Renal Parathyroid Hormone-dependent Adenylate Cyclase in Vitamin D-deficient Rats

INHIBITION BY HYDROXYLATED VITAMIN D3 METABOLITES*

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The adenylate cyclase activity by bovine synthetic parathyroid hormone (bPTH) (1-34) was studied in vitro in kidney plasma membranes from D-deficient (D-Mb) or normal (D-Mb) rats. In D-Mb, the apparent affinity of parathyroid hormone (PTH) for membranes (170 ± 30 nm) was significantly higher than that measured in D-Mb (55 ± 5 nm). The maximum velocity of the PTH-stimulated adenylate cyclase was significantly higher in D-Mb than in D-Mb (163.0 ± 13.7) and 93.4 ± 6.7 pmol of cAMP/mg of protein/min, respectively. The stimulation of adenylate cyclase by PTH was then studied in vitro in D-Mb and D-Mb. In D-Mb, 25-hydroxyvitamin D3, 24,25-, and 1, 25-di hydroxyvitamin D3 significantly inhibited cAMP production in the presence of 0.87 μM of bPTH. Vitamin D3 had no effect. Maximal inhibition (86%) was observed for 25-hydroxyvitamin D3. 1,25-Dihydroxyvitamin D3 decreased the maximum velocity of PTH-stimulated adenylate cyclase but did not modify the bPTH apparent affinity for D-Mb. The vitamin D3 metabolites tested did not modify the cyclase stimulation by isoproterenol, sodium fluoride, or 5′-guanylylimidodiphosphate. The presence of 1,25-dihydroxyvitamin D3 or 25-hydroxyvitamin D3 did not increase the (NaK)-ATPase or the phosphodiesterase activities. In the presence of 1,25-dihydroxyvitamin D3 and bPTH, the apparent affinity of ATP for the catalytic moiety was not modified. The maximum velocity was decreased. These results suggest an in vitro interaction between hydroxylated vitamin D metabolites and kidney membranes PTH receptor.

The effects of vitamin D1 or its metabolites on renal phosphate elimination are not well defined. At a low dose, 25(OH)D3,2 the hepatic metabolite of vitamin D3, has no effect on urinary phosphorus excretion in either vitamin D-deficient rats (1) or normal (2) thryoparathyroidectomized rats, whereas it lowers phosphaturia when perfused at a higher concentration (2-4). On the other hand, physiological amounts of 1,25(OH)2D3, the hormonal form of vitamin D3, correct the decrease in urinary phosphorus excretion following thryoparathyroidectomy in rats (5, 6), 1,25(OH)2D3 may control blood phosphorus homeostasis by correcting both hypophosphatemia and hyperphosphatemia in growing rats (7). The relationship between vitamin D metabolites and parathyroid hormone (PTH) at the renal level is unclear; this could explain why the renal effects of vitamin D metabolites are not well defined. In thryoparathyroidectomized rats, 25(OH)D3 lowers phosphaturia when perfused in the presence of small amounts of PTH (1, 2, 8). Popovtzer and Robinette (8) suggested that 25(OH)D3 decreases the PTH-dependent renal cAMP, thus resulting in decreased renal phosphorus excretion.

With these data in mind, we looked for a possible direct in vitro interaction between vitamin D3 metabolites and the PTH-dependent renal cortical adenylate cyclase prepared from either normal or vitamin D-deficient rats.

**METHODS AND MATERIALS**

**Chemicals—**Synthetic bovine PTH (1-34) (bPTH) was purchased from Beckman (Bioproducts, Geneva, Switzerland) (Batch No. D 0414; specific activity, 3820 international units/mg). Crystalline 25(OH)D3 and vitamin D3 were given by Roussel Uclaf Laboratories (Paris). Crystalline 1,25(OH)2D3 and 24,25(OH)2D3 were provided by Dr. Uskokovic (Hoffmann La Roche, Nutley, N.J.). They were not repurified before use. Other reagents were reagent grade and were purchased from local sources.

**Animals—**Male weaning C-D Cobs rats were obtained from Charles River Breeding Laboratories (Rouen, France) and immediately placed on either a normal or a vitamin D-deficient diet (Centre National de la Recherche Scientifique, La Miniere, France) for 4 weeks. Plasma calcium in vitamin D-deficient rats at the time of experiments was 1.4 ± 0.2 mmol/liter (mean ± S.E., n = 12), versus 2.6 ± 0.4 mmol/liter (mean ± S.E., n = 4) in normal rats.

**Plasma Membrane Preparation—**A subcellular fraction enriched in plasma membranes was prepared from the renal cortex from either normal rats (D-Mb) or vitamin D-deficient rats (D-Mb) according to the procedure of Heidrich et al. (9), omitting the free-flow electrophoresis step. The plasma membranes obtained were suspended in a buffer containing Tris (50 mm), dimethylsulfoxide (10% v/v), pH 7.4, and stored in small portions (protein concentration, 5 to 10 mg/ml) in liquid nitrogen until used.

**Adenylate Cyclase Assay—**The incubation medium for plasmic membrane adenylate cyclase activity contained Tris-HCl (50 mm, pH 7.4), MgCl2 (6.5 mm), theophylline (9 mm), KCl (30 mm), EDTA (1 mm), ATP (2.5 mm), phosphocreatine (25 mm), creatinine kinase (2 mg/ml), bovine serum albumin (0.1%), and plasma membranes (25 to 50 μg/tube). When necessary, NaF (10 mm), PTH, isoproterenol (0.1 μmol/liter), Gpp(NH)p (0.1 mm), cholecalciferol metabolites, or vehicle (ethanol) were added. In order to avoid their absorption along tube walls, the cholecalciferol metabolites were dissolved in ethanol (final concentration, 1%). In preliminary experiments, such an amount of
ethanol was shown not to modify the adenylate cyclase activity. The final incubation volume was always 70 μl. The tubes were incubated at 37°C for 15 min and the reaction was stopped by boiling for 3 min. The cAMP produced was measured according to the method of Gilman (10). In preliminary experiments, ATP at concentrations of up to 8 mM was shown not to interfere with the assay.

Additional Procedures—The protein content of plasma membranes was determined by the method of Lowry et al. (11) with bovine serum albumin as standard. Phosphodiesterase activity in the presence of cAMP (1 μM) was measured according to the method of Thompson and Appleman (12). (Na-K)ATPase activity was measured by colorimetric assay of P, release from ATP (13). The reaction medium (0.4 ml) contained Tris-HCl (40 mM, pH 7.4), NaCl (0.1 M), KCl (10 mM), MgCl₂ (5 mM), ATP (4 mM), and 0.15 to 0.2 mg of membrane protein per tube. Incubation time was 10 min at 30°C.

Statistical analysis was performed using Student’s paired t test. Adenylate cyclase activity was expressed as picomoles of cAMP released/min/mg of membrane protein (pmol mg⁻¹ min⁻¹). Phosphodiesterase activity was expressed as picomoles of cAMP hydrolyzed/min/mg of membrane protein. (Na-K)-ATPase activity was expressed as nanomoles of inorganic phosphorus produced/min/mg of membrane protein.

RESULTS

The dose-response curves in the presence of increasing amounts of PTH of the PTH-dependent adenylate cyclase from normal and vitamin D-deficient rat kidney are shown in Fig. 1. The double reciprocal plots from the experimental data yielded an apparent Kₐ of bPTH for D'Mb adenylate cyclase of 55 ± 5 nM (mean ± S.E., n = 4) that was significantly different (p < 0.001) from the apparent Kₐ obtained in D Mb (170 ± 30 nM). The Vₐ max obtained in D'Mb (163.6 ± 13.7 pmol mg⁻¹ min⁻¹) was significantly higher (p < 0.001) than the Vₐ max obtained in D'Mb (93.4 ± 6.7 pmol mg⁻¹ min⁻¹). The basal and NaF-stimulated adenylate cyclase activities were not significantly different in D Mb and D'Mb.

D'Mb or D'Mb were exposed to various concentrations of 25(OH)D₃ in the absence or in the presence of 0.87 μM bPTH (submaximal concentration). In D'Mb, the bPTH-stimulated adenylate cyclase activity was significantly decreased in the presence of 10 μM or more of 25(OH)D₃ (Fig. 2). Maximal inhibition was noted when the concentration in incubation medium was 100 pm. 25(OH)D₃ had no inhibitory effect in D'Mb. Basal cyclase activity was not modified by the presence of 25(OH)D₃ in either D'Mb or D'Mb. Similarly, the presence of 10 μM or higher concentrations of 1,25(OH)D₃ or 24,25(OH)₂D₃ decreased the adenylate cyclase stimulation by bPTH (0.87 μM) in D'Mb but not in D'Mb (Fig. 3). A significant decrease in adenylate cyclase activity was observed with dihydroxyvitamin D₃ metabolites at concentrations 1 order of magnitude lower than that of 25(OH)D₃. The maximal adenylate cyclase inhibition (86%) was noted in the presence of 1,25(OH)₂D₃ (100 μM) and was significantly greater (p < 0.001) than the maximal inhibition in the presence of 24,25(OH)₂D₃ (56%) or of 25(OH)D₃ (62%). As shown in Fig. 4, in D'Mb, 1,25(OH)₂D₃ (50 pm) decreased the bPTH-stimulated adenylate cyclase Vₐ max from 90.2 ± 1.8 (mean ± S.E., n = 4) to 25.7 ± 0.7 pmol mg⁻¹ min⁻¹ (p < 0.001) but did not modify the apparent Kₐ. Vitamin D₃ (6.1 pm to 10 nm) had no effect on either D'Mb or D'Mb (Fig. 3).

Incubation of D'Mb with 1,25(OH)₂D₃ for 10 min at 37°C prior to incubation with bPTH (0.87 μM) did not result in a stronger cyclase inhibition.

In order to test whether or not inhibition in the presence of 1,25(OH)₂D₃ is reversible, in some experiments, D'Mb were removed from incubation medium containing 1,25(OH)₂D₃ (50 pm) by centrifugation for 2 min at 2,000 × g. They were then reincubated in a 1,25(OH)₂D₃-free medium. In these membranes, the bPTH-stimulated adenylate cyclase was similar to that of membranes incubated with ethanol.

The effect of cholecalciferol metabolites (10 μM or 1 nm) on the adenylate cyclase stimulation by other effectors than bPTH was measured. None of these metabolites inhibited the cyclase stimulation by NaF (10 mM), Gpp(NH)p (0.1 mM) or isoproterenol (0.1 mM) in either D'Mb or D'Mb.

In D'Mb incubated with bPTH (0.87 μM) together with increasing amounts of ATP (up to 8 mM), the presence of 1,25(OH)₂D₃ (50 pm) decreased the adenylate cyclase Vₐ max from 90.8 to 31.0 pmol mg⁻¹ min⁻¹. The Kₐ for ATP (0.2 mm) was not modified.

The cAMP-dependent phosphodiesterase activity was determined in the absence or in the presence of 1,25(OH)₂D₃ or
**PTH-dependent Cyclase Inhibition by Vitamin D Metabolites**

*Effect of 1,25(OH)_{2}D_{3} and 25(OH)D_{3} (100 pM) on the phosphodiesterase and (Na-K)-ATPase activities from normal and vitamin D-deficient rat kidney plasma membranes*

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<thead>
<tr>
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<th>Phosphodiesterase activity</th>
<th>(Na-K)-ATPase activity</th>
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<tbody>
<tr>
<td>D(^{-})*Mb</td>
<td></td>
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<tr>
<td>Control*</td>
<td>(62.6 \pm 6.2)(\mu)M</td>
<td>(1.40 \pm 0.14)</td>
</tr>
<tr>
<td>1,25(OH)<em>{2}D</em>{3}</td>
<td>(64.6 \pm 3.8)(\mu)M</td>
<td>(1.38 \pm 0.13)</td>
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<tr>
<td>25(OH)D_{3}</td>
<td>(59.3 \pm 4.1)(\mu)M</td>
<td>(1.43 \pm 0.15)</td>
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*Expressed as picomoles of cAMP hydrolyzed/mg of protein/min (initial CAMP concentration, 1 \(\mu\)M).

<table>
<thead>
<tr>
<th></th>
<th>D(^{-})*Mb</th>
<th>D(^{-})*Mb</th>
<th>D(^{-})*Mb</th>
<th>D(^{-})*Mb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(55.3 \pm 0.5) (\mu)M</td>
<td>(1.83 \pm 0.20)</td>
<td>(1.40 \pm 0.14)</td>
<td>(1.32 \pm 0.15)</td>
</tr>
<tr>
<td>1,25(OH)<em>{2}D</em>{3}</td>
<td>(57.0 \pm 2.9) (\mu)M</td>
<td>(1.38 \pm 0.13)</td>
<td>(1.43 \pm 0.15)</td>
<td>(1.42 \pm 0.13)</td>
</tr>
<tr>
<td>25(OH)D_{3}</td>
<td>(50.2 \pm 1.7) (\mu)M</td>
<td>(1.32 \pm 0.15)</td>
<td>(1.42 \pm 0.13)</td>
<td>(1.42 \pm 0.13)</td>
</tr>
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</table>

*Expressed as nanomoles of inorganic phosphorus produced/mg of protein/min.

*Control values obtained in the presence of ethanol (final concentration, 1%).

*Mean \(\pm\) S.E. of three experiments.

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**DISCUSSION**

These in vitro experiments demonstrate that the maximal velocity of renal cortical plasma membrane PTH-dependent adenylate cyclase is lower in vitamin D-deficient than in normal rats. Similarly, the PTH apparent affinity for kidney membranes is lower in the vitamin D-deficient animals. It should be noted that such an affinity could be overestimated in both vitamin D-deficient and normal rats, since the bPTH used in these experiments is slightly contaminated with other peptides. The basal and fluoride-stimulated cyclase activities are similar in the kidney membranes prepared from normal or vitamin D-deficient rats, respectively. These results are in good agreement with those published by Forte et al. (14, 15) and Kakuta et al. (16). The reasons why kidney PTH-dependent adenylate cyclase kinetics differs in normal and vitamin D-deficient rats are unclear. In the latter animals, higher circulating PTH level secondary to vitamin D deficiency could explain these differences. As a matter of fact, high plasma levels of other polypeptides, such as insulin or growth hormone, decrease the number of their cellular receptors (17, 18). However, in the case of PTH, this hypothesis needs to be demonstrated further since elevated levels of other hormones such as glucagon or vasopressin increase the responsiveness of their specific adenylate cyclase in liver or kidney, respectively (19, 20). Another possibility is that chronic vitamin D deficiency explains per se the different adenylate cyclase response to PTH between normal and vitamin D-deficient rats, since vitamin D is known to modify the composition of certain plasma membranes (21, 22). Finally, this difference could be due to chronic calcium depletion as suggested by Kakuta et al. (16).

Our results demonstrate that in vitamin D-deficient rats, the adenylate cyclase stimulation by bPTH (0.87 \(\mu\)M) is dramatically decreased in the presence of 25(OH)D_{3} (100 pM), 1,25(OH)_{2}D_{3} (10 pm), or, 24,25(OH)_{2}D_{3} (10 pm). The maximal inhibition (86%) is obtained with 1,25(OH)_{2}D_{3} (100 pm) and is reversible. This inhibition by hydroxylated vitamin D metabolites appears to be specific for PTH, since these metabolites did not modify the adenylate cyclase stimulation by NaF, Gpp(NH)p, or isoproterenol. In addition, 1,25(OH)_{2}D_{3} did not activate the membrane cAMP-dependent phosphodiesterase or the (Na-K)-dependent ATPase. The ATP affinity for the adenylate cyclase catalytic moiety was not modified by the
interaction was not demonstrable in normal rats. The presence of 1,25(OH)2D3. Taken together, these results suggest that vitamin D metabolites, as well as other steroid hormones (26, 27), may have membrane effects that are independent of a nuclear mechanism. A modification of the PTH-dependent adenylate cyclase activity could be the result of such a membrane effect of vitamin D metabolites. As a matter of fact, in kidney slices from parathyroidectomized rats, Popovtzer and Wald (28) have demonstrated that 25(OH)D3 inhibits the PTH-dependent adenylate cyclase activity. In cultured chick renal cortical cells, Henry (29) has observed a modulation of the cAMP production in response to PTH by 1,25(OH)2D3. Data obtained in vitro also suggest an interaction between the PTH-mediated renal adenylate cyclase and vitamin D metabolites. In thyroparathyroidectomized rats, 25(OH)D3 decreases the urinary cAMP elimination in response to PTH infusion (8). In man (30), oral administration of 1,25(OH)2D3 results in a decrease of urinary cAMP production in response to PTH stimulation (31, 32). Results obtained in various tissues (21, 22, 25) suggest that vitamin D metabolites, as well as other steroid hormones, modify adenylate cyclase activity (35, 36).

Our results provide direct in vitro evidence for such an interaction at the kidney membrane level. The interaction between vitamin D hydroxylated metabolites and PTH membrane receptor could be related to the modification in phosphorus elimination in response to these metabolites. PTH exerts its phosphaturic effect via the renal cAMP production (31). The antiphosphaturic action of 25(OH)D3 given to thyroparathyroidectomized rats infused withPTH (8) could result from the inhibition of the PTH-dependent adenylate cyclase activity that is herein demonstrated. The antiphosphaturic effect of 1,25(OH)2D3 in normal rats (32) or in the presence of PTH could be the result of the same mechanism. However, in the absence of PTH (33) or in normal rats (34), a phosphaturic effect of 1,25(OH)2D3 has been reported. This last effect could be the result of a different mechanism, independent of the PTH-vitamin D interaction at the membrane level. Another possible consequence of this PTH-vitamin D interaction could be a modification of the 1α-hydroxylase responsiveness to PTH, since 1α-hydroxylase stimulation by PTH seems to involve renal adenylate cyclase activation (35, 36).

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REFERENCES