Islet Insulin Release and Net Calcium Retention In Vitro in Vitamin D–Deficient Rats

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SUMMARY

In our previous studies, perifused islets from vitamin D–deficient (D-def) rats showed marked impairment of glucose-induced biphasic release, accounted for at least in part by a decrease in food intake. In studies reported here, we test whether D-def rat islets have an impaired response to 5.6 mM glucose or tolbutamide, (T), and if so, whether this impairment is related to a decrease in food intake or a defect in islet calcium metabolism. We isolated islets of normal rats, D-def rats, and rats pair fed (PF) to D-def rats. Biphasic insulin release from perifused islets and net ^{45}Ca retention in lot-incubated islets were measured in response to 5.6 mM glucose, 0.37 mM T, or both.

Compared with secretion from normal islets, biphasic insulin release from islets of both D-def rats and PF rats was diminished by >50% in response to 5.6 mM glucose alone or 5.6 mM glucose plus T. Insulin secretion was not significantly different between islets of D-def rats and islets of PF rats. In contrast, net calcium retention in islets of D-def rats was decreased to 68% of retention in islets of PF rats. However, net calcium retention in islets of both PF and D-def rats increased in response to T.

The pair-feeding experiments suggest that the decrease in insulin release from islets of D-def rats is due to the decrease in food intake associated with the D-def state. On the other hand, the defect in calcium retention in islets of D-def rats raises the possibility that vitamin D may have a specific effect on islet calcium metabolism. In this case, the mechanism of impaired insulin release in islets of D-def rats would be different from that in islets of PF rats and would involve a defect in intracellular calcium handling. DIABETES 1986; 35:771–75.

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Recent studies showing the presence of a cytosolic receptor protein for 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$],[1] the localization of tritiated $1,25(OH)_2D_3$ to the nucleus of rat pancreatic β-cells,[2] and presence of vitamin D–dependent calcium-binding proteins in chick β-cells[3] suggest that the islet β-cell is a target site of action for vitamin D. This is an attractive hypothesis because vitamin D stimulates calcium transport,[4,5] and cytosolic calcium plays a critical role in insulin release.[6]

In our previous studies,[7] we reported impairment of glucose-induced insulin release from islets of vitamin D–deficient (D-def) rats. Islets from D-def rats also had a defect in net calcium retention in response to glucose. Further studies in which normal rats were pair fed to D-def rats suggested the defect in insulin secretion and net calcium retention were not specific for vitamin D deficiency but related to an associated decrease in food intake.

To further evaluate the relation between vitamin D deficiency and impaired insulin release, we tested *1*) whether the defect was specific for glucose or also occurred in response to another secretagogue and, if so, the relation of the defect to food intake and *2*) the relation of the defect in insulin release to calcium retention. For both purposes, we used tolbutamide, a calcium ionophore[8,9] that stimulates insulin release by stimulating calcium influx.[10] Hence, tolbutamide provided us with a tool to study both impaired insulin release and the defect in cellular calcium handling in the D-def rat.

MATERIALS AND METHODS

The model for vitamin D deficieny is described in our previous article.[7] Normal rats were fed a rat chow diet. To produce vitamin D deficiency, rats at weaning were fed a synthetic D-def diet containing 0.47% calcium and 0.3% phosphorus prepared by H. F. D. (standard diet #11) as described by Suda et al.[11] Rats pair fed to D-def rats were repleted by adding 83 μg of vitamin D_3 to 1 kg of diet. In our laboratory,

TABLE 1
Effect of glucose, 5.6 mM, and tolbutamide 100 μg/ml (0.37 mM), on net ^{45}Ca retention and insulin release from islets of normal, pair-fed, and vitamin D–deficient rats

Group	Exp.	Serum calcium (mg/dl)	Insulin (μU/islet, 3–60 min)	Calcium retention (pmol/islet, 90 min)
A	Normal		14.4 ± 3.9 (7)	6.57 ± .40 (36) [63.3 ± 5.9]
B	Normal/T		36.4 ± 6.2† (7)	7.43 ± .51 (38)
C	Pair fed	11.6 ± .5	5.3 ± 1.10 (5)	8.06 ± 1.10 (24) [37.4 ± 7.0]
D	Pair fed/T		15.3 ± 5.6* (5)	12.29 ± 1.11† (26)
E	D-def	5.1 ± .4	6.3 ± 1.8 (5)	5.47 ± .50 (36) [35.5 ± 5.7]
F	D-def/T		15.1 ± 3.2† (5)	12.60 ± 1.63‡ (27)

Cumulative insulin secretion from normal islets was more than twofold secretion from pair-fed and D-def islets in the absence (analysis of variance, $P < .05$) or presence (analysis of variance, $P < .01$) of tolbutamide (T). Tolbutamide stimulated insulin release from normal (B vs. A), pair-fed (D vs. C), and D-def (F vs. E) islets, but this effect was not significantly different between pair-fed and D-def islets (F vs. D). Net ^{45}Ca retention was less in islets from D-def rats than islets from pair-fed rats (E vs. C, $P < .05$). Tolbutamide stimulated net calcium retention in pair-fed (D vs. C) and D-def (F vs. E) rats, but this effect was not different between pair-fed and D-def islets (F vs. D).
The normal serum calcium in our laboratory is 10.0 ± .14. Numbers in parentheses are number of experiments of five islets each for calcium retention and number of paired perifusions for insulin release. The glucose concentration was 5.6 mM during the perifusion or incubation. *$P < .05$; †$P < .01$; ‡$P < .001$ for significant difference between groups B vs. A, D vs. C, or F vs. E.
For comparison, calcium retention data in response to 16.7 mM glucose obtained in our previous studies[7] are shown in brackets. Comparisons with low glucose data may not be quantitatively completely relatable because they were performed at different times with different islets.

in studies comparing the effects of diet, biphasic insulin secretion does not differ significantly between perifused isolated islets of rats fed rat chow and islets of rats fed vitamin-replete diet #11.

Islet isolation, perifusion, culture, and methods of assaying insulin have been previously described.[7,11,12] Fifty islets were placed in control and test chambers and perifused during each experiment. After a 60-min basal perifusion with Krebs-Ringer bicarbonate buffer containing 2.8 mM glucose, a test perifusion was performed with buffer containing concentrations of 5.6 mM glucose with and without 0.37 mM (100 μg/ml) tolbutamide. Because the potency of tolbutamide is dependent on the concentration of glucose, a concentration of 5.6 mM glucose was used during the test period to ensure biphasic insulin release in response to tolbutamide, as well as to test the effects of vitamin D deficiency on insulin release in response to a low stimulatory concentration of glucose alone. Samples were collected throughout the perifusion and radioimmunoassayed for insulin at the time points indicated in the figure. Cumulative insulin secretion on exposure to glucose or test agent was determined for the first (3–8 min) or second (8–60 min) phase by calculating the area under the curve. Perifusion experiments were designed to be tested for statistical significance by the paired *t* test, and experiments on net calcium retention were designed to be tested for significance by the group *t* test. Analysis of variance within and between groups was used to test for significant differences in insulin secretion between three groups (normal, D-def, and pair fed).

As in previous experiments,[7] we studied the relationship of food intake to the defect in insulin release and net calcium retention. We compared tolbutamide-induced insulin release and net calcium retention in islets of D-def rats to insulin release and net calcium retention in islets of normal rats that were pair fed to D-def rats. If the defects in insulin release and calcium retention in vitamin D deficiency are secondary to diminished food intake, then similar findings should be evident in studies with pair-fed rat islets. Pair feeding of normal rats was accomplished by feeding normal rats an amount of food equal to that consumed by D-def rats during the preceding 24 h for a 4- to 6-wk period after weaning. Weight of food eaten was measured daily during the pair feeding and body weight measured every 2 or 3 days.

The methods for studying calcium have been previously described.[13,14] Islets were incubated in media containing ^{45}Ca for 90 min at 37°C. After incubation, islets in batches of five were removed, washed, and placed in liquid scintillation fluid. Experiments included from 2 to 10 batches of five islets each, depending on the number of islets studied that day. Experiments were repeated on 4 or 5 different days with islets from different rats. The mean ^{45}Ca uptake, expressed in picomoles per islet, was calculated from the ^{45}Ca uptake and the specific activity of ^{45}Ca in the medium.

RESULTS

Effects of vitamin D deficiency on insulin release in response to 5.6 mM glucose. In 5.6 mM glucose alone (in the absence of tolbutamide), insulin secretion from D-def and pair-fed islets was less than normal. Total insulin release from normal islets was more than twofold the secretion from pair-fed or D-def islets. Insulin secretion was not different between pair-fed and D-def islets (Table 1, Figure 1).

Effects of vitamin D deficiency on insulin release in response to tolbutamide 0.37 mM. With 5.6 mM glucose, tolbutamide stimulated biphasic insulin release from D-def rat islets. As seen with glucose alone, insulin release from normal islets was increased more than twofold when compared with secretion from pair-fed and D-def islets, but insulin secretion was not significantly different between islets of pair-fed and D-def rats. In this experiment, despite comparable food intake, D-def rats weighed significantly less than pair-fed controls (D-def, 279 ± 9 g; pair fed 332 ± 6 g, $P < .05$). This observation suggests that D-def rats do not gain as much weight per gram of food ingested as D-replete rats, perhaps absorbing less of their food or utilizing their nutrients less efficiently. A similar observation is evident on the analyses of data on food intake and weight gain in D-def and D-replete rats originally published by Steenbock and Herting.[15]

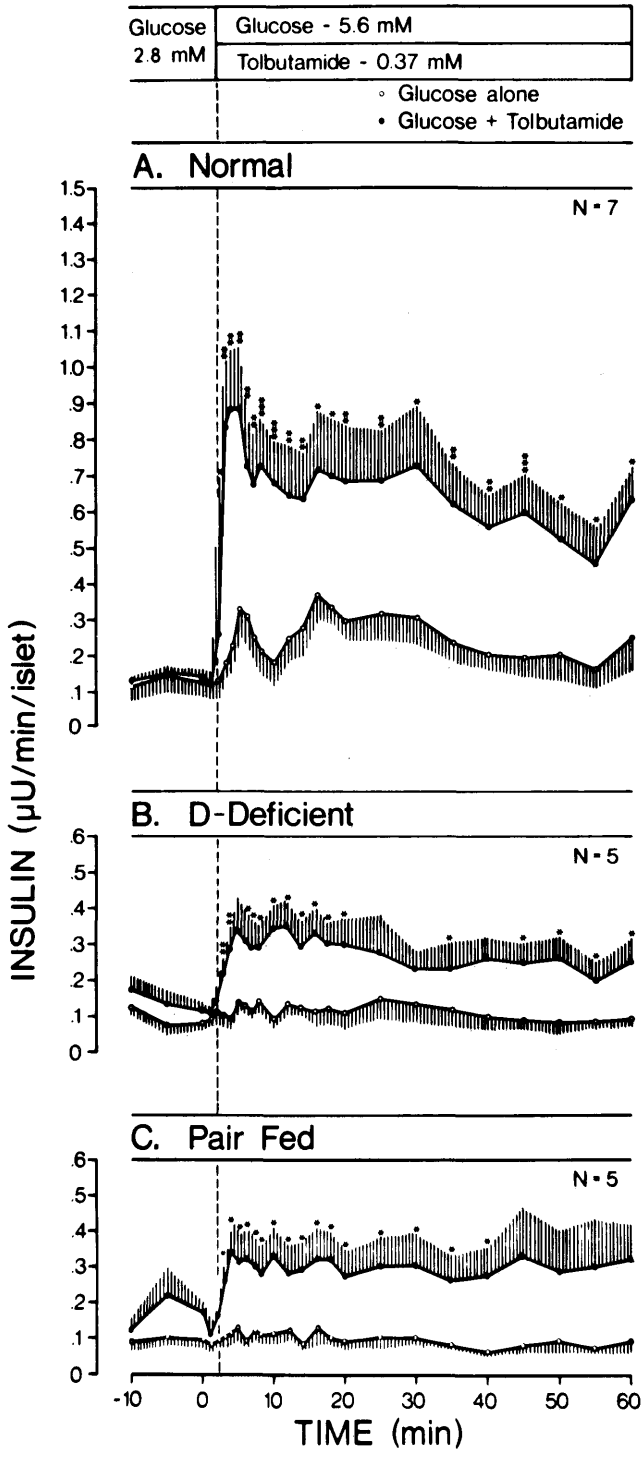

FIGURE 1. Effect of tolbutamide on biphasic insulin release in vitro from islets of normal (*A*), vitamin D–deficient (D-def; *B*), and pair-fed (*C*) rats. During pretreatment or washout period, islets were perifused for 60 min in basal media containing 2.8 mM glucose. At time 0, media was changed to test media. *Horizontal bar* at *top* indicates concentration of glucose, tolbutamide, or both. Insulin was assayed at points indicated from 10 min before time 0 and for 60 min thereafter. Islets from both D-def and pair-fed rats showed decreased first- and second-phase insulin release in response to glucose alone or glucose plus tolbutamide compared with normal islets. However, differences between islets of D-def and pair-fed rats were not significantly different from each other. SEM is shown by *hatched lines*. N, number of experiments. *$P < .05$; †$P < .01$; ‡$P < .001$ for significant differences between control and test islets.

Effects of vitamin D deficiency on net calcium retention in response to 5.6 mM glucose. Net calcium retention in response to 5.6 mM glucose was much less than that observed in previous studies[7] with 16.7 mM glucose. In contrast to previous studies,[7] net calcium retention in response to 5.6 mM glucose in islets of D-def rats was not diminished compared with islets of normal rats. However, when compared with appropriate control islets, i.e., islets from pair-fed rats, the interpretation is different. Islets from pair-fed rats had an increase in uptake compared with islets from normal rats. When uptake in islets of D-def rats is compared with uptake in islets of pair-fed rats, ^{45}Ca uptake in response to 5.6 mM glucose in islets of D-def rats was diminished to 68% of that from islets of pair-fed control rats (Table 1, group E vs. group C).

Effects of tolbutamide on calcium retention. With 5.6 mM glucose, 0.37 mM tolbutamide significantly increased net calcium retention in islets of pair-fed and D-def rats. The responses between islets of pair-fed and D-def rats were not significantly different.

DISCUSSION

Several questions regarding the impairment of insulin release are: *1*) Does vitamin D deficiency specifically impair insulin release, or are the effects of D deficiency related to decreased food intake? *2*) What is the relation between the defects in calcium retention and impaired release? and *3*) Is the response to tolbutamide specifically impaired or related to the impaired response to glucose?

Regarding the first question, the mechanism for decreased insulin release in response to both glucose and tolbutamide may be the same for D-def and pair-fed rats. Decreased food intake in the D-def rat and food restriction in the pair-fed rat could conceivably lead to similar abnormalities in calcium uptake, glucose metabolism, or cAMP production, which, in turn, would impair insulin release.

Our previous studies[7] suggested that defects in glucose-induced insulin secretion from islets of D-def rats were not specific but related to changes in food intake. In those studies, insulin responses to 16.7 mM glucose from islets of pair-fed and D-def rats were decreased similarly. Restriction of food intake prevented the increase in insulin secretion observed after treatment of D-def rats with $1,25(OH)_2D_3$. Our findings reported here are similar for tolbutamide-induced insulin secretion; i.e., secretion from islets of pair-fed and D-def rats was decreased similarly in response to tolbutamide.

Regarding the second question, about the relation of the defect in calcium retention to the impairment of insulin release, we have demonstrated defects in net calcium retention in islets of D-def rats at 5.6 and 16.7 mM glucose and in islets of pair-fed rats at 16.7 mM glucose. (See Table 1 for previous results.) Because insulin secretion is calcium dependent,[16] these defects in net calcium retention could impair insulin release. On the other hand, because impairment of insulin release with decreased food intake may be related to abnormalities other than the handling of calcium,[17–19] these defects in net calcium retention in both pair-fed and D-def rats may be coincidental and unrelated to the observed impairment of insulin release.

Unlike previous studies at 16.7 mM glucose,[7] at 5.6 mM

glucose, islets of pair-fed rats did not have a defect in calcium retention. The reason for the difference in net ^{45}Ca retention in islets of pair-fed rats at 5.6 and 16.7 mM glucose is not known. Studies of fasting rats and mice show either decreased,[20] unchanged,[20] or increased[21] retention of calcium, depending on the duration of fasting, the concentration of glucose in the medium, and the duration of islet incubation. Because the experimental protocols in our studies were identical except for the glucose concentration, the difference in calcium retention at 16.7 and 5.6 mM glucose in islets of pair-fed rats must be due to the decrease in glucose concentration. The effects of pair feeding on cellular calcium retention and insulin release may under certain conditions but not others mimic the effects of D deficiency per se.

Regarding the third question, we cannot say whether the response to tolbutamide is specifically impaired or related to the impaired response to glucose. Although the absolute level of insulin release in response to tolbutamide was decreased, the increase relative to glucose alone was similar among D-def, pair-fed, and normal rats. The similar increase, when expressed as a percent of the response to glucose alone, raises the possibility that the islets secrete insulin at a low rate because of a decreased functional pool or content of insulin, decreased glucose metabolism, or a defect in calcium handling, and, as a result, the response to tolbutamide is nonspecifically impaired.

The presence of a defect in calcium retention in response to glucose[7] suggested that a similar defect might be evident in response to tolbutamide. On the contrary, we found that tolbutamide increased net calcium retention in islets of D-def rats. If tolbutamide increases calcium retention by stimulating calcium uptake,[10,16] the increase in net calcium retention in response to tolbutamide indicates that the D-def islet can transport calcium across its cell membrane.

Our observations indicate that vitamin D deficiency is associated with marked impairment of insulin release. The impairment of insulin release occurs not only in response to 16.7 mM glucose stimulation but also to 5.6 mM glucose and tolbutamide. Our studies on insulin release from islets of pair-fed rats again suggest that the effects of vitamin D deficiency may not be related directly to vitamin D, per se, but may be attributed in part indirectly to the decrease in food intake associated with the D-def state. On the other hand, a defect in islet net calcium retention in the D-def state may be unrelated to the associated decrease in food intake, may result directly from vitamin D deficiency, and may cause impairment of insulin release. Others,[22] also with pair-fed rats, have suggested that the effects of vitamin D deficiency on insulin secretion are specific. However, despite pair feeding, the weights of pair-fed rats, although not statistically significant, appeared to be greater than D-def rats, and the changes in food intake and weight gain with $1,25(OH)_2D_3$ treatment were not given. This difference in weight gain between D-def and pair-fed rats was observed in our study.

As discussed in our previous studies,[7] D-def and pair-fed rats differ in their eating habits. Vitamin D–deficient rats eat less over 24 h. Pair-fed rats were fed in one serving, ate it rapidly within a few hours, and were without food for the remaining 24 h. We do not know whether these two types of eating patterns would affect insulin release from isolated islets differently in rats already eating reduced amounts of food. It is also possible that the differences in eating habits may affect calcium uptake. We also observed that pair-fed rats had a higher serum calcium than normal rats, which may or may not affect insulin secretion or calcium uptake. Although our studies have pointed out certain limitations and difficulties in separating out possible effects of vitamin D deficiency from effects of pair feeding, future carefully designed studies measuring dynamic changes in islet calcium influx and efflux at the cellular and subcellular level in response to stimuli[23] should demonstrate differences between the effects of vitamin D deficiency and pair feeding. This information may provide further insight into the mechanism of vitamin D action in insulin secretion and an explanation for the defects in insulin release from islets of D-def rats.

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