preparations from both the PsH and Abnormal Adenylate Cyclase Activity in Pseudohypoparathyroidism 1223
control kidneys. Furthermore, the magnitude of the response was not significantly different at each concentration of PTH employed.

In contrast, in subsequent studies alteration in substrate concentration resulted in a significantly different response to PTH of the PsH membrane adenylyl cyclase activity compared to the controls. At a subsaturating concentration of ATP (0.1 mM) the maximum PTH-stimulated adenylyl cyclase activity (50 µg/ml PTH) was 113.3±3.8 in the controls, whereas that in the PsH preparation was significantly less, 57.7±4.9 (P < 0.05). In these studies the basal enzyme activity in the controls was 51.4±6.0, whereas that from the PsH kidney was 24.8±1.7 (P < 0.05). Furthermore, the alteration in PTH responsiveness was evident over the entire dose-response range for the hormone (Fig. 2). Although significant stimulation of adenylyl cyclase activity was initially detectable, in the PsH and control preparations at a concentration of 10 µg/ml PTH the magnitude of the response, in the PsH preparation, was decreased at each point on the dose-response curve.

The apparent abnormality of the PsH enzyme was substantiated by measuring adenylyl cyclase activity

![Figure 1](image1.png)

**Figure 1** Dose response of adenylyl cyclase activity to PTH in the PsH and a control preparation at a saturating concentration of ATP (1.5 mM). The data displayed are from a representative experiment and each measurement represents the mean of triplicate determinations. The adenylyl cyclase activity was measured as described in Methods. In this experiment the basal enzyme activity of control 1 was 136.7 ±15.0 and that of the PsH enzyme was 121.8±2.5.

![Figure 2](image2.png)

**Figure 2** Dose response of adenylyl cyclase activity to PTH in the PsH and a control preparation at a subsaturating concentration of ATP (0.1 mM). The data displayed are from a representative experiment and assays were performed as described in Methods with the exception that the ATP concentration in the reaction mixture was 0.1 mM. In this experiment the basal enzyme activity of control 1 was 48.4±3.5 and that of the PsH enzyme was 21.3±2.5.

![Figure 3](image3.png)

**Figure 3** Effect of substrate concentration on PTH-stimulated adenylyl cyclase activity in the control and PsH preparations. Assays were performed as described in Methods with the exception that the ATP concentration in the reaction mixture was varied over the range of 0.075–1.0 mM. In these experiments 50 µg/ml PTH (a maximum stimulating concentration) was added to the reaction mixtures. The results of the representative studies illustrated in this figure are shown as Lineweaver-Burke plots (1/ATP vs. 1/PTH-stimulated adenylyl cyclase activity [picomoles of cyclic AMP formed per milligram of protein per minute]) in which lines have been calculated by the method of least squares. Although two experiments were performed on each of the control and PsH preparations, single representative studies, in which the $K_m$ for ATP was determined, are shown. The apparent $K_m$ for controls 1, 2, and 3 averaged 0.20±0.035, 0.25±0.06, and 0.18±0.03, respectively, whereas that for the PsH preparation was 0.53±0.05. The apparent $K_m$ of the PsH preparation is significantly greater (P < 0.01) than the average apparent $K_m$ for the controls 0.21±0.027.
at varying concentrations of ATP (0.075–1.0 mM). Lineweaver-Burke plots were prepared from the data and the apparent $K_m$ for both control and PsH enzyme activity calculated (Fig. 3). In these studies the apparent $K_m$ for the control enzyme activity was 0.21 ±0.027, and that for the PsH preparation significantly elevated at 0.53±0.05 ($P < 0.01$).

The possible role of a defective nucleotide regulatory site in the genesis of the abnormal enzyme activity was investigated by comparing the PTH-stimulated adenylate cyclase activity, in the control and PsH preparations, in the absence and presence of GTP (Table I). In a reaction mixture containing 0.1 mM ATP, with or without 15 μg/ml PTH, diminished basal and PTH-stimulated activity of the PsH enzyme, in comparison to the controls, was again evident. Indeed the basal and PTH-stimulated enzyme activity, obtained in this series of studies, agrees favorably with that noted above and in Fig. 2. Moreover, addition of GTP (1 μM–0.1 mM) to the incubation mixture resulted in normalization of the responsiveness of the PsH enzyme. At each concentration of GTP the basal and PTH-stimulated enzyme activity, in the controls and PsH preparations, were not significantly different. Thus, whereas addition of GTP to the reaction mixture enhanced basal and PTH-stimulated adenylate cyclase activity in the controls ≥30%, an increase of >200% in PsH enzyme activity occurred.

### DISCUSSION

The data presented confirm the presence of a defect in the PTH-receptor-adenylate cyclase complex of the kidney membranes in our patient with PsH. Moreover, the results support the hypothesis that the biochemical abnormality in the enzyme complex is related to an abnormality of the nucleotide regulatory site. In a series of recent studies Rodbell et al. (3, 17) and Londos et al. (18) have established that purine nucleotides (other than ATP as substrate), acting at a nucleotide regulatory site, are required for hormonal stimulation of adenylate cyclase. Whether the nucleotide regulatory site is absolutely specific for guanine nucleotides remains uncertain. Although it has been observed that ATP apparently satisfies the requirement for nucleotide (3), the concentration of ATP required in this process is at least three orders of magnitude higher than that of GTP suggesting that the effect may be secondary to contamination of the ATP with guanine nucleotides. Therefore, in the present study demonstrable PTH-stimulated adenylate cyclase activity in the control and PsH preparations, in the absence of

### TABLE I

Effect of GTP on PTH-Stimulated Adenylate Cyclase Activity in the Control and PsH Enzyme Preparations

<table>
<thead>
<tr>
<th>GTP (μM)</th>
<th>Control 1</th>
<th>Control 2</th>
<th>Control 3</th>
<th>Controls (average)</th>
<th>Pseudohypoparathyroidism (PsH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80.4±5.5</td>
<td>72.0±4.5</td>
<td>70.3±3.5</td>
<td>74.2±3.2</td>
<td>30.4±2.7*</td>
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<tr>
<td></td>
<td>(48.5±4.9)</td>
<td>(42.3±4.0)</td>
<td>(49.3±4.7)</td>
<td>(46.7±2.2)</td>
<td>(20.0±1.6)*</td>
</tr>
<tr>
<td>1 μM</td>
<td>83.2±4.7</td>
<td>71.6±6.3</td>
<td>82.9±4.4</td>
<td>79.2±3.8</td>
<td>87.8±0.3</td>
</tr>
<tr>
<td></td>
<td>(50.3±3.8)</td>
<td>(53.2±4.2)</td>
<td>(56.4±4.7)</td>
<td>(53.3±1.8)</td>
<td>(55.4±4.2)</td>
</tr>
<tr>
<td>10 μM</td>
<td>111.5±6.8</td>
<td>106.7±5.3</td>
<td>95.7±6.0</td>
<td>104.6±4.7</td>
<td>101.1±3.1</td>
</tr>
<tr>
<td></td>
<td>(69.0±3.2)</td>
<td>(72.0±4.3)</td>
<td>(66.3±3.9)</td>
<td>(69.1±1.7)</td>
<td>(62.4±4.2)</td>
</tr>
<tr>
<td>0.1 mM</td>
<td>92.9±8.4</td>
<td>84.0±7.6</td>
<td>103.3±5.8</td>
<td>93.4±5.6</td>
<td>86.9±1.2</td>
</tr>
<tr>
<td></td>
<td>(64.8±5.6)</td>
<td>(60.5±6.2)</td>
<td>(53.2±4.9)</td>
<td>(59.5±3.4)</td>
<td>(54.3±3.7)</td>
</tr>
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</table>

Control and PsH membranes were incubated in reaction mixtures containing 0.1 mM ATP with or without PTH (15 μg/ml). Where indicated, GTP (1 μM–0.1 mM) was added to the incubation mixture. Both PTH-stimulated and basal activity (in parentheses) are shown. In each individual control the data represent the mean±SEM of two experiments and the PsH data the mean±SEM of three experiments. In each experiment the data points were the mean of triplicate determinations. The controls (average) data is the mean±SEM of the pooled control data and thus represents the results of six experiments. The adenylate cyclase activity in the PsH preparation, in the absence of GTP, is significantly less than that in the controls (average). In contrast, the adenylate cyclase activity in the PsH preparation is not significantly different from the controls (average) when GTP is present in the reaction mixture.

* $P < 0.05$. 

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added GTP, suggests that ATP (or GTP contaminating the ATP preparation) can subserve the role of GTP at the nucleotide regulatory site in the renal membranes. However, comparison of PTH effects on control and PsH adenylate cyclase activity reveals different patterns of response at saturating and substracting concentrations of ATP. Although there was no observable difference in PTH responsiveness between the preparations at an ATP concentration of 1.5 mM, PTH elicited a significantly decreased response to adenylate cyclase activity in the PsH preparation at an ATP concentration of 0.1 mM. In addition, consistent with this observation, the calculated apparent Kₐ for ATP of the PTH-stimulated adenylate cyclase of the PsH preparation is ≈2.5-fold greater than that of the controls. Moreover, addition of GTP to the reaction mixture restores normal responsiveness to the PsH enzyme system. The normal activity of the PsH enzyme system at a saturating concentration of ATP and the restoration of normal responsiveness, upon addition of GTP, are consistent with the hypothesis that the nucleotide receptor site is defective, requiring greater concentrations of nucleotide (than the controls) to effect maximal activation of enzyme activity. Such a defect may result in "partial uncoupling" of the PTH receptor and adenylate cyclase, thereby rendering the organ refractory to hormone stimulation.

Our results are compatible with previously reported data. The calculated Kₐ for ATP of the control preparations (0.21±0.027 mM) agrees favorably with values previously reported for the enzyme in mammalian kidney (0.1–0.2 mM) (19, 20) and that from a variety of normal tissues (0.08–0.25 mM) (21, 23) as well. In addition, the limited effect of GTP on PTH enhancement of adenylate cyclase activity in the control preparations is consistent with previous observations made in mammalian kidney (24, 25) and bone (25) where GTP likewise had only a small potentiating effect on PTH stimulation. Thus, the apparent abnormalities of the PsH enzyme, marked by a Kₐ for ATP of 0.53±0.05, and an inordinate enhancement of PTH-stimulated adenylate cyclase activity by GTP, are not only significantly different from the controls in this study, but are in sharp contrast with previous observations in other normal tissues. Moreover, our results are consistent with the previous observations of Marcus and Aurbach (4) and, in addition, provide explanation of the apparently enigmatic demonstration of PTH-stimulated adenylate cyclase activity (at saturating concentrations of ATP) in their PsH kidney preparation.

Thus, our data indicate that the defect in the renal cortical membranes of our patient with PsH is an abnormal nucleotide regulatory unit in the adenylate cyclase complex. The results also exclude other possible abnormalities in the membrane complex, such as absence of the PTH receptor or the adenylate cyclase itself (the adenylate cyclase response is normal at saturating concentrations of ATP). Nevertheless, although we consider it unlikely, the possibility that the abnormalities observed are secondary to a relative deficiency of "contaminating" GTP or increased GTPase activity in the PsH membrane preparation cannot be totally excluded. In any case, the relationship of the demonstrated enzyme abnormality in vitro to the expressed defect characteristic of PsH (a blunted increase in PTH-stimulated urinary cyclic AMP excretion) remains to be determined. The reported tissue levels of ATP are sufficient to saturate the PsH enzyme thus negating the apparent defect. However, the possibility that ATP itself does not activate the nucleotide regulatory site (i.e., guanine nucleotide contaminants subserve this role) suggests that the intracellular concentration of GTP, which is several fold less than ATP, may be central to the expression of the abnormality. Moreover, the presence of a high tissue level of ATP cannot be extrapolated to approximate the cytosolic concentration of this nucleotide because ATP is compartmentalized with extremely high amounts in mitochondria (26). Nevertheless, further study of the proposed defect in the renal cortical membrane of patients with PsH, exploration of the possible heterogeneity of the defect, and correlation with the physiological abnormality await the availability of additional affected kidney tissue.

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