Effect of 1,25 Dihydroxyvitamin D_3 on Insulin Secretion

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SUMMARY

Recent autoradiographic studies demonstrated that B-cells concentrate 1,25 $(OH)_2$ D_3 in their nuclei, suggesting a genomic action on B-cell function. This study was undertaken to investigate the effects of 1,25 $(OH)_2$ D_3 on insulin secretion in vitamin D-deficient rats. Mature vitamin D-deficient rats were injected with 1,25 $(OH)_2$ D_3 or the ethanol-isotonic saline vehicle. Administration of 1,25 $(OH)_2$ D_3 to 10 rats resulted in a 17 μU/ml (113%) increase in insulin levels and 0.9 mg/dl (16%) increase in plasma calcium. No changes were found in insulin or calcium levels in 5 control rats given vehicle alone. A group of vitamin D-deficient rats with plasma calcium levels of 5.4 ± 0.1 mg/dl had insulin levels that were the same as those observed in a group of vitamin D-deficient rats with plasma calcium levels of 6.3 ± 0.1 mg/dl. The difference in calcium levels between these two groups is similar to the increase in plasma calcium found after 1,25 $(OH)_2$ D_3 administration. The results of these studies indicate that 1,25 $(OH)_2$ D_3 action on pancreatic B-cells affects insulin secretion. Since insulin increases synthesis of 1,25 $(OH)_2$ D_3, the existence of a feedback loop between B-cells and kidney proximal tubule cells is suggested. DIABETES *30*:382–386, May 1981.

The effect of 1,25 dihydroxyvitamin D_3 (1,25 $(OH)_2$ D_3) has been associated with calcium and phosphate handling in intestine, bone, and kidney. It is well accepted that these three tissues are acted on by 1,25 $(OH)_2$ D_3, since receptors for 1,25 $(OH)_2$ D_3 have been reported to exist in these tissues.[1–3] The thaw-mount autoradiographic technique[4] has been successfully used in our laboratory to identify target tissues of other steroid hormones. Recently, we have employed this technique to identify organs, and the specific cells in these organs, that are targets of 1,25 $(OH)_2$ D_3. We thus confirmed the biochemical evidence for nuclear binding in intestine,[1] parathyroid,[5] and kidney[3] and further identified the specific target cells in these heterogenous tissues. Moreover, we have identified a large number of target organs that were previously unrecognized; among these are stomach, skin, pituitary,[6] brain,[7] and the endocrine pancreas.[8]

Support for a physiologic significance of 1,25 $(OH)_2$ D_3 action at these new sites is forthcoming. As a result of the autoradiographic demonstration of skin as a target of 1,25 $(OH)_2$ D_3, studies were undertaken that have demonstrated a physiologic role for 1,25 $(OH)_2$ D_3 action in the skin.[9] A double technique of autoradiography and immunocytochemistry has been used to identify the target cells of 1,25 $(OH)_2$ D_3 in the pituitary as thyrotropes, and in the pancreas as B-cells.[10] The present study provides evidence for a physiologic role of 1,25 $(OH)_2$ D_3 in B-cell function.

MATERIALS AND METHODS

Animals and treatment. Twenty-one-day male Holtzman Sprague-Dawley rats were individually housed and fed, ad libitum, a vitamin D-deficient diet[11] for 8 wk. The deficient state was evaluated by monitoring weight gain and plasma calcium levels. After 5 wk blood samples were obtained to determine insulin and calcium levels after an 8-h fast. At 7 wk blood samples were taken to establish insulin and calcium values in 15 vitamin D-deficient rats before any injections. These 15 deficient rats (average body weight 199 g) were divided into two groups. Group I consisted of 10 rats given i.p. injections of 1,25 $(OH)_2$ D_3 (W. E. Scott, Hoffmann-La Roche) dissolved in 75% ethanol-isotonic saline, at 40 h (0.7 ng/g body wt, i.e., 1.68 pmol/g) and at 64 h (16.8 pmol/g body wt) after the non-treatment blood samples were obtained. Group II consisted of 5 control rats receiving equal volumes (1.0 ml/kg) of vehicle at 40 h. Blood samples were obtained 8 h after injection.

Six untreated vitamin D-deficient rats remained on the vitamin D-deficient diet; after 12 wk on the diet blood samples were obtained from 4 surviving deficient rats. Additional

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samples were obtained from 5 rats to compare calcium levels from tail tip samples with calcium levels of blood samples obtained from the carotid artery.

Blood samples and assays. On the days samples were taken, the rats were fasted from 8 a.m. to 4 p.m. and blood was collected at 4 p.m. Injections, when made, were at the start of the fast (8 a.m.). Blood was collected from the tail tip in heparinized hematocrit microcapillary tubes. The blood samples were placed on ice and subsequently centrifuged 3 min in an ultracentrifuge. The heparinized plasma was then transferred to small vials and stored at −30°C. Plasma immunoreactive insulin was determined using Phadebas insulin radioimmunoassay kit (Pharmacia, Inc.); intra-assay variability was 4.4 ± 1.3%. Insulin levels of the 5-wk and 7-wk samples were measured with kits of the same lot to avoid interassay variability. Plasma calcium was determined with an autoanalyzer (Precision Systems), courtesy of Dr. T. C. Peng. Results were analyzed using Student's *t* test or paired *t* test (values given as mean ± SEM).

RESULTS

The immunoreactive insulin levels in 34 fasted vitamin D-deficient rats after 5 wk on diet were 18 ± 1 μU/ml. The calcium values were 5.9 ± 0.1 mg/dl. Fasting insulin levels after 7 wk on the vitamin D-deficient diet were similar to those at 5 wk. In plasma collected 8 h after the administration of 1,25 $(OH)_2$ D_3, insulin levels increased 17 μU/ml (113%) to 32 ± 3 μU/ml. Concurrently, the plasma calcium (Ca) increased 0.9 ± 0.1 mg/dl. After the second injection of 1,25 $(OH)_2$ D_3 on the following day, fasting insulin levels showed no further increase (Table 1). The insulin levels after 2 days of 1,25 $(OH)_2$ D_3 treatment were elevated 13 μU/ml over the control values of these rats. This reflects a small, although not significant decrease in insulin levels from the previous day. In contrast, Ca levels showed a continued increase to 7.8 ± 0.3 mg/dl from the 5.7 mg/dl pretreatment Ca levels ($P < 0.001$), and the 6.6 mg/dl levels of the previous day ($P < 0.005$).

In the 5 control rats before the administration of vehicle, fasting Ca and insulin values were 6.2 ± 0.5 mg/dl and 19 ± 2 μU/ml, respectively. These animals were given 75% ethanol-isotonic saline i.p., and the fasting Ca decreased insignificantly to 5.8 ± 0.7 mg/dl, while circulating insulin levels remained essentially unchanged (Table 2). Four rats fed the deficient diet for 12 wk had Ca and insulin levels of 4.9 ± 0.5 mg/dl and 18 ± 2 μU/ml, respectively, and showed no increase in weight in the last 4 wk on diet.

TABLE 2
Plasma calcium and insulin levels in vitamin D-deficient rats: response to varied treatment

Treatment	N	Ca (mg/dl)*	Insulin (μU/ml)*
5-wk deficient diet†	34	5.9 ± 0.1	18 ± 1
7-wk deficient diet	10	5.7 ± 0.1	15 ± 2
7-wk deficient + 1,25 $(OH)_2$ D_3	10	6.6 ± 0.1	32 ± 3
7-wk deficient diet	5	6.2 ± 0.5	19 ± 2
7-wk deficient + vehicle	5	5.8 ± 0.7	19 ± 4
12-wk deficient diet	4	4.9 ± 0.5	18 ± 2

* Mean ± SEM.
† 0.47% Ca and 0.3% phosphorus.

The total calcium levels in these rats after 5 and 7 wk on vitamin D-deficient diet were 1.0 to 1.5 mg/dl higher than those previously observed for rats on this diet.[11] This may be due to different methods of blood collection. Therefore, 5 rats were used to compare Ca levels in samples (plasma) obtained from the tail tip with values obtained from samples (serum) collected from the carotid artery at the time of killing. Samples from the tail tip gave values 0.81 ± 0.2 mg/dl higher than those from the carotid artery.

DISCUSSION

A relationship between function of B-cells and vitamin D metabolism and action has previously been observed. Experimentally diabetic rats have calcium deficiencies that can be corrected with 1,25 $(OH)_2$ D_3[12] or insulin.[13] Insulin treatment also normalized the low 1,25 $(OH)_2$ D_3 levels found in these rats.[14] The low 1,25 $(OH)_2$ D_3 levels in diabetic rats have been attributed to the decreased activity of 25 hydroxyvitamin D3-1α-hydroxylase.[15,16] Defective metabolism of vitamin D, calcium, or phosphorus was not evident

TABLE 1
Plasma calcium and insulin levels in vitamin D-deficient rats: response to 1,25 $(OH)_2$ D_3 treatment

	Day 1 (0 h)		Day 2 (48 h)		Day 3 (72 h)	
Rat	Calcium (mg/dl)	Insulin (μU/ml)	Calcium (mg/dl)	Insulin (μU/ml)	Calcium (mg/dl)	Insulin (μU/ml)
1	5.7	9	7.3	39	9.1	33
2	6.0	18	6.7	19	8.4	25
3	5.4	7	6.6	26	7.9	19
4	6.2	20	7.2	34	8.0	32
5	5.4	20	5.8	35	6.6	34
6	5.6	10	6.2	21	7.8	23
7	5.0	5	6.6	28	7.9	24
8	5.8	18	6.9	47	6.7	23
9	5.9	22	6.8	30	8.6	40
10	5.9	20	6.3	36	6.9	30
Mean ± SEM	5.7 ± 0.1	15 ± 2	6.6 ± 0.1*	32 ± 3†	7.8 ± 0.3†	28 ± 2†

48-h sample taken 8 h after 1,25 $(OH)_2$ D_3 injection of 0.7 ng/g body wt.
72-h sample taken 8 h after second 1,25 $(OH)_2$ D_3 injection.
* $P < 0.02$ versus pretreatment levels.
† $P < 0.001$ versus pretreatment levels.

in one study of human diabetics,[17] while other studies have observed altered phosphate[18,19] and calcium metabolism.[20–22]

Administration of 1,25 $(OH)_2$ D_3 to vitamin D-deficient rats in the present study increased circulating insulin levels, probably by acting directly on B-cells. The small increase of blood calcium levels that was observed may also be involved in the stimulation of B-cell activity and resultant increased circulating insulin levels. To determine whether total plasma calcium levels correlate with insulin levels, control values from 34 rats during the fifth week and 10 rats during the seventh week were separated into two groups according to calcium levels. Insulin levels from rats with calcium levels less than 6 mg/dl comprised one group, and calcium levels greater than or equal to 6 mg/dl made up the second group (N = 22 per group). Calcium levels of 5.4 ± 0.1 mg/dl were associated with insulin levels of 17 ± 1 μU/ml, and calcium levels of 6.3 ± 0.1 mg/dl were associated with insulin levels of 18 ± 1 μU/ml. While calcium levels are significantly different ($P < 0.001$, paired *t* test), there is no significant difference in insulin levels between the two groups. Similarly, when insulin and calcium levels are evaluated in 12-wk-deficient rats, calcium levels are down to 4.9 mg/dl, while insulin levels are not different. Thus, it would seem that a plasma total calcium increase of 0.9 mg/dl, after 1,25 $(OH)_2$ D_3 administration, would not account for the large increase in circulating insulin levels. An increase in ionized calcium is, however, a possible factor.

In autoradiographic studies we have demonstrated that the endocrine pancreas is a target of 1,25 $(OH)_2$ D_3 and that the hormone is concentrated and retained in nuclei of B-cells.[8,10] Thus, we identified specific cells of the pancreas that contain the 1,25 $(OH)_2$ D_3 receptors that have been isolated from cytosol of pancreatic homogenates.[3] In view of the autoradiographic data, the increased insulin levels observed after 1,25 $(OH)_2$ D_3 administration indicate a direct effect of 1,25 $(OH)_2$ D_3 on B-cell function. Evidence present in the literature supports our results. Vitamin D-dependent calcium binding protein found in the pancreas[23,24] has been shown to be located in the islets,[25] and morphologic studies show ultrastructural changes in B-cells after administration of 1,25 $(OH)_2$ D_3.[26]

The results of experiments performed with dogs are also consistent with a proposed role of 1,25 $(OH)_2$ D_3 in B-cell function. Dogs with hypophosphatemia induced by dietary manipulation had significantly higher insulin responses than normal dogs. The ionized calcium of both groups was similar, and calcium infusions that increased serum-ionized calcium 0.7–1 mg/dl had no effect on insulin responses.[27] It is known that phosphate deprivation and the resultant hypophosphatemia stimulates the synthesis of 1,25 $(OH)_2$ D_3 and inhibits 24,25 $(OH)_2$ D_3 production.[28,29] The increased 1,25 $(OH)_2$ D_3 levels, which can reasonably be expected in these dogs, may be an important factor in the increased insulin responses.

Similar associations between 1,25 $(OH)_2$ D_3 levels and augmented insulin secretion are evident in humans. Insulin levels and responses are greater in patients with primary hyperparathyroidism than in normal individuals or the same patients after surgical treatment.[30–32] Mild hypercalcemia induced by PTH administration in normal subjects reproduced the heightened insulin response to glucose, while calcium infusion-induced hypercalcemia did not. There is no effect of PTH on insulin release in vitro,[33,34] however, the fact that PTH administration to normal men elicited changes implicates an indirect effect of PTH that apparently was not related to serum calcium.[30] In primary hyperparathyroidism serum, 1,25 $(OH)_2$ D_3 levels are elevated well above normal[34,35] and PTH administration to normal men would conceivably increase 1,25 $(OH)_2$ D_3 production, thus increased 1,25 $(OH)_2$ D_3 levels may reasonably be implicated in the augmented insulin responses reported.

The present study reports insulin levels for rats in which the vitamin D and calcium status is abnormal. There are two previous studies of insulin in hypocalcemic rats with altered vitamin D states. In 1974, Pento et al.[36] demonstrated that hypocalcemic parathyroidectomized rats had an insulin response to glucose that was 40% of that observed in normocalcemic sham-operated controls. This group concluded that the reduced insulin response resulted from the hypocalcemia. It should be noted, however, that the lack of parathyroid hormone tends to result in decreased 1,25 $(OH)_2$ D_3 and increased 24,25 $(OH)_2$ D_3 levels.[37] More recently, Norman et al. reported that the perfused pancreas of hypocalcemic vitamin D-deficient rats responded to a glucose-arginine stimulus by secreting 40–50% less insulin than the calcium- and vitamin D-replete controls. These authors concluded that their results implicate a direct effect of vitamin D in optimizing conditions for B-cell response.[38] The results of these studies and the present one are in agreement, in that all three indicate that hypocalcemia and vitamin D abnormalities in rats affect insulin release. In each of these studies low serum calcium remains a factor and hypocalcemia has been implicated in reduced insulin responses to glucose or arginine.[39,40] Is the effect of vitamin D, then, a direct one, or is it mediated by blood calcium levels? To demonstrate a direct effect of a vitamin D metabolite on insulin secretion, evidence must be provided that indicates the receptor status of the B-cells, the metabolite for which the receptor is specific, and a physiologic effect of the specified metabolite.

Receptor proteins for 25 (OH) D_3 and 1,25 $(OH)_2$ D_3 have been isolated from homogenates of whole chick pancreas.[3] This can hardly be construed to be representative of the endocrine pancreas, which represents 1% of the pancreas.[41] Although a physiologic effect is indicated in the study of Norman et al.,[38] one remains uncertain, on the basis of the data presented, whether this is a direct or indirect effect, and which of the metabolites of vitamin D produced the proposed response. It has been suggested that 25 (OH) D_3 produces hypoglycemia by an extrapancreatic action.[42]

Based on our identification of B-cells as targets of 1,25 $(OH)_2$ D_3 and supportive literature, we previously proposed that 1,25 $(OH)_2$ D_3 has a direct, genomic effect on B-cell functions, including insulin secretion.[8,10] The proposed 1,25 $(OH)_2$ D_3 stimulation of insulin secretion reported in this communication may involve a genomic action affecting intracellular vitamin D-dependent calcium binding protein levels, 1,25 $(OH)_2$ D_3-associated cell membrane permeability,[43–45] and mitochondrial handling of calcium.[46,47] Alterations of mitochondrial calcium content appear to be of considerable importance to the secretory response of the

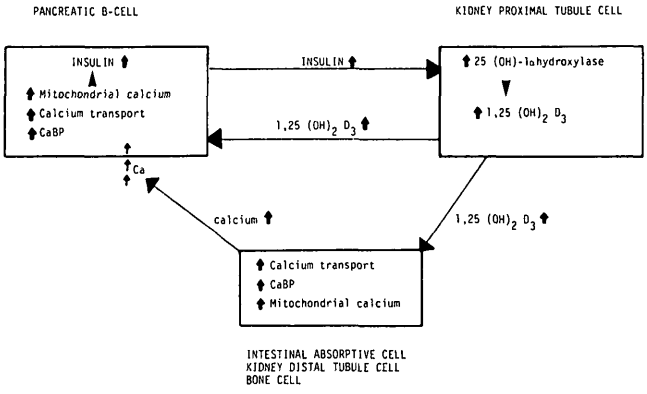

FIGURE 1. Proposed model of relationships between kidney proximal tubule cells and pancreatic B-cells. Insulin affects the 25 (OH)-1α-hydroxylase enzyme of the kidney proximal tubule cells. 1,25 $(OH)_2$ D_3 produced by this enzyme has a direct action on intestinal absorptive cells and kidney distal tubule cells, which yields increased blood calcium. 1,25 $(OH)_2$ D_3 also acts directly on B-cells, affecting the calcium handling important to insulin release. The effects of these two hormones suggest a "feedback" relationship in which each hormone acts to maintain the release or production of the other.

B-cell.[48,49] It is of interest, therefore, that 1,25 $(OH)_2$ D_3 administration to mice affects the calcium content of B-cell mitochondria.[26] A role for 1,25 $(OH)_2$ D_3 in B-cell calcium handling is further indicated by results obtained from in vitro studies of isolated islets of vitamin D-deficient rats. In these islets, the acute phase of glucose-stimulated insulin release is virtually absent.[50] The acute phase of glucose-stimulated release is dependent on intracellular calcium and independent of extracellular calcium,[51] thus, the greatly reduced first phase insulin release in vitamin D-deficient islets suggests that intracellular B-cell calcium pools are depleted or altered. Therefore, in vitamin D-deficient rats, 1,25 $(OH)_2$ D_3 probably modulates intracellular calcium stores by mitochondrial accumulation and stimulated synthesis of vitamin D-dependent calcium binding protein. The increase in available calcium then facilitates insulin release. Insulin, on the other hand, increases 1,25 $(OH)_2$ D_3 levels in diabetic rats[14] and permits parathyroid hormone stimulation of 1,25 $(OH)_2$ D_3 production in kidney cell cultures.[52]

The effect of insulin on 1,25 $(OH)_2$ D_3 production, in conjunction with the present demonstration of 1,25 $(OH)_2$ D_3 on insulin secretion, suggests a positive feedback relationship between kidney proximal tubule cells and B-cells (Figure 1). The positive feedback of 1,25 $(OH)_2$ D_3 to B-cells may be similar to that proposed for skin. In skin, 1,25 $(OH)_2$ D_3 increases the production of 7-dehydrocholesterol, which is then available for vitamin D_3 production.[9] In B-cells, 1,25 $(OH)_2$ D_3 may act by increasing calcium binding protein and mitochondrial calcium levels, and, thus, increase calcium pools available for response. Insulin may, in turn, sensitize the proximal tubule cells so they may respond to parathyroid hormone or other stimuli. This feedback is limited by the activity of 25 hydroxy-1α-hydroxylase, which is inhibited by rising calcium, phosphorus, and 1,25 $(OH)_2$ D_3 levels.[37]

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