Effect of vitamin D₃ on the pancreatic secretion of insulin and somatostatin

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Abstract. To clarify the role of vitamin D in the regulation of the endocrine pancreas, we have studied insulin and somatostatin secretion in vitamin D deficient rats (reared on a vitamin D deficient diet), D-replete rats (reared on a vitamin D deficient diet and given 160 IU of vitamin D₃ sc twice a week) and control rats (reared on laboratory chow), using the isolated perfused rat pancreas.

In the vitamin D deficient rats, the perfusate insulin induced by 16.7 mM glucose was only 35% of the secretion in the control rats. In the D-replete rats, the insulin release was restored to that of the controls. Similarly, the plasma insulin level in the vitamin D deficient rats was very low and the level in the D-replete rats was also restored to the level of the controls. The perfusate somatostatin response to glucose was not significantly different in any of the three groups. In addition, since the plasma calcium level in the vitamin D deficient rats was very low and in the D-replete rats was still lower, compared to normal rats, we suggest that vitamin D acts not only via the plasma calcium level but possibly also directly on the B cell.

It is well known that vitamin D is metabolized to 25-hydroxy vitamin D (25 OHD) and subsequently to several derivatives of the dihydroxylated forms, of which 1,25-dihydroxy vitamin D (1,25(OH)₂D) and 24,25-dihydroxy vitamin D (24,25(OH)₂D) appear to be the most important (DeLuca 1979). It is widely believed that 1,25(OH)₂D is the most active hormonal form of vitamin D, and that it stimulates intestinal calcium transport (Holick et al. 1971) and bone mobilization (Raisz et al. 1972).

Recently, Christakos et al. (1979) have presented evidence for the existence of specific high affinity binding proteins/receptors for 1,25(OH)₂D₃ in the chick pancreas (Christakos & Norman 1981) as well as in the intestine, bone, and parathyroid gland (Wecksler et al. 1977, 1979; Kream et al. 1977). Although a few reports (Clark et al. 1981; Norman et al. 1980) have suggested an interrelationship between vitamin D and endocrine pancreatic function, the details are still unclear. In order to clarify the effect of vitamin D on pancreatic B and D cells, insulin and somatostatin release in response to glucose was measured using the isolated perfused D-deplete and D-replete rat pancreas.

Materials and Methods

Animals

Three week old weanling male Wistar rats were divided into three groups. Group I (control) was reared on a nutritionally complete diet containing 1.2% calcium (Ca), 0.8% phosphate (P) and 5.3 IU vitamin D/g (Laboratory chow, Oriental Yeast Co. Ltd., Japan). Group II (vitamin D-deficient) was fed a rachitogenic vitamin D deficient diet containing 0.02% Ca and 0.8% P.

Group III (vitamin D-replete) was given 160 IU of vitamin D₃ sc, twice a week, in addition to the vitamin D deficient diet described above. Control rats and vitamin D deficient rats were injected twice a week with vehicle alone. All animals were housed in a conditioned room with 12/12 h light/dark cycle and an ambient tempera-
ture of 25°C and were fed ad libitum and given free access to water. After 7–8 weeks, the rats were studied according to the perfusion system described below.

Perfusion system
The technique of Grodsky et al. (1963), with minor modifications as previously reported (Goto et al. 1978) was used. The pancreas preparation included the attached segment of the duodenum. The splenic veins were ligated, and the left gastric branches of the right and left gastro-epiploic artery and vein were ligated and transected collective in small groups. All perfusions were accomplished with 4.4 mM glucose in Krebs-Ringer bicarbonate buffer supplemented with 0.25% bovine serum albumin, 2.5 mM Ca, and 4.6% dextran (mean vol wt 70,000).

The medium was gassed with 95% O2 and 5% CO2 and maintained at pH 7.4 at 37°C. The flow rate was kept constant at 1.9 ml/min. After an equilibration period of 20 min, glucose was introduced over a 20 min period through a side arm pump to provide a final concentration of 16.7 mM. The animals were fasted overnight and blood was collected from the cervical vein before they were killed. The blood was chilled in ice, centrifuged, and stored at −20°C until the time of assays for 25 OHD, calcium, glucose, and insulin. Plasma calcium levels were determined with an autoanalyzer and plasma glucose levels by the glucose oxidase method.

Competitive protein binding assays and radioimmunoassay
Plasma 25 OHD was measured by competitive protein binding assay, using normal rat serum as the binding protein (Shimotsuji & Seino 1980). The minimum detectable level was 0.25 ng/tube. Somatostatin was measured by specific radioimmunoassay, using antiseraum T-316

Fig. 1.
Data taken from fasting animals immediately before isolation of the pancreas. Vertical bars represent mean ± SE of control, D-deficient and D-replete rat.
and synthetic cyclic somatostatin as standard, as previously reported (Arimura et al. 1975). The minimum detectable quantity was 10 pg/ml. Insulin was measured by radioimmunoassay by the polyethylene glycol method (Desbuquois & Aurbach 1971) using rat insulin as standard.

Statistical analysis was performed using Student’s t-test for unpaired groups (values given as mean ± SEM).

Results

Body weight, plasma 25 OHD, calcium, glucose, and insulin levels

As noted in Fig. 1, the body weight of the vitamin D deficient rats was 135 ± 10 g, significantly lower than the controls (265 ± 9 g) (P < 0.001). The body weight of the D-replete rats was 185 ± 28 g, significantly higher than the D-deficient rats (P < 0.05) but still lower than the controls (P < 0.02). The plasma concentration of 25 OHD in the D-deficient rats decreased to an undetectable level (below 2 ng/ml), whereas the plasma 25 OHD level in the D-replete rats (20.5 ± 3.4 ng/ml) was slightly but not significantly higher than in the normal controls (15.2 ± 2.1 ng/ml). The vitamin D deficient rats were severely hypocalcaemic (Ca: 4.1 ± 0.2 mg/dl). The plasma calcium level in the D-replete rats increased to 7.9 ± 0.4 mg/dl, significantly higher than in the D-deficient rats (P < 0.001), but remained lower than in the control levels (9.4 ± 0.3 mg/dl) (P < 0.005).

The plasma insulin level in the D-deficient rats was markedly low (6 ± 2 µU/ml), and the level in the D-replete rats (16 ± 2 µU/ml) increased to the control level (17 ± 4 U/ml). No significant difference in blood glucose among the three groups was noted (control: 110 ± 5, D-deficient: 122 ± 5, D-replete: 112 ± 7 mg/dl).

Perfusate insulin and somatostatin

In the presence of 16.7 mM glucose, a biphasic pattern of insulin release was observed in the control rats, with a first peak of 183 ± 21 µU/ml and a second peak of 151 ± 25 µU/ml. In the vitamin D-deficient rats, both the first phase (mean peak value of 33 ± 12 µU/ml) and the second phase (mean peak value of 105 ± 19 µU/ml) of insulin release were markedly impaired and significantly lower than the corresponding values of controls (P < 0.005, P < 0.05, respectively). On the other hand, in the D-replete rats, both the first and second phase of insulin release increased to the same level as controls. There was no significant difference in the somatostatin secretion in the presence of 16.7 mM glucose among the three groups (Fig. 2).

As shown in Fig. 3, the glucose-stimulated insulin secretions expressed as integrated increments from basal levels were 707 ± 120 µU/20 min in the D-deplete rats, significantly lower than the values of 2100 ± 510 µU/20 min (P < 0.01) in the controls and 2200 ± 710 µU/20 min in the D-replete rats (P < 0.01).

The glucose-stimulated somatostatin secretions, expressed as the sum of increments above the basal levels, were not significantly different between the three groups.
Fig. 3.
Insulin and somatostatin responses to 16.7 mM glucose from the isolated perfused pancreas, expressed as the sum of the increments above basal levels during 16.7 mM glucose injection. Abbreviations are shown as $\Sigma\Delta$IRI and $\Sigma\Delta$IRS. Vertical bars represent mean ± SE.

Discussion

Several investigators have recently suggested the presence of a cytosol receptor for 1,25(OH)$_2$D$_3$ (Christakos & Norman 1981) and of a vitamin D dependent calcium binding protein (CaBP) (Christakos et al. 1979) in the pancreas. It would be interesting to know whether these have an effect on pancreatic function. There are, however, few reports on the role of vitamin D and its metabolites in the endocrine pancreas.

Clark et al. (1981) have reported that administration of 1,25(OH)$_2$D$_3$ to vitamin D-deficient rats resulted in a large increase (113%) in insulin levels in spite of a small increase (16%) in plasma calcium. They have demonstrated also that the small increase in plasma calcium levels did not affect the significant increase in insulin levels, suggesting that 1,25(OH)$_2$D$_3$ increases plasma insulin directly. Norman et al. (1980) have demonstrated that the insulin release from the isolated vitamin D-deficient rat pancreas is 40–50% less than from the D-replete pancreas, indicating that vitamin D deficiency inhibited insulin secretion. The present study has confirmed these observations in D-replete rats.

In the study by Norman et al. (1980) vitamin D$_3$ repletion was conducted for only 3 days and the pancreas was stimulated with 10 mM arginine. In our study, vitamin D repletion was conducted for 7–8 weeks after weanling while the rats were reared on a D-deficient diet, and the pancreas was stimulated with 16.7 mM glucose. Glucose is the strongest physiological stimulus of the B cells, and the role of intracellular and extracellular Ca in glucose-stimulated biphasic insulin release has been
documented in rat islets. Wollheim et al. (1978) and Kikuchi et al. (1979) have reported that intracellular Ca stores were the major regulatory control of first phase insulin release, and that intracellular Ca and increased uptake of extracellular Ca contributed to the second phase of glucose-induced insulin release. The present study has shown that the integrated increments of insulin release in response to glucose from the D-deficient rat pancreas exhibits a marked reduction compared to that from the D-replete pancreas, especially in the first phase. The first and second phase of insulin release from the D-deficient pancreas were 18 and 60%, respectively, of the release from the D-replete pancreas. It seems likely, therefore, that vitamin D deficiency induces the impaired insulin secretion which may be caused by a reduction of vitamin D dependent CaBP or a decrease in intracellular Ca storage in the islet.

The possibility that the effect of vitamin D is mediated by other factors should be considered. First, the improved insulin secretion in the D-replete rats might be mediated by the increase of plasma Ca levels in vivo. It is well known that insulin release in vitro is dependent on an acute change in Ca2+ (Curry et al. 1968). However, it must be emphasized that the in vitro pancreatic experiments with depleted and replete animals were performed with the same Ca concentration, and the perfusate insulin secretion and plasma insulin level in the D-replete rats were restored to normal levels despite significantly lower than normal plasma calcium levels.

Second, inhibition of insulin release in the D-deplete state may be due to malnutrition (Seino et al. 1980). Vitamin D-deficient rats gain little weight after 4 weeks of feeding with the D-deficient diet and become markedly emaciated. However, this possibility is rendered less likely by the following facts. Although D-replete rats gain more weight than D-deplete, it is still significantly less than normal and they are moderately emaciated. The insulin release from the D-replete rat pancreas and the plasma insulin levels are equal to those in normal rats in spite of lesser weight. The plasma calcium levels and nutritional conditions are, of course, important in insulin secretion but we believe that vitamin D has some direct effect on insulin release from the pancreas.

In the present study, somatostatin secretion was not significantly different in any of the three groups. The reason for this, though not clear at present, deserves consideration. Recent autoradiographic studies (Clark et al. 1980) have demonstrated that B-cells concentrate 1,25(OH)2D3 in their nuclei and have suggested that the target cells in the islets for 1,25(OH)2D3 are the B-cells. Other investigators (Kanatsuka et al. 1981) have indicated that calcium plays an important role in the regulation of the secretion of somatostatin as well as of insulin, and have suggested that B and D cells differ in their sensitivity to the calcium ion. This tends to support the possibility suggested by our own finding that the D-cell is less sensitive than the B-cell to the effects of vitamin D.

Finally, we conclude that the role of vitamin D is mediated, at least in part, by a direct action on the B cells in the regulation of insulin secretion.

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References


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