Insulin modulates the stimulation of renal 1,25-dihydroxyvitamin D₃ production by parathyroid hormone

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Abstract. Previous studies have shown that there is an impairment in renal production of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), the major biologically active metabolite of vitamin D₃, in diabetes. This impairment is not due to a deficiency in the parathyroid hormone (PTH), a major stimulator of renal 1,25(OH)₂D₃ production. Therefore, we have investigated the capacity of PTH to stimulate 1,25(OH)₂D₃ production in insulin deficiency and with insulin replacement. Experiments were performed in rats fed a 0.6% calcium, vitamin D sufficient diet for 2 weeks. Thyroparathyroidectomy was performed on all rats. Rats to be rendered diabetic were injected with streptozotocin immediately after surgery. In non-diabetic rats, PTH administration significantly increased renal 1,25(OH)₂D₃ production (11 ± 2 vs 46 ± 5 pg/min/g; P < 0.05). In diabetic rats, however, PTH caused only a modest increase in 1,25(OH)₂D₃ production (11 ± 1 vs 19 ± 4 pg/min/g; P < 0.05). With insulin replacement, PTH stimulation of 1,25(OH)₂D₃ production was markedly increased over that seen in diabetic rats (48 ± 12 vs 19 ± 4 pg/min/g; P < 0.05). PTH was equally effective in raising serum calcium, depressing serum phosphorus and tubular reabsorption of phosphate in non-diabetic as well as in diabetic rats. These results demonstrate that insulin is necessary for the maximal stimulation of renal 1,25(OH)₂D₃ production by PTH. However, insulin is not necessary for PTH action in terms of renal handling of phosphate and inducing hypercalcaemia. These results suggest multiple pathways for the action of PTH, only some of which are insulin requiring.

Previous studies have shown that plasma levels of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), the biologically active form of vitamin D₃, are low in diabetic rats (Schneider et al. 1977; Horst et al. 1979; Hough et al. 1982). The catabolism of 1,25(OH)₂D₃ in diabetic rats is not accelerated (Spencer et al. 1980) suggesting a decrease in the production rate. Recently, we have found that there is an impairment in renal production of 1,25(OH)₂D₃ in diabetes and this impairment is corrected by insulin treatment (Wongsurawat et al. 1983).

One of the major regulators of the renal production of 1,25(OH)₂D₃ is the parathyroid hormone (PTH) (Garabedian et al. 1972; Henry et al. 1974). Thyroparathyroidectomy has been shown to markedly reduce renal 1,25(OH)₂D₃ production (Garabedian et al. 1972). The administration of parathyroid extract to thyroparathyroidectomized (TPTX) rats significantly increases 1,25(OH)₂D₃ production. In diabetes, impairment of 1,25(OH)₂D₃ production is unlikely to be due to PTH deficiency since serum PTH levels are elevated compared with the control (Schneider et al. 1974). However, presence of insulin seems to be an important factor for PTH stimulation of renal 1,25(OH)₂D₃ production (Henry 1981).

The purpose of this report was to compare the effects of PTH on renal production of 1,25(OH)₂D₃ in diabetic and non-diabetic rats. The effects of PTH in diabetic rats with and without insulin treatment were also compared. The effects of PTH on serum calcium, phosphorus and tu-
bular reabsorption of phosphate (TRP) were measured to determine the extent of impairment of PTH action in diabetes.

Material and Methods

Male F344 rats 4 weeks of age (Charles River Breeding Laboratories, Wilmington, MA, USA) were fed a diet containing 3 IU vitamin D/g diet, 0.6% calcium and 0.6% phosphorus and deionized water ad libitum. After 2 weeks on the diet, all rats were thyroparathyroidectomized to remove endogenous PTH. Surgery was performed under sodium pentobarbital anaesthesia. Parathyroid glands were identified and removed under a dissecting stereomicroscope and most of thyroid glands were then electrocauterized. To reduce the possibility of incomplete parathyroidectomy, only rats with both parathyroid glands identifiable were used. Rats, successfully thyro-parathyroidectomized, demonstrated a significant drop in serum calcium 18 h after surgery. On the fifth post-operative day PTH administration was initiated to half of the rats by sc injection (trichloroacetic acid extract from bovine parathyroid glands, Sigma Chemical Co., St. Louis, MO, USA, dissolved in a solution of 1 mM acetic acid, 16 µl/ml glycerol, and 0.25 µl/ml phenol, pH 4.0). Rats were dosed with 0.33 U PTH/g body weight in 0.1 ml solution at 48 h, 24 h, and 2 h prior to sacrifice. The other half of the rats (control group) were injected with vehicle only. Rats were fed up to the time of sacrifice which was about 10.00 a.m.

In some experiments, rats were rendered diabetic immediately following thyroparathyroidectomy by a single ip injection of 65 mg/kg body weight streptozotocin (Sigma Chemical Co., St. Louis, MO, USA) freshly dissolved in citrate buffer, pH 4.5). Criteria for the establishment of diabetes were polyuria, glycosuria and hyperglycaemia. The rats were then divided into three treatment groups. The first group received PTH injection at 48 h, 24 h and 2 h prior to sacrifice. The second group received vehicle injections only. The third group was given daily sc injections of 3 U NHP insulin at 08.00 a.m. daily for 3 days before sacrifice. This group of insulin-treated rats also received PTH injections. The dosage and schedule of PTH injections were the same as described above.

One day before the animals were killed, urine was collected over a 24 h period for the measurement of volume, creatinine, glucose and phosphorus. At the time of sacrifice, rats were anaesthetized with diethyl ether, and the abdominal cavity was exposed. Blood was withdrawn from the inferior vena cava for the measurement of calcium, phosphorus, creatinine and glucose.

The renal production of 1,25(OH)2D3 was measured in vitro using isolated renal cortical slices as previously described (Armbrcht et al. 1981a) with some modification. To increase sensitivity, unlabelled 25-hydroxyvitamin D3 (25OHD3) was used as substrate and a competitive binding assay was used to detect 1,25(OH)2D3 (Gray & Napoli 1983). Briefly, kidney was removed from the rat, bisected in the sagittal plane and cortical slices (50–75 mg/slice) were prepared using a Stadie-Riggs microtome. Slices were weighed and placed in plastic vials (two slices per vial) containing 1 ml Krebs-Ringer bicarbonate buffer (pH 7.4). The vials were gassed with 95% O2/5% CO2 for 1 min and stoppered. The buffer contained 5 µM unlabelled 25OHD3 (a gift from Dr. J. C. Babcock, Upjohn Co., Kalamazoo, MI, USA). The buffer concentration of calcium was 2.5 mmol/l and the phosphorus concentration was 1.2 mmol/l.

After 1 h of incubation at 37°C in a shaking water bath, slices were extracted. [3H]1,25(OH)2D3 (approximately 5000 CPM, 92 Ci/mmol) in 25 µl of 95% ethanol was added to each extract to monitor recovery of the product. Samples were extracted using acetonitrile, and the 1,25(OH)2D3 was partially purified using C-18 Sep-Pak followed by silica Sep-Pak (Water Associates, Inc., Milford, MA, USA) as previously described (Reinhardt et al. 1984). The final fraction containing 1,25(OH)2D3 was dried under a stream of nitrogen and redissolved in ethanol. In some experiments, the final fraction was analyzed for products of 25OHD3 metabolism using high pressure liquid chromatography. Chromatography on a Zorbax Sil column (0.6 x 25 cm) equilibrated with methylene chloride: isopropanol (95:5) demonstrated that greater than 90% of the product in this fraction co-migrated as a single peak with authentic 1,25(OH)2D3. This solvent system separates out the major products of 25OHD3 metabolism by mammalian cells, including 19-nor-10-keto-25-hydroxyvitamin D3 (Lester et al. 1984).

The 1,25(OH)2D3 in the collected fraction was measured using radioligand assay modified from Eisman et al. (1976). Test sample, binding protein, and [3H]1,25(OH)2D3 were equilibrated together for 10 min at 0°C and 60 min at 25°C. Unbound [3H]1,25(OH)2D3 was separated out from bound label using dextran-coated charcoal. Binding protein was obtained from duodena of male New Zealand white rabbits extract (Duncan et al. 1983). This assay could detect as little as 5 pg of 1,25(OH)2D3 and was linear between 5 and 50 pg using a Logit transformation. The binding protein was specific for 1,25(OH)2D3 as opposed to 25OHD3, vitamin D3 and 24,25(OH)2D3. Using this sensitive binding assay allows the measurement of 1,25(OH)2D3 production in animals fed diets containing adequate amounts of calcium and vitamin D.

Results

The effects of thyroparathyroidectomy and subsequent administration of PTH were examined first in non-diabetic TPTX rats (Table I). As shown
Table 1.
Effects of PTH in TPTX non-diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PTH</th>
</tr>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>initial</td>
<td>82 ± 5</td>
<td>89 ± 7</td>
</tr>
<tr>
<td>final</td>
<td>109 ± 3</td>
<td>113 ± 5</td>
</tr>
<tr>
<td>Serum (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose</td>
<td>97 ± 8</td>
<td>89 ± 7</td>
</tr>
<tr>
<td>calcium</td>
<td>5.3 ± 0.1</td>
<td>11.1 ± 0.5*</td>
</tr>
<tr>
<td>phosphorus</td>
<td>13.1 ± 0.5</td>
<td>7.8 ± 0.2*</td>
</tr>
<tr>
<td>TRP (%)</td>
<td>84 ± 3</td>
<td>71 ± 4*</td>
</tr>
<tr>
<td>1,25(OH)₂D₃ (pg/min/g)</td>
<td>11 ± 2</td>
<td>46 ± 5*</td>
</tr>
</tbody>
</table>

Table entries are mean ± SEM of 7–9 rats per group. *Significantly different from control (P < 0.05).

previously (Garabedian et al. 1972; Armbrecht et al. 1982), TPTX rats demonstrated reduced serum calcium, elevated serum phosphorus and a low rate of renal production of 1,25(OH)₂D₃. The administration of PTH to TPTX rats demonstrated the expected effects of PTH, namely a significant rise in serum calcium, a fall in serum phosphorus, a decrease in tubular reabsorption of phosphorus (TRP) and a marked increase in renal production of 1,25(OH)₂D₃.

The effects of PTH administration were then examined in insulin-deficient (diabetic) rats (Table 2). Parathyroid hormone, with or without insulin replacement, was effective in terms of raising serum calcium and depressing serum phosphorus and TRP. There was no difference in these parameters between rats given PTH alone or in conjunction with insulin treatment. Parathyroid hormone had no significant effects on serum glucose or urinary output. Insulin replacement corrected diabetic hyperglycaemia and polyuria (Table 2).

The conversion of 25OHD₃ to 1,25(OH)₂D₃ was studied in diabetic rats, diabetic rats treated with PTH and diabetic rats treated with PTH plus insulin (Fig. 1). Diabetic rats show a very low rate of renal 1,25(OH)₂D₃ production (11 ± 1 pg/min/g). Administration of PTH to diabetic rats caused a modest but significant increase in the renal production of 1,25(OH)₂D₃ (19 ± 4 pg/min/g). In insulin-treated diabetic rats, PTH administration caused a marked increase in the renal 1,25(OH)₂D₃ produc-

Table 2.
Effects of PTH in diabetic and insulin-treated diabetic rats (all rats were thyroparathyroidectomized).

<table>
<thead>
<tr>
<th></th>
<th>DM</th>
<th>DM + PTH</th>
<th>DM + PTH + I</th>
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</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>initial</td>
<td>166 ± 9</td>
<td>152 ± 7</td>
<td>170 ± 6</td>
</tr>
<tr>
<td>final</td>
<td>143 ± 5</td>
<td>137 ± 4</td>
<td>182 ± 7a,b</td>
</tr>
<tr>
<td>Serum (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose</td>
<td>495 ± 59</td>
<td>504 ± 51</td>
<td>54 ± 7a,b</td>
</tr>
<tr>
<td>calcium</td>
<td>8.88 ± 0.07</td>
<td>10.7 ± 0.3a</td>
<td>11.6 ± 0.7a</td>
</tr>
<tr>
<td>phosphorus</td>
<td>15.3 ± 1.4</td>
<td>10.8 ± 0.5a</td>
<td>9.7 ± 0.4a</td>
</tr>
<tr>
<td>Urine (mg/24 h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose</td>
<td>1230 ± 70</td>
<td>1244 ± 63</td>
<td>8.2 ± 2a,b</td>
</tr>
<tr>
<td>phosphorus</td>
<td>20.1 ± 3.98</td>
<td>46.69 ± 4.32</td>
<td>30.13 ± 4.01a,b</td>
</tr>
<tr>
<td>creatinine</td>
<td>4.36 ± 1.55</td>
<td>5.70 ± 0.82</td>
<td>5.47 ± 0.98</td>
</tr>
<tr>
<td>TRP (%)</td>
<td>77.0 ± 3.7</td>
<td>60.8 ± 8.3a</td>
<td>57.5 ± 7.4a</td>
</tr>
</tbody>
</table>

Table entries are the mean ± SEM of 7–9 rats per group. a Significantly different from diabetic (DM); P < 0.05.

b Significantly different from DM + PTH; P < 0.05.

I denotes insulin-treated.
tion (48 ± 12 pg/min/g). This rate of production was similar to that produced by non-diabetic TPTX rats administered with PTH (46 ± 5 pg/min/g; Table 1). Results are given as mean ± SEM. Student's two-tailed t-test was used for comparison of difference. A confidence level of 95% or greater was considered to be significant.

Discussion

These results demonstrate that the response to PTH in terms of renal 1,25(OH)_{2}D_{3} production is markedly reduced in insulin-deficient (diabetic) rats (Fig. 1). The precise mechanism by which PTH modulates renal 25OHD_{3} metabolism is unknown, but some evidence has been presented for the involvement of adenylate cyclase, cyclic AMP and protein kinase activity (Horiuchi et al. 1977; Rost et al. 1981; Armbrecht et al. 1984). Renal resistance to PTH in streptozotocin-induced diabetic rats has been suggested as the mechanism by which the 1α-hydroxylase is depressed (Sulimovic et al. 1981). This was based on the finding that the diabetic rats excreted significantly less nephrogenous cyclic AMP compared with the control or the insulin-treated diabetics. However, the kidneys of streptozotocin-induced diabetic rats elicited a normal cyclic AMP response to PTH (Wongsurawat et al. 1983). This indicates that the renal cyclic AMP generation in response to PTH was not responsible for the decreased effect of PTH in terms of 1,25(OH)_{2}D_{3} production in diabetes.

Recently, it has been shown that liver post-mitochondrial supernatant from diabetic rats showed a decrease in [3H]cyclic AMP binding activity which was associated with a decrease in the number of cyclic AMP binding sites (Srivastava 1983). The diabetic supernatants showed a selective reduction in the regulatory subunit of type I cyclic AMP-dependent protein kinase without any change in the regulatory subunit of type II cyclic AMP-dependent protein kinase (Srivastava 1983). It is possible that this decrease in the cyclic AMP binding activity of the regulatory subunit of type I cyclic AMP-dependent protein kinase also exists in the diabetic kidney and is, at least in part, responsible for the dampened effect of PTH-stimulated renal 1,25(OH)_{2}D_{3} production.

Our studies show that insulin replacement normalizes the renal production of 1,25(OH)_{2}D_{3} in response to PTH (Fig. 1). The results do not establish, however, that insulin acts directly on the renal 1α-hydroxylase. Insulin apparently has permissive effect on PTH stimulation of 1,25(OH)_{2}D_{3} production in cultured chick kidney cells (Henry 1981). These findings suggest that insulin deficiency is responsible for the decreased 1,25(OH)_{2}D_{3} production. However, insulin is not an absolute requirement in 1α-hydroxylation. It is apparent from our results (Fig. 1) that PTH can induce a modest increase in 1,25(OH)_{2}D_{3} production in
diabetic state. Diabetic rats, when fed a low (0.02%) calcium diet, can also significantly increase serum 1,25(OH)₂D₃ concentration compared with diabetic rats fed a normal (0.5%) calcium diet (Wilson et al. 1982).

Diabetic TPTX rats were able to respond normally to PTH administration, with or without insulin, in terms of serum calcium and phosphorus and TRP (Table 2). The results suggest, at least on short term basis, that insulin has no significant role in modulating the effect of PTH on calcaemic and phosphaturic response. The results also suggest that the pathways of PTH action in the kidney are different, since diabetes inhibits the PTH stimulation of 1,25(OH)₂D₃ production but not the TRP.

The relative refractoriness to PTH in terms of 1,25(OH)₂D₃ production in diabetes is similar to that observed with aging. The older animals demonstrate a significant decreased 1,25(OH)₂D₃ production in response to PTH both in vivo and in vitro (Armbrecht et al. 1982). However, other renal functions mediated by PTH, such as phosphate diuresis in response to a low calcium diet, do not change with age (Armbrecht et al. 1981b). It may be that in both diabetes and aging there is a biochemical defect distal to the generation of cyclic AMP which affects 1,25(OH)₂D₃ production but does not affect renal handling of calcium and phosphorus.

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References


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