

Dietary Vitamin D Is Essential for Normal Insulin Secretion from the Perfused Rat Pancreas

Seizo Kadowaki and Anthony W. Norman
Department of Biochemistry, University of California,
Riverside, California 92521

Abstract. We have reported previously that arginine-induced insulin secretion was impaired in the vitamin D-deficient rat pancreas, and that it was improved by dietary vitamin D repletion (Norman, A. W., B. J. Frankel, A. M. Heldt, and G. M. Grodsky, 1980, *Science [Wash. DC]*. 209:823-825). In this study, we evaluate in the perfused rat pancreas system whether the effects of vitamin D and its metabolites on insulin secretion are direct in action on the pancreas and limited to the secretagogue arginine, or whether they are secondary to the hypocalcemia or reduced caloric and calcium intake associated with vitamin D deficiency. In an experiment where vitamin D-replete (+D) rats were pair-fed to D-deficient (-D) rats fed ad lib., the secretion of insulin in response to arginine infusion in the +D perfused rat pancreas was threefold higher than in the -D control. In a second experiment, the serum calcium level was elevated from the characteristic hypocalcemic level of -D rats (4.9 ± 0.1 mg/dl) to a normal calcemic level (10.0 ± 0.3 mg/dl) by feeding the rats a -D diet with dietary calcium levels ranging from 0.4 to 4%. In these -D rats, the pancreatic perfusion study with the secretagogue arginine showed a similar blunted insulin secretion response in all groups in spite of the significant differences of serum calcium levels. In a third experiment, insulin secretion in response to the separate administration of arginine (10 mM), glucose (16.9 mM), and tolbutamide (0.37 mM) was found to be significantly higher in pair-fed, normocalcemic +D rats than in -D rats with normal calcium

levels. These results indicate that vitamin D or its metabolites are essential for normal insulin secretion and that the dietary intake of calcium and the resulting serum calcium levels play a lesser role than vitamin D availability in mediating insulin secretion.

Introduction

In recent years, there have appeared several reports which suggest that the endocrine pancreas is also a target tissue for the hormonally active form of vitamin D₃, 1,25-dihydroxyvitamin D₃[1,25-(OH)₂-D₃]¹, along with the classical vitamin D target organs: the intestine, bone, and kidney (1). These observations include: (a) the presence of a cytosol receptor protein for 1,25-(OH)₂-D₃ in the chick pancreas (2-4); (b) the presence of immunoreactive vitamin D-dependent calcium binding protein (CaBP) in the chick (4), pig (5), and rat (6) pancreas by radioimmunoassay and its dependency on dietary vitamin D₃ (7); (c) immunohistochemical demonstration of CaBP in the endocrine B cells of chick (8), cat (9), dog (9), mice (9), and rat (9); (d) demonstration of the localization of [³H]1,25-(OH)₂-D₃ in the nucleus of the rat pancreas B cells by autoradiography (10). Collectively, these results strongly support the involvement of vitamin D and its metabolites in calcium metabolism relevant for the endocrine pancreatic B cells, and raises the possibility that vitamin D metabolites regulate endocrine B cell function including insulin secretion. In this respect, a direct interrelationship between pancreatic B cell function and vitamin D has been reported (11); vitamin D deficiency was found to inhibit insulin but not glucagon secretion in response to arginine in the isolated perfused rat pancreas, and dietary vitamin D repletion markedly improved insulin secretion. Subsequently, in results compatible to that of Norman et al. (11), Clark et al. (12) reported that administration in vivo of 1,25-(OH)₂-D₃ to vitamin D-deficient (-D) rats elevated the peripheral blood levels of insulin.

A preliminary report of these studies was presented at the Endocrine Meetings, June 1983, San Antonio, TX. This is paper LII in a series entitled "Studies on the Mode of Action of Vitamin D."

Address all correspondence to Dr. Norman.

Received for publication 24 June 1983 and in revised form 26 October 1983.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/84/03/0759/08 \$1.00

Volume 73, March 1984, 759-766

1. Abbreviations used in this paper: 1,25-(OH)₂-D₃, 1,25-dihydroxyvitamin D₃; 25-OH-D₃, 25-hydroxyvitamin D₃; CaBP, calcium binding protein; +D, vitamin D-replete; -D, vitamin D-deficient; PTH, parathyroid hormone.

However, it remains unknown which vitamin D-related factor(s) may play a major role in the insulin secretion mechanism, since several nutritional, metabolic, and biochemical changes ensue after the vitamin D depletion. These include: (a) a decreased dietary intake of both calories and total calcium; (b) a decrease in serum calcium levels; (c) a decrease in islet vitamin D-dependent CaBP levels; (d) changes in the hormonal environment (1); and (e) changes in cell membrane characteristics (13), etc. In this communication, we report the results of experiments where we have normalized the serum calcium of -D rats and have controlled the caloric intake of D-replete (+D) rats in an effort to determine the level of involvement of vitamin D status in the secretion of insulin from the isolated perfused rat pancreas.

Methods

Male weanling rats, obtained from the Holtzman Co. (Madison, WI), were raised for 6-7 wk on a synthetic -D diet (0.4% Ca, 0.35% P) (14). All animals were housed individually in stainless steel cages with a 12-h light, 12-h dark cycle free of UV light and were allowed free access to the diet and water. These animals were used in the following separate experiments. When vitamin D₃ was provided, it was dissolved in 0.1 ml of a solvent (95% ethanol:1,2-propanediol, 1:1 vol) containing appropriate concentrations of vitamin D₃, and subcutaneously injected. Vehicle alone (0.1 ml) was administered to the control group.

Experiment 1. This experiment was designed to investigate the effect of vitamin D₃ (4.55 nmol, 3 times per week) on the arginine (10 mM)-induced insulin secretion from the isolated perfused pancreas of rats that were fed either ad lib. or pair-fed with -D control rats given vehicle alone. Vitamin D supplementation with or without pair-feeding was continued at least for 3 wk after 5 wk of vitamin D depletion before starting the pancreatic perfusion study, according to the method described below.

The daily dietary intake of -D control rats was measured gravimetrically every day by using a commercial rat food device (Wahmann Manufacturing Co., Timonium, MD) and the mean dietary intake of these animals was given to the +D rats (scheduled to be pair-fed) the next day. Preliminary studies indicated that the daily dietary intake (mean±SEM) was 15±2, 30±4, and 15±3 g/d in the -D control rats

fed ad lib. (n = 10), +D rats fed ad lib. (n = 10), and +D rats pair-fed with -D rats (n = 10), respectively.

Experiment 2. This experiment was designed to evaluate how differing serum levels of calcium might affect the arginine-induced insulin secretion from the isolated perfused pancreas of the -D rat. Several levels of serum calcium from low (4.9 mg/dl) to normal (10.0 mg/dl) were established in -D rats by manipulating the dietary calcium composition (group 1, 0.4%; group 2, 1%; group 3, 2%; group 4, 4%) at the expense of cellulose. Also, the dietary composition of D-glucose (53.5%) was made constant among these groups. Groups 2-4 were pair-fed with group 1 according to the method described in experiment 1 until the day of perfusion.

Experiment 3. This experiment was designed to evaluate the insulin secretion in response to the separate administration of the secretagogues arginine, glucose, or tolbutamide in the isolated perfused pancreas of +D rats (vitamin D₃, 975 pmol/d subcutaneous) pair-fed with -D rats with a normal serum calcium effected by manipulating the dietary calcium composition (4% with pair-feeding). Vitamin D₃ or vehicle alone was administered for 3-4 wk until the day of pancreatic perfusion. According to the results from experiment 2, normal serum calcium levels could be obtained in -D rats placed on the -D diet containing 4% calcium.

Perfusion system. The pancreas was isolated and perfused by the procedure described by Grodsky et al. (15) with minor modifications. Overnight fasted animals were anesthetized with chloral hydrate (300 mg/kg body weight intraperitoneal) and the surgical preparation was carried out according to the method described previously (16). All experiments were performed between 9:00 a.m. and 4:00 p.m. by using a pancreatic perfusion apparatus (KM-IA Type, Kokenrika Co., Ltd., Osaka, Japan). The entire surgical operation was finished within 20 min, and the pancreas was perfused at a flow rate of 2 ml/min with a Krebs-Ringer bicarbonate buffer, pH 7.4, containing 0.25% bovine serum albumin and 4.6% Dextran (Sigma Chemical Co., St. Louis, MO) equilibrated with 95% O₂:5% CO₂ at 37°C. After 15 min equilibration time with this medium, the insulin secretagogue (arginine, glucose, or tolbutamide) was introduced for 20 min from a side syringe to maintain a final concentration of 10 mM, arginine; 16.7 mM, glucose; or 0.37 mM, tolbutamide, respectively. In experiment 3, the secretagogues arginine (10 mM), glucose (16.7 mM), or tolbutamide (0.37 mM) were separately used to stimulate insulin secretions. The portal effluent was collected in fractions of 2.0-min duration and frozen until assayed for insulin. Blood samples (3 ml) were obtained from all rats undergoing the perfusion experiment through the jugular vein just before removal

Table I. Parameters of +D Rats Fed ad lib. or Pair-fed with D Control Rats (Experiment 1)

Group	n	Body weight	Pancreas weight	Blood					Insulin secretion		
				Calcium	Phosphorus	Glucose	25-OH-D ₃	1,25-(OH) ₂ -D ₃	1st phase	2nd phase	Total
		g	g	mg/dl	mg/dl	mg/dl	ng/ml	pg/ml	ng	ng	ng
1, -D a.l.*	10	185±17	1.29±0.07	4.9±0.2	8.6±0.3	113±6	≤0.5	<5	40±6	70±11	110±16
2, +D p.f.*	11	210±7	1.41±0.08	10.4±0.5‡	10.4±0.5‡	120±9	12±1.5‡	320±50*	120±20*	150±15‡	280±40‡
3, +D a.l.*	18	260±10§¶	1.60±0.10‡¶	10.2±0.2‡	6.3±1.0§¶	123±6	19±3‡	490±60¶	260±20‡¶	390±59‡¶	650±70‡¶

Results are expressed as mean±SEM of the indicated numbers. +D, 4.55 nmol of vitamin D₃ was injected subcutaneously three times per week for 3-4 wk until pancreatic perfusion. -D, vehicle alone was administered according to the same schedule as +D group. * a.l., ad lib., p.f., pair-fed to group 1. ‡, § Significant difference from group 1: ‡ p < 0.001, § = P < 0.05. ¶¶ Significant difference from group 2: ¶ = P < 0.05, ¶¶ = P < 0.001.

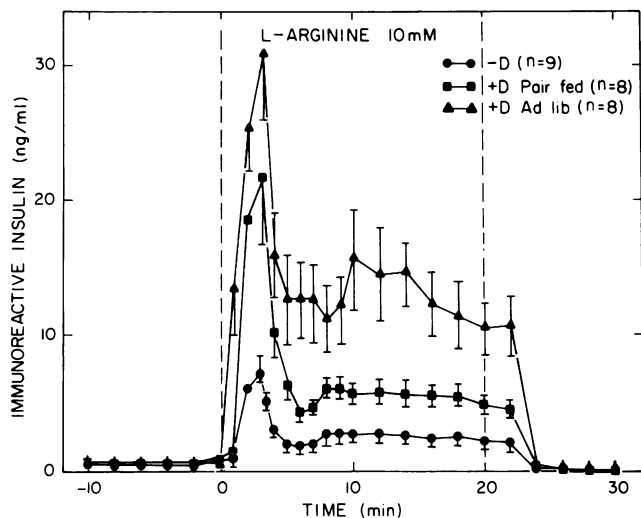


Figure 1. Effect of vitamin D repletion on arginine (10 mM)-glucose (5 mM)-induced insulin secretion from the isolated perfused pancreas of +D rat fed ad lib (—▲—) or pair-fed (—■—) with -D (—●—) control rats. All pancreases were perfused in the presence of 5.5 mM glucose throughout the experiment (-15 to +30 min). Arginine was introduced from 0 to 20 min to maintain a final concentration of 10 mM. The means±SEM of the indicated number of experiments are shown.

of the pancreas, and were collected in chilled heparinized tubes. After centrifugation, plasma was stored at -20°C until it was assayed for glucose, calcium, phosphate, and vitamin D metabolites.

Analytical method. Immunoreactive insulin was measured by a radioimmunoassay procedure by using antibody raised against porcine insulin, and rat insulin (Novo Research Institute, Copenhagen, Denmark) was used as a standard. The separation of free and bound hormone was carried out via utilization of dextran charcoal (17). Plasma glucose was measured by glucose oxidase method (18). Plasma total calcium value was determined by an atomic absorption method (19) and ionized calcium was measured by utilization of an ionized calcium analyzer Space-state 20 (Orion Research Inc., Cambridge, MA). Plasma phosphate value was

determined by the method of Chen et al. (20). Measurement of plasma 25-hydroxyvitamin D₃ (25-OH-D₃) and 1,25-(OH)₂-D₃ were performed as previously described (21).

Results

Parameters of -D and +D rats (Table I). Table I tabulates the typical values of relevant parameters for -D and +D rats used in these pancreatic perfusion studies. In the -D group (group 1), plasma vitamin D metabolite levels including 25-OH-D₃ and 1,25-(OH)₂-D₃ were not detectable, and low serum calcium levels were observed. Vitamin D repletion completely normalized serum calcium levels and increased serum vitamin D metabolite levels in both groups 2 and 3. Body weight and pancreatic weight were significantly lower in -D group, while serum glucose levels were not different amongst these groups. Body weight and pancreatic weight in group 2 (+D, pair-fed with group 1) were significantly reduced compared with group 3 (+D, ad lib.), but not significantly different from group 1.

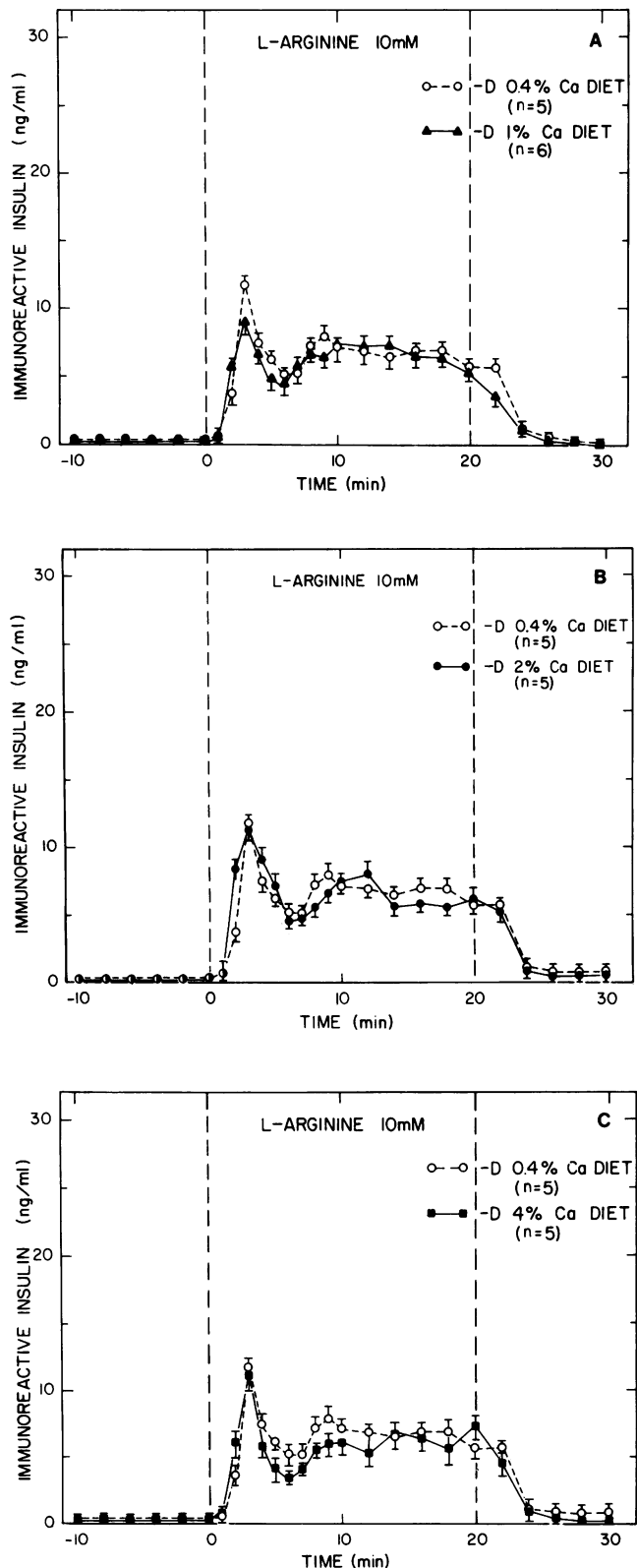
Effect of vitamin D repletion on arginine (10 mM)-glucose (5 mM)-induced insulin secretion from the isolated perfused pancreas of +D rat fed ad lib. or pair-fed with -D control rats (Fig. 1, Table I). Fig. 1 presents the results of arginine (10 mM)-glucose (5 mM)-induced insulin secretion from the pancreas of groups 1 (-D, fed ad lib.), group 2 (+D, pair-fed to -D), and group 3 (+D, fed ad lib.); all groups had typical biphasic responses of insulin secretion in response to the infusion of arginine. In each group, the insulin response reached a maximum within 3 min of arginine-glucose stimulation followed by transient decreases towards 6 min (first phase) and after that, a sustained insulin release was observed until the end of stimulation (second phase).

The maximum value of insulin secretion in the first phase of groups 1, 2, and 3 was 7.0±1.2, 21.5±4.2, and 31±4.0 ng/ml, respectively (group 1 vs. 2, $P < 0.005$; group 2 vs. 3, $P < 0.05$). Insulin secretion in the second phase was also highest in group 3 followed by group 2 and group 1. Cumulative insulin secretion in the first and second phases was 40±6 and 70±11

Table II. Parameters of -D Rats Raised on Different Dietary Calcium Levels (Experiment 2)

Group	n	Dietary Ca %	Body weight g	Pancreas weight g	Total Ca mg/dl	Ionized Ca ⁺⁺ mg/dl	Insulin secretion		
							1st phase ng	2nd phase ng	Total ng
1	8	0.4	166±4	1.30±.06	4.9±0.1	2.2±0.1	76±6	190±10	260±10
2	6	1	167±5	1.29±.05	5.1±0.2	2.5±0.1*	64±6	180±10	250±20
3	6	2	169±6	1.28±.06	6.8±0.6*§	3.3±0.4*§	85±9	170±20	250±20
4	6	4	168±3	1.31±.05	10±0.3‡¶¶	5.2±0.1‡¶¶	63±9	190±10	250±20

Results are expressed as means±SEM of the indicated numbers. Groups 2-4 were pair-fed with group 1. Ionized Ca (Ca⁺⁺) and total Ca were measured in the same sample. * $P < 0.05$, ‡ $P < 0.01$, significant difference from group 1. § $P < 0.05$, ¶ $P < 0.01$, significant difference from group 2. ¶¶ $P < 0.001$, significant difference from group 3.



ng in group 1, 120 ± 20 and 150 ± 15 ng in group 2, and 260 ± 20 and 390 ± 50 ng in group 3 (first phase: group 1 vs. 2, $P < 0.005$; group 2 vs. 3, $P < 0.005$; second phase: group 1 vs. 2, $P < 0.001$; group 2 vs. 3, $P < 0.001$). The same results were noted with respect to the total amount of insulin secreted (Table I). These results demonstrate that arginine-glucose-induced insulin release was clearly enhanced in +D rats but not brought to the control values of the ad lib. rats, even when the dietary intake of calories and calcium was restricted to the amount consumed by the -D control rats.

Effect of varying serum calcium level on arginine (10 mM)-glucose (5 mM)-induced insulin secretion from the isolated perfused pancreas of -D rat (Table II, Fig. 2). Dietary calcium manipulation (from 0.4 to 4%, effected by substituting equal amounts of cellulose) resulted in several different serum calcium levels in -D rats pair-fed with a -D group fed ad lib. as tabulated in Table II. The serum calcium level increased as the dietary calcium levels were elevated. Ionized calcium levels measured in the same plasma sample were found to be about 50% of the total calcium level in each group. Body weight and pancreatic weight were similar among these groups. As shown in Fig. 2 A, the arginine (10 mM)-glucose (5 mM)-elicited insulin secretion presented a typical biphasic pattern. The peak value in the first phase of groups 1, 2, 3, and 4 was 11.7 ± 0.9 , 9.0 ± 1.0 , 11.3 ± 0.8 , and 11.2 ± 1.7 ng/ml, respectively, which were not significantly different from each other. The same results were noted also in the case of the second phase of insulin secretion. Cumulative insulin secretion in the first and second phases showed no difference among these groups (Table II). These results indicate that differences in the prevailing serum calcium levels prior to pancreas perfusion does not affect arginine-glucose-induced in vitro insulin secretion from the isolated perfused pancreas.

Effect of vitamin D repletion on separate arginine (10 mM), glucose (16.7 mM), and tolbutamide (0.37 mM)-induced insulin secretion from the isolated perfused pancreas of the rats pair-fed with -D control rats with normocalcemia (Table III, Fig. 3). This experiment employed normocalcemic rats with or without vitamin D-repletion under conditions of pair-feeding. As shown in Table II, -D rats raised on a 4% dietary calcium levels (group 4) had normal serum calcium levels. Vitamin D repletion of these rats also produced normocalcemia (Table III). Then, the pancreas response of insulin secretion to the separate

Figure 2. Effect of varying serum calcium levels on arginine (10 mM)-glucose (5 mM)-induced insulin secretion from the isolated perfused pancreas of the -D rat. Arginine-glucose-induced insulin secretion was compared between the group with (A) 0.4% Ca (---○---) and 1% Ca (—▲—), (B) 0.4% Ca (---○---) and 2% Ca, (—●—), and (C) 0.4% Ca (---○---) and 4% Ca (—■—), respectively. All pancreases were perfused in the presence of 5.5 mM glucose throughout the experiment (-15 to +30 min). Arginine was introduced from 0 to 20 min to maintain a final concentration of 10 mM. The means \pm SEM of the indicated number of experiments are shown.

Table III. Parameters of -D and +D Animals whose Pancreas Were Perfused with Different Insulin Secretagogues (Experiment 3)

Group	Vitamin D status	n	Secretagogue stimulus	Body weight	Total	Insulin secretion		
						1st phase	2nd phase	Total
			%	g	mg	ng	ng	ng
1	-D	5	arginine, 10 mM	188±4	117±4	63±9	186±8	250±16
2	+D	5	arginine, 10 mM	183±3	117±3	200±10*	380±40‡	640±40*
3	-D	4	glucose, 16.7 mM	187±6	120±8	21±2	38±10	60±13
4	+D	4	glucose, 16.7 mM	193±10	109±6	72±3	220±30*	300±30*
5	-D	5	tolbutamide, 0.3 mM	191±5	119±4	21±3	25±4	46±6
6	+D	5	tolbutamide, 0.3 mM	187±6	118±4	39±2‡	21±5	61±6‡

Results are expressed as mean±SEM of the indicated numbers. +D animals were pair-fed with -D animals for 3-4 wk until the time of pancreatic perfusion. +D, 975 pmol of vitamin D₃ was injected subcutaneously every day for 3-4 wk until the time of pancreatic perfusion. -D, vehicle alone (0.2 ml of 1,2-propanediol) was administered according to the same schedule as the +D group. * $P < 0.001$, † $P < 0.05$, significant difference from -D group in each pair-fed experiment.

secretagogues arginine (10 mM), glucose (16.7 mM) or tolbutamide (0.37 mM), each in the presence of basal glucose, was compared.

Arginine-stimulated insulin secretion was significantly higher in the +D group than the -D group (Fig. 3A). The peak values of the first phase of insulin secretion in the +D group was threefold higher than in the -D group (32±3.5 vs. 11.2±1.7, $P < 0.001$). Insulin release in the second phase also was twofold higher in the +D rats. These differences were also reflected in the cumulative insulin secretion as tabulated in Table III.

In the instance of glucose stimulation, insulin response also exhibited a typical biphasic pattern. The peak value in the first phase appeared at 3 min, which was 2.3-fold higher in the +D group (13.5±2.1 vs. 5.2±0.5 ng/ml; $P < 0.001$). The differences of insulin secretion were more marked in the second phase; here, the cumulative insulin secretion in the first and second phase were about threefold and fivefold higher in +D groups (Table III; Fig. 3B).

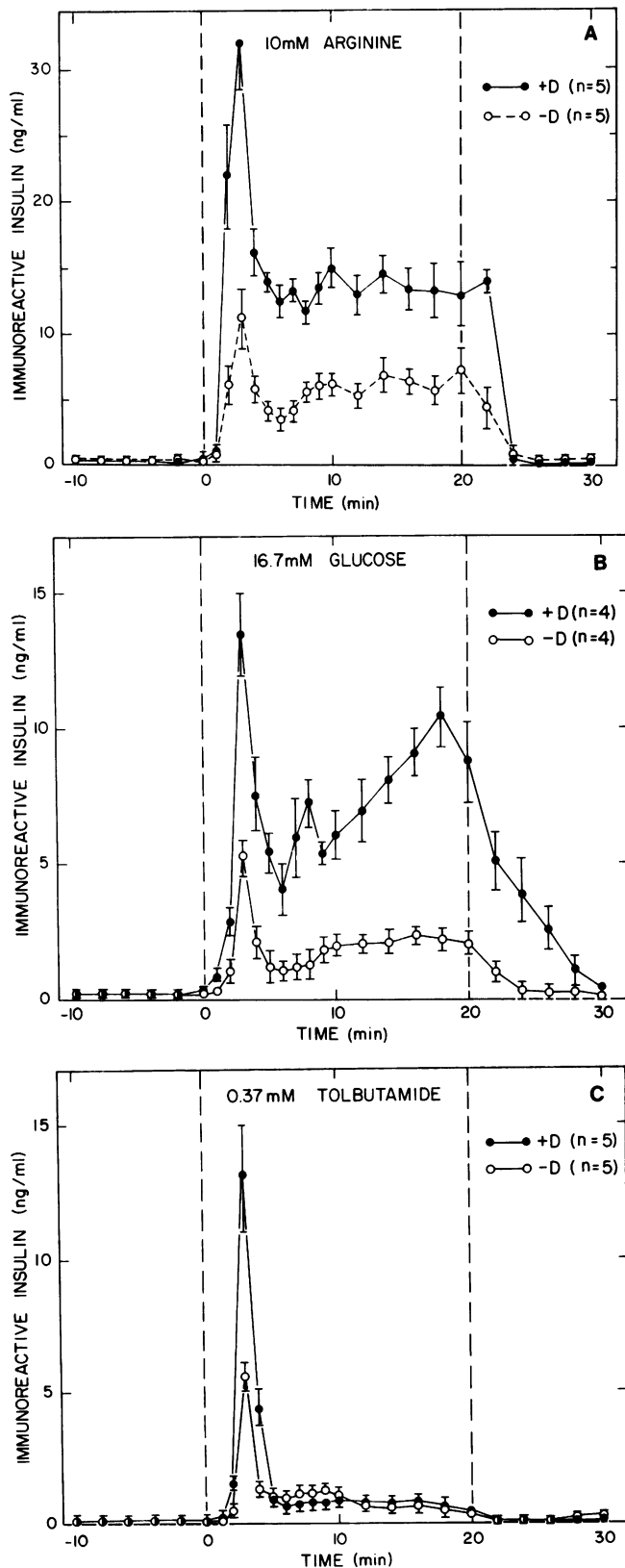
Tolbutamide stimulation demonstrated a typical monophasic insulin response showing peak value at 3 min. This peak value was also 2.3-fold higher in the +D group (13.1±0.8 vs. 5.6±1.0 ng/ml, $P < 0.001$), while no significant difference was found in the second phase of insulin secretion. The cumulative insulin secretion in the first phase also showed a twofold higher value in the +D group, while no difference was found in the second phase (Table III; Fig. 3C).

Discussion

The present results indicate (a) that normalization of serum calcium in -D rats does not normalize arginine-glucose-induced insulin secretion and (b) that equalization of dietary caloric intake between -D and +D rats does not abolish the effect of

vitamin D on mediating insulin secretion. Thus, the present findings confirm our previous report (11) that arginine-glucose-induced insulin secretion from the isolated perfused pancreas was significantly decreased in vitamin D deficiency and improved by dietary vitamin D repletion.

There are several direct and indirect factors known to affect insulin secretion after vitamin D repletion; these include: (a) increased dietary caloric and calcium intake; (b) increased levels of serum total and ionized calcium; (c) changes of hormonal environment, especially parathyroid hormone (PTH); (d) a genomic effect of the vitamin D metabolite, 1,25-(OH)₂-D₃, on pancreatic B cell, involving the production of vitamin D-dependent CaBP, which may influence intracellular calcium metabolism and enzyme activation; and (e) putative membrane effects of vitamin D metabolite on pancreatic B cell. It is generally accepted that insulin release in response to glucose (22-24) and amino acids (25, 26) is decreased by fasting. Since a twofold higher dietary intake was found in the +D group than in the -D control group (30±4 vs. 15±2 g/d, $P < 0.05$) as described in Results, experiment 1 was carried out under pair-feeding conditions to eliminate the difference of dietary intake between -D and +D groups. The results in experiment 1 demonstrated a significantly higher insulin secretion in response to arginine-glucose in the +D rats than the -D controls, suggesting the involvement of other factors rather than dietary factors in this mechanism. Moreover, experiment 2 demonstrated that establishment of various levels of serum calcium in -D rats before pancreatic perfusion resulted in no significant difference in the arginine-glucose-induced insulin secretion in vitro. Even when serum calcium was normalized (total Ca equals 10.0±0.3 mg/dl and ionized Ca equals 5.2±0.1 mg/dl; group 4, Table II), arginine-induced insulin secretion was not improved. It is possible that a portion of the blunting of the response to arginine-



glucose may be due to an impairment in glucose recognition by the pancreas in the -D animal.

As shown in Figure 3 A, B, and C, similar results were obtained with the insulin secretagogues arginine, glucose, and tolbutamide, all studied separately. For all three secretagogues, there was observable a clear-cut effect of vitamin D status on insulin secretion even when the prevailing serum Ca levels were normalized in vivo as well as in vitro by perfusion of the pancreas with a solution containing 2.5 mM Ca. Thus, our present results support the conclusion that vitamin D status plays an important role in insulin secretion irrespective of the dietary caloric, dietary calcium, or previously prevailing serum calcium levels.

It is generally accepted that Ca^{++} plays a crucial role in insulin secretion and a rise in pancreatic B cell intracellular Ca^{++} concentration is now considered to be a major controlling influence in the stimulus-secretion coupling response (27, 28). These intracellular Ca^{++} levels are believed to be influenced not only by the Ca^{++} influx from extracellular sites into the cells, but also by the uptake and release of Ca^{++} by the B cell's intracellular organelles. Thus, Kikuchi et al. (29) have shown that glucose-induced insulin release was enhanced in the Ca^{++} -loaded islet preincubated in high phosphate (5 mM) media as compared with normal (1 mM) phosphate media, when both islets were perfused under low Ca^{++} concentrations (0.1 mM) to minimize the effect of extracellular calcium.

The results of Kikuchi et al. (29) also indicated that the handling of intracellular calcium is primarily responsible for the first phase of insulin secretion, whereas both intracellular and extracellular calcium are involved in the second phase. In the present studies, both phases of insulin secretion in response to glucose were more clearly enhanced in +D pancreas than -D pancreas (Fig. 3 B), even if the extracellular calcium concentration both in vivo and in vitro was kept similar. This result may suggest that the reduced insulin secretion associated with the vitamin D deficiency of this study may be related to a reduced intracellular calcium storage, which in turn leads to a reduction in the level of intracellular calcium available for insulin secretion. Thus, vitamin D status may determine the pancreatic B cell levels of stored calcium.

The relationship between the calciotropic hormonal environment and B cell function should also be considered, since PTH and calcitonin are inevitably changed before and after vitamin D administration. It is known that the plasma PTH level is elevated in vitamin D deficiency in the pig (30) and rat (31), and it is possible that chronic PTH secretion may inhibit

Figure 3. Effect of vitamin D repletion on separate (A) arginine (10 mM), (B) glucose (16.7 mM), and (C) tolbutamide (0.37 mM)-induced insulin secretion from the isolated perfused pancreas of the rats pair-fed with -D control rats with normocalcemia. All pancreases were perfused in the presence of 5.5 mM glucose throughout the experiment (-15 to +30 min). The insulin secretagogue was introduced from 0 to 20 min to maintain the appropriate final concentration (see Methods). The means \pm SEM of the indicated number of experiments are shown.

B cell function during vitamin D deficiency. However, Rebodello (32) found no effect of PTH status on the secretion of insulin by the isolated perfused rat pancreas.

An obvious explanation for the involvement of vitamin D and its metabolites in pancreatic insulin secretion relates to the reports of the presence of a vitamin D-dependent CaBP in the pancreas of the rat (6), chick (4), pig (5), cat (9), dog (9), and mouse (9).

In this respect, recent investigation by Roth et al. (8) has clearly demonstrated the cellular localization of CaBP in the chick pancreas by the immunohistochemical protein-A-gold technique. These results have shown that pancreatic CaBP is exclusively localized in the endocrine B cells of the chick pancreas and not in the A (glucagon) cells and D (somatostatin) cells. In the B cells, CaBP was not only found in the cytosol, but also in subcellular organelles, such as secretory granules (Norman, A. W., unpublished observations), suggesting a possible relationship between CaBP and B cell function to secrete insulin.

At the present time, the biochemical function or physiological role of the vitamin D-dependent CaBP is not known even in sites of its highest cellular concentration, e.g., the intestinal epithelial cell (33) and the kidney (34). Also, this same CaBP has been found in other locations, including the cerebellum (7), and central nervous system's Perkinge cells (35). It is to be anticipated that further study of the exact mechanisms by which vitamin D or its metabolites improve insulin secretion may well provide insight into the general biological function of CaBP.

Acknowledgments

This work was supported in part by grant A-82-71 from the Kroc Foundation and by U. S. Public Health Service grant Am-09012-019.

References

1. Norman, A. W., J. Roth, and L. Orci. 1982. The vitamin D endocrine system: steroid metabolism, hormone receptors and biological response (calcium binding proteins). *Endocrine Reviews*. 3:331-366.
2. Christakos, S., and A. W. Norman. 1981. Studies on the mode of action of calciferol XXIX. Biochemical characterization of 1,25-dihydroxyvitamin D₃ receptors in chick pancreas and kidney cytosol. *Endocrinology*. 108:140-149.
3. Pike, J. W., L. L. Gooze, and M. R. Haussler. 1980. Biochemical evidence for 1,25-dihydroxyvitamin D₃ receptor-like macromolecule in parathyroid, pancreatic, pituitary and placental tissue. *Life Sci*. 26:407-416.
4. Pike, J. W. 1981. Receptors for 1,25-dihydroxyvitamin D₃ in chick pancreas: a partial physical and functional characterization. *J. Steroid Biochem*. 16:385-395.
5. Arnold, B. M., M. Kuttner, D. M. Willis, J. W. Hitchman, J. E. Harrison, and T. M. Murray. 1975. Radioimmunoassay studies of intestinal calcium-binding protein in the pig. II. The distribution of intestinal CaBP in pig tissues. *Can. J. Physiol. Pharmacol*. 53:1135-1140.
6. Best, L., and W. J. Malaisse. 1983. Phosphatidylinositol and phosphatidic acid metabolism in rat pancreatic islets in response to neurotransmitter and hormonal stimuli. *Biochim. Biophys. Acta*. 750:157-163.
7. Christakos, S., E. J. Friedlander, B. R. Frandsen, and A. W. Norman. 1979. Studies on the mode of action of calciferol XIII. Development of a radioimmunoassay for vitamin D-dependent chick intestinal calcium binding protein and tissue distribution. *Endocrinology*. 104:1495-1502.
8. Roth, J., S. Bonner-Weir, A. W. Norman, and L. Orci. 1982. Immunohistochemistry of vitamin D-dependent calcium binding protein in chick pancreas: exclusive localization in B-cells. *Endocrinology*. 116:2216-2218.
9. Morrissey, R. L., T. J. Bucci, R. N. Empson, and E. G. Lufkin. 1975. Calcium-binding proteins: its cellular localization in jejunum, kidney and pancreas. *Proc. Soc. Exp. Biol. Med*. 148:56-60.
10. Stumpf, W. E., M. Sar, and H. F. DeLuca. 1981. Sites of action of 1,25(OH)₂-vitamin D₃ identified by thaw mount autoradiography. In *Hormonal Control of Calcium Metabolism*. D. V. Cohn, R. V. Talmadge, and J. L. Matthews, editors. Excerpta Medica, Amsterdam. 222-229.
11. Norman, A. W., B. J. Frankel, A. M. Heldt, and G. M. Grodsky. 1980. Vitamin D-deficiency inhibits pancreatic secretion of insulin. *Science (Wash. DC)*. 209:823-825.
12. Clark, S. A., W. E. Stumpf, and M. Sar. 1981. Effect of 1,25-dihydroxyvitamin D₃ on insulin secretion. *Diabetes*. 30:382-386.
13. Nemere, I., and A. W. Norman. 1982. Vitamin D and cell membranes. *Biochim. Biophys. Acta*. 694:307-327.
14. Steenbock, H., and D. C. Herting. 1955. Vitamin D and growth. *J. Nutr*. 57:449-468.
15. Grodsky, G. M., A. A. Batts, L. L. Bennet, C. Viella, N. B. McWilliams, and D. H. Smith. 1963. Effects of carbohydrates on secretion of insulin from isolated rat pancreas. *Am. J. Physiol*. 205:638-644.
16. Kadowaki, S., T. Taminato, T. Chiba, K. Mori, H. Abe, Y. Goto, Y. Seino, S. Matsukura, M. Nozawa, and T. Fujita. 1979. Somatostatin release from isolated perfused rat pancreas—possible role of endogenous somatostatin on insulin release. *Diabetes*. 28:600-603.
17. Herbert, V., K. S. Lau, C. W. Gottlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab*. 25:1375-1384.
18. Raabo, E., and T. C. Terkildsen. 1960. On the enzymatic determination of blood glucose. *Scand. J. Clin. Lab. Invest*. 12:402-411.
19. Perkin-Elmer Instruction Manual. 1973. Analysis of serum: determination of calcium. Perkin-Elmer Corp., Norwalk, CT. BC-1R.
20. Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem*. 28:1756-1758.
21. Bishop, J. E., A. W. Norman, J. W. Coburn, P. A. Roberts, and H. L. Henry. 1980. Studies on the metabolism of calciferol. XVI. Determination of the concentration of 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D in a single two milliliter plasma sample. *J. Min. Elec. Metab*. 3:181-189.
22. Cahill, G. F., Jr., M. G. Herrera, A. P. Morgan, J. S. Soeldner, J. Steinke, P. L. Levy, G. A. Reichard, Jr., and D. M. Kipnis. 1966. Hormone-fuel interrelationships during fasting. *J. Clin. Invest*. 45:1751-1769.
23. Vance, J. E., K. D. Buchanan, and R. H. Williams. 1968. Effect of starvation and refeeding on serum immunoreactive glucagon and insulin levels. *J. Lab. Clin. Med*. 72:290-297.
24. Malaisse, W. J., F. Malaisse-Lagae, and P. H. Wright. 1967. Effect of fasting upon insulin secretion from in the rat. *Am. J. Physiol*. 213:843-848.
25. Ashcroft, S. J. 1976. The control of insulin release by sugars. *Ciba Found. Symp*. 41:117-139.
26. Matschinsky, F. M., C. Rujanaveck, A. S. Pagliara, and W. T. Norfleet. 1980. Adaptation of α - and β -cells of rat and mouse pancreatic

islets to starvation, to refeeding after starvation, and to obesity. *J. Clin. Invest.* 65:207-218.

27. Douglas, W. W. 1968. Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. The First Caddum Memorial Lecture, Cambridge, September 1967. *Br. J. Pharmacol.* 34:451-478.

28. Wallheim, C. B., M. Kikuchi, A. E. Renold, and G. W. G. Sharp. 1978. The role of intracellular and extracellular Ca^{++} in glucose-stimulated biphasic insulin release. *J. Clin. Invest.* 62:451-458.

29. Kikuchi, M., C. B. Wallheim, E. G. Siegel, A. E. Renold, and G. W. G. Sharp. 1979. Biphasic insulin release in rat islets of Langerhans and the role of intracellular Ca^{++} stores. *Endocrinology.* 105:1013-1019.

30. Pointillart, A., M. Thomasset, and J. M. Garel. 1979. Effect of vitamin D-deficiency on calcium-binding protein (CaBP) and on plasma calcitonin (CT) and parathyroid hormone (PTH) levels in growing pigs. In *Vitamin D: Basic Research and its Clinical Application*. A. W. Norman, K. Schaefer, D. von Herrath, H.-G. Grigoleit, J. W. Coburn, H. F. DeLuca, E. B. Mawer, and T. Suda, editors. Walter de Gruyter & Co., Berlin. 275-278.

31. Holtrop, M. E., M. F. Holick, and D. L. Carnes. 1982. Mineralization of bones as a reflection of serum calcium and serum phosphorus levels in rats on vitamin D-deficient diets. In *Vitamin D: Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism*. A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. von Herrath, editors. Walter de Gruyter & Co., Berlin. 197-202.

32. Rebolledo, O. 1975. Failure of parathyroid hormone to affect insulin and glucagon release from the isolated perfused rat pancreas. *Horm. Metab. Res.* 7:287-290.

33. Thorens, B., J. Roth, A. W. Norman, A. Perrelet, and L. Orci. 1982. Immunohistochemical localization of the vitamin D-dependent calcium-binding protein in chick duodenum. *J. Cell Biol.* 94:201-206.

34. Roth, J., D. Brown, A. W. Norman, and L. Orci. 1983. Localization of the vitamin D-dependent calcium binding protein in mammalian kidney. *Am. J. Physiol.* 243:243-252.

35. Garcia-Segura, L. M., D. Baetens, J. Roth, A. W. Norman, and L. Orci. Immunohistochemical mapping of the calcium-binding protein immunoreactivity in the rat central nervous system. *Brain Res.* In press.