The Endocrine Pancreas in Pyridoxine Deficient Rats

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Toyota, T., Kai, Y., Kakizaki, M., Ohtsuka, H., Shibata, Y. and Goto, Y. The Endocrine Pancreas in Pyridoxine Deficient Rats. Tohoku J. exp. Med., 1981, 134 (3), 331-336 — Because the supplementation of pyridoxine (vitamin B<sub>6</sub>) improves the glucose tolerance in gestational diabetes and adult onset diabetes, pyridoxine deficiency has been considered to be one of the factors that cause diabetes mellitus. We produced pyridoxine deficient rats by giving pyridoxine-free food with deoxypridoxine which inhibits competitively the activity of pyridoxal phosphate. In these pyridoxine deficient rats plasma insulin during the glucose tolerance test was significantly low as compared with controls. In vitro experiments of pancreas perfusion showed that secretion of insulin and glucagon was impaired in the pyridoxine deficiency. Since the restriction of diet-calorie caused a decrease in arginine-induced secretion of insulin and glucagon from the isolated pancreas, the impairment of the endocrine pancreas may depend on malnutrition. Pyridoxine deficiency is surely one of the factors that impair the endocrine pancreas by multifactorial derangement of metabolism besides the tryptophan-nicotinic acid pathway.

As a result of dietary pyridoxine deficiency xanthurenic acid, which forms the complex with insulin and inhibits glucose uptake into fat cells, is increased (Kotake and Inada 1953a; Kotake et al. 1968, 1975; Kotake and Murakami 1971; Murakami and Kotake 1972). By-products from tryptophan, i.e. quinaldic acid and hydroxyquinaldic acid inhibit synthesis of proinsulin in the islet cells of rats (Noto and Okamoto 1978). In diabetic patients a large amount of xanthurenic acid is excreted into the urine (Kotake and Inada 1953a; Rosen et al. 1955). Because the supplementation of pyridoxine lowers the blood level of glucose in gestational diabetes (Schultz 1963), kynurenine metabolites, by-products of tryptophan-nicotinic acid metabolism, would play an etiologic role in human diabetes.

The present study was carried out to investigate the endocrine pancreas in pyridoxine deficient rats. Using in vitro perfusion experiments of the isolated pancreas, we tried to elucidate a problem that the pyridoxine deficiency could cause any deterioration in insulin and glucagon secretion.

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METHODS AND MATERIALS

Pyridoxine deficient rats were fed on Test Meal A which did not contain pyridoxine but deoxypyridoxine (10 mg/100 g). In principle, activity of pyridoxine is competitively inhibited by deoxypyridoxine.

Male albino rats of the Wistar strain were used for this study. Feeding on Test Meal A was started 5 weeks after birth. Thirty-four days after it, pyridoxine deficiency became evident. The skin dried and hair turned yellow and coarse (Group A).

Another group of rats (Group B) was given a test meal consisting of corn starch (38%), vitamin free casein (10%), alpha starch (8%), linolate salad oil (6%), granule sugar (5%), salt (6%), vitamin A (1000 IU), B1 (2.4 mg), B12 (0.001 mg), C (60 mg), D2 (200 IU), E (10 mg), K3 (10.4 mg), biotin (0.04 mg), niacin (12 mg), inositol (12 mg) and choline hydrochloride (5 mg). These rats were furnished restricted calories so as to keep almost all the same body weight as Group A.

Control rats (Group C) were given ad libitum the conventional chow purchased from Oriental Kobe Co. Thirty-four days after the start of experiments, body weights of the rats in Groups A, B and C were 126±5, 158±8 and 230±15 g, respectively. After an overnight fast, the glucose tolerance test (GTT) was carried out in the three groups. Glucose solution, 2 g/kg body weight, was given orally and blood samples were obtained from tail vessels every 30 min for 2 hr for the determination of glucose and insulin.

The pancreas was isolated by the procedure described by Grodsky et al. (1963) with some modifications (Toyota et al. 1978). Under anesthesia with pentobarbital sodium, 45 mg/kg body weight, the abdominal cavity was opened. The stomach and spleen were removed without bleeding. The pancreas was isolated together with the proximal portion of the duodenum. After placed in an incubator, the pancreas was perfused with perfusion medium through a plastic cannula which had been inserted into the celiac artery. The perfusion medium was Krebs-Ringer bicarbonate buffer containing dextran (5%) and glucose (3.8 mM). It was gassed with a mixture of 95% O2 and 5% CO2, resulting in pH 7.4. Arterial pO2 was 300-350 mmHg and portal pO2 was 150-200 mmHg. The approximate difference in oxygen tension across the pancreas was 100 mmHg. The flow rate was maintained constantly between 2.8 and 3.0 ml/min and the pressure was about 80 mm Hg. In all experiments perfusate samples, 3 ml each, were collected from a cannula in the portal vein at suitable intervals as indicated in Figs. 1 and 2. Immediately after sampling, the tubes containing 3 ml of the perfusate and 0.1 ml of aprotinin (Trasylol, Bayer, GFR) were kept at 4°C and stored at -20°C until analysis.

A 15-min period of perfusion with Krebs-Ringer bicarbonate buffer containing 3.8 mM glucose was allowed before the start of the experiments. The pancreas was stimulated with 11.1 mM glucose for 10 min and after a 15-min interval in the presence of 3.8 mM glucose, the pancreas was stimulated again with arginine hydrochloride solution, 20 mM, for 10 min.

Insulin and glucagon were measured by a charcoal separation method for radioimmunoassay. 125I-labeled pork insulin purchased from Dainabott Radioisotope Laboratory, Tokyo, and rat insulin from Novo Research Institute, Copenhagen, Denmark, were used for insulin assay. 125I-labeled pork glucagon purchased from Nuclear Medical Laboratory, Dallas, Texas, USA, and antiserum to glucagon (30 K) were used for glucagon assay. Statistical comparison was performed using Student’s t-test.

RESULTS

Pyridoxine deficient rats, Group A, were characterized by growth retardation and dermatitis. Their fasting plasma insulin was 11.6±0.8 µU/ml (mean±s.e.) which was significantly lower than 20.3±1.9 µU/ml of controls. Each average of plasma insulin at 30, 60 and 90 min after glucose ingestion was low as compared with controls (Table 1).
The pancreas isolated from the pyridoxine deficient rats responded to a continuous infusion of glucose or arginine with a monophasic pattern of insulin secretion. As shown in Fig. 1, insulin release stimulated by glucose, and especially by arginine, at intervals of 15 min was deteriorated remarkably in the pyridoxine deficient rats. The sum of insulin values was 14.6±5.7 ng/ml/10 min during arginine

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<tr>
<th>TABLE 1. Oral glucose tolerance test</th>
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<tr>
<td>Pyridoxine deficient rats</td>
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<tr>
<td>Blood glucose (mg/100 ml) 71±8 157±15 141±11 99±4† 92±4</td>
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<tr>
<td>Plasma insulin (μU/ml) 11.6±0.8† 21.0±1.9* 13.3±1.3* 11.2±0.5† 11.8±1.0</td>
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<td>Calorie-restricted rats</td>
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<td>Blood glucose (mg/100 ml) 74±2 105±2† 116±3† 118±3 101±4</td>
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<tr>
<td>Plasma insulin (μU/ml) 18.9±1.7 20.6±1.1* 21.6±1.1 17.1±1.5 15.0±0.7</td>
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<tr>
<td>Control rats</td>
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<tr>
<td>Blood glucose (mg/100 ml) 79±4 129±2 143±3 118±4 101±4</td>
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* p<0.05, † p<0.02, and †† p<0.01 v.s. control.

Each value is presented as means of five experiments±s.e. Ratios of the sum of plasma insulin to that of blood glucose are calculated. The ratios in pyridoxine deficient rats and calorie-restricted rats are 0.135±0.009 and 0.181±0.006, respectively. The ratios are significantly lower than 0.203±0.015 of normal rats (p<0.01).

![Fig. 1. Insulin and glucagon secretion from the pancreas isolated from pyridoxine deficient rats (Group A). Sums of insulin values during glucose infusion and arginine infusion are 63.8±16.4 and 14.7±5.7 ng/ml/10 min, respectively. Controls are 131±26.5 and 68±23.3 ng/ml/10 min (Group C). Sum of glucagon values during arginine infusion is 24.4±9.4 ng/ml/10 min in Group A and 84.2±16.6 ng/ml/10 min in Group C. •• Group A (n=4); o o Group C (n=4).](image-url)
infusion in Group A, while the sum of insulin values was 68±23.3 ng/ml/10 min in controls (Group C).

Glucagon release was also suppressed in the pyridoxine deficient rats. Fig. 1 shows that Group A responded to arginine infusion with a monophasic pattern of glucagon secretion as in the case of insulin. The sum of glucagon values during arginine infusion in Group A was 24.4±9.4 ng/ml/10 min which was significantly lower than 84.2±16.6 ng/ml/10 min in Group C. These results may imply that pyridoxine deficiency disturbs the endocrine pancreas. However, from the fact that the calorie-restricted rats (Group B) were also insufficient in the function of the endocrine pancreas (Fig. 2), we can hardly deduce the conclusion that the reduction of insulin and glucagon secretion is not due to malnutrition but due to an increase in kynurenine metabolites consequent to pyridoxine deficiency.

The present results showed that in Group B the insulin level during GTT was below the normal level. The ratio of the sum of insulin values to that of blood glucose during GTT in Group B was 0.181±0.006 which was significantly lower than 0.203±0.015 in Group C (p<0.01).

**DISCUSSION**

Pyridoxine deficiency has been produced in experimental animals by administration of tryptophan rich diet, glucocorticoids and contraceptive steroid
(Altman and Greengard 1966; Price et al. 1967; Rose and Braidman 1971). We produced pyridoxine deficient rats by giving pyridoxine-free food with deoxy-
pyridoxine which inhibits competitively the activity of pyridoxal phosphate. In
pyridoxine deficiency a large amount of xanthurenic acid is excreted in urine
because the main pathway of tryptophan breakdown is blocked owing to lack of
pyridoxine.

Pyridoxal phosphate (vitamin B₆) is required as coenzyme by kynureninase
which catalyzes the main pathway of kynurenine and hydroxykynurenine formed
from tryptophan into anthranilic acid and hydroxyanthranilic acid, respectively

Focussing our attention on the pancreatic beta cells, we are interested in the
fact that quinaldic acid and hydroxyquinaldic acid inhibit insulin secretion from
the pancreatic islet cells (Okamoto 1975). If pyridoxine deficiency causes an
increase in quinaldic acid and hydroxyquinaldic acid, the impairment of insulin
secretion would be attributed, as shown in Fig. 1, to an increase in kynurenine
metabolites owing to derangement of tryptophan-nicotinic acid metabolism.

It is of interest to note that the pancreas isolated from the pyridoxine deficient
rats responded to arginine infusion with a monophasic pattern of glucagon secre-
tion as in insulin secretion and that the glucagon level was remarkably lower
than that of control rats. Although there has been no available information
concerning effects of kynurenine metabolites on the pancreatic alpha cells, pyridoxine
deficiency possibly affects not only insulin but also glucagon secretion.

Is it true that the reason why pyridoxine deficiency inhibits the endo-
crine pancreas is because kynurenine metabolites are increased? Since the
restriction of diet-calorie caused a decrease in the arginine-induced insulin and
glucagon secretion as illustrated in Fig. 2, the impairment of the endocrine pancreas
may depend on malnutrition in a broad sense. Pyridoxine deficiency is known to
deteriorate not only protein but also fat metabolism, resulting in deficiency of
essential fatty acid (Mueller and Iacono 1963; Huber et al. 1968).

Early clinical studies showed that the supplementation of pyridoxine im-
proved blood glucose in gestational diabetes (Bennink and Schreurs 1975) and
adult onset diabetes (Schultz 1963). However, recent works could recognize no
significant alteration in the glucose tolerance in diabetic patients by the treatment
with pyridoxine (Perkins 1977; Gillmer and Maboko 1979; Rao et al. 1980).
Although pyridoxine deficiency is surely one of the factors that impair the
endocrine pancreas, it is difficult to conclude that diabetic state is produced by
pyridoxine deficiency alone. Further study is necessary in the future for clarifying
the relationship between onset of diabetes mellitus and pyridoxine deficiency.

References

1) Altman, K. & Greengard, O. (1966) Tryptophan pyrrolase induced in human liver by


