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## Tyrosine hydroxylase activity in the endocrine pancreas: changes induced by short-term dietary manipulation

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### Abstract

**Background:** Tyrosine hydroxylase (TH) activity and its possible participation in the control of insulin secretion were studied in pancreatic islets of adult Wistar rats fed a standard commercial diet (SD) or carbohydrates alone (CHD) for one week. TH activity, norepinephrine (NE) content, and glucose-induced insulin secretion were assessed. Blood glucose and insulin levels were measured at the time of sacrifice.

**Results:** CHD rats had significantly higher blood glucose and lower insulin levels than SD rats ( $114.5 \pm 6.7$  vs  $80.7 \pm 7.25$  mg/dl,  $p < 0.001$ ;  $20.25 \pm 2.45$  vs  $42.5 \pm 4.99$   $\mu$ U/ml,  $p < 0.01$ , respectively). Whereas TH activity was significantly higher in CHD isolated islets ( $600 \pm 60$  vs  $330 \pm 40$  pmol/mg protein/h;  $p < 0.001$ ), NE content was significantly lower ( $18 \pm 1$  vs  $31 \pm 5$  pmol/mg protein), suggesting that TH activity would be inhibited by the end-products of catecholamines (CAs) biosynthetic pathway. A similar TH activity was found in control and solarectomized rats ( $330 \pm 40$  vs  $300 \pm 80$  pmol/mg protein/h), suggesting an endogenous rather than a neural origin of TH activity. CHD islets released significantly less insulin in response to glucose than SD islets ( $7.4 \pm 0.9$  vs  $11.4 \pm 1.1$  ng/islet/h;  $p < 0.02$ ).

**Conclusions:** TH activity is present in islet cells; dietary manipulation simultaneously induces an increase in this activity together with a decrease in glucose-induced insulin secretion in rat islets. TH activity – and the consequent endogenous CAs turnover – would participate in the paracrine control of insulin secretion.

### Background

The presence of monoamines in the endocrine pancreas has been reported by several authors in different animals using either histochemical or chemical procedures [1–3], even in islets obtained from chemically sympathectomized mice [4].

Islet cells have also been shown to contain enzymes involved both in catecholamine (CAs) synthesis (tyrosine hydroxylase, TH [5–7], and dihydroxyphenylalanine, DOPA, decarboxylase [6–9]) and inactivation (monoamine oxidase, MAO [10–12]). The increase in islet CAs content in animals previously treated with precursors of CAs

synthesis [13] would suggest that these enzymes could be actively involved in CAs turnover in islet cells.

The increment of islet CAs – induced by the administration of their precursors – significantly modified the release of insulin, suggesting that endogenous CAs can affect islet function [14]. In support of this assumption we have recently reported that: a) Glucose induces a selective release of CAs by isolated islets, b) TH inhibition by a specific inhibitor increased the glucose-induced insulin secretion from isolated islets in fresh and precultured islets, and c) Addition of  $\alpha$  and  $\beta$  adrenergic receptor blockers significantly affected the secretion of insulin elicited by glucose [15]. To assess the physiological significance of these results it remains to be elucidated whether they can be reproduced at least partially *in vivo*.

The present study was designed to gain further insight into the possible role of islet TH in CAs synthesis and insulin secretion *in vivo*. Therefore, we tested whether dietary manipulation of normal rats fed only with carbohydrates can modify TH activity, CA content, as well as content and insulin release from isolated islets.

## Materials and Methods

### Chemicals and drugs

Collagenase was obtained from Serva Feinbiochemica (Heidelberg, Germany); bovine serum albumin fraction V (BSA) and other reagents of the purest available grade were from Sigma Chemical Co. (St. Louis, MO, USA).

### Animals and diets

Normal male Wistar rats (180–200 g) were maintained in a temperature-controlled environment (23°C) with a fixed 12 h light:12 h darkness cycle. The animals were divided into two groups: the intervention group received a carbohydrate diet (CHD) containing 44% glucose plus 56% starch (starch composition = carbohydrates:proteins:lipids 27:3.1:0.4) for a week, whereas control rats received a commercial standard diet (SD) containing carbohydrates:proteins:lipids 45:43:12 for the same period. The animals had free access to food and water throughout the experiment. Both weight and food intake were recorded for each individual animal every 48 h during the experimental period.

### Serum glucose and insulin determinations

Blood samples from both groups of animals were obtained from the retro-orbital plexus under light ether anesthesia early in the morning. Serum glucose was determined by the glucose-oxidase GOD-PAP method (Roche Diagnostics, Mannheim, Germany) and insulin levels were determined by radioimmunoassay (RIA) [16].

### Islet isolation and incubation

Pancreatic islets from SD and CHD rats were isolated by the collagenase method of Lacy and Kostianovsky [17]. Groups of five islets from both groups were incubated for 60 min at 37°C in 600  $\mu$ l Krebs-Ringer-bicarbonate buffer with 1% (w/v) BSA, Trasylol™ (400 IU/ml), and either 3.3 or 16.7 mM glucose [18]. The medium had been previously gassed with a mixture of CO<sub>2</sub>/O<sub>2</sub> (5%/95%) (v/v) to adjust the pH to 7.4; aliquots were obtained from the medium at the end of the incubation period and kept frozen until insulin determination by RIA [16].

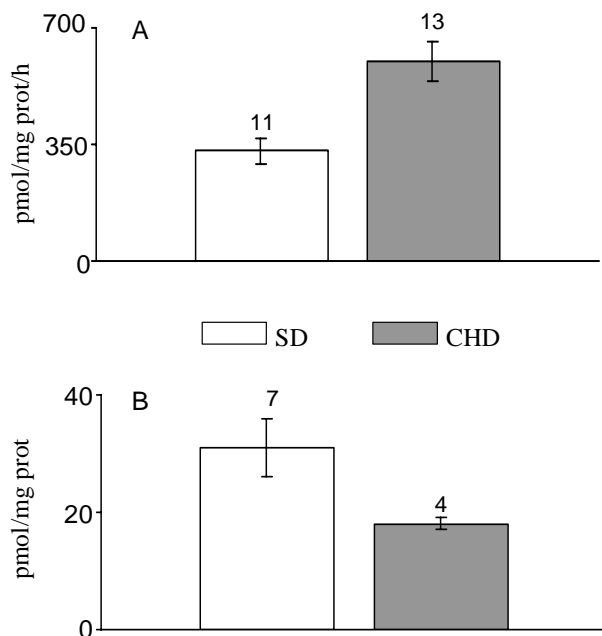
### Islet insulin content

Groups of 20 isolated islets were placed in 400  $\mu$ l H<sub>2</sub>O. After disruption by ultrasound for 15 s, the samples were diluted with two volumes of 30% (v/v) acetic acid. Suitable further aliquots at appropriate dilutions were then used to measure insulin levels by RIA [16].

### TH activity assay

SD and CHD islets were placed in a tube containing Locke's solution and then disrupted with a glass homogenizer in a 150 mM KCl solution (1/10 [w/v]) in the cold. Of this homogenate, 150  $\mu$ l were used as the source of enzyme in all experiments. The incubation medium had the following composition (in a total volume of 0.5 ml): 400 mM sodium-acetate buffer, pH 6.1; 1.0 mM FeSO<sub>4</sub> 40 mM 2-mercaptoethanol; 100  $\mu$ M or 2.0 mM DMPH<sub>4</sub>(2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride); 2.0 mM sodium phosphate; 15 units/ml DOPA decarboxylase; 10  $\mu$ M pyridoxal phosphate; 100  $\mu$ M [1-<sup>14</sup>C]L-tyrosine (10 Ci/mol, New England Nuclear Corporation, Boston, USA). Blanks were obtained by performing the assay in the presence of 2 mM of the inhibitor 3-iodotyrosine in the incubation medium. The reaction was carried out for 30 min at 37°C with continuous shaking in a 10 ml Erlenmeyer flask covered with a rubber injection-vial stopper from which a plastic well was suspended containing a folded paper impregnated with 0.2 ml NCS (Nuclear Chicago solubilizer) in order to trap the <sup>14</sup>CO<sub>2</sub> formed in the decarboxylation step of the assay [19].

The reaction was stopped by injecting 0.5 ml of 10% (v/v) trichloroacetic acid into the flask. The incubation was next resumed for 2 h at 37°C with continuous shaking to trap the <sup>14</sup>CO<sub>2</sub> released by the enzymic reactions. The plastic wells were then removed, wiped with absorbent tissue, and placed in 10 ml toluene scintillation fluid containing 0.6 g 1,4-bis-[2 (5-phenyloxazolyl)]benzene (POPOP), 4 g 2,5-diphenyloxazole (PPO), and 5 ml ethanol per liter of toluene. The samples were finally counted in a liquid-scintillation spectrometer.



**Figure 1**  
 Effect of CHD upon TH activity (pmol/mg protein/h) [panel A], and NE content (pmol/mg prot) [panel B] of normal rat isolated islets of Langerhans (SD, open bars; CHD, hatched bars). Each bar represents the mean ± SEM. n, above the corresponding bar. P values: TH activity, p < 0.001; NE content, p < 0.05.

**CA quantitation**

NE was partially purified by batch alumina extraction, separated by reverse-phase HPLC on a 4.6 X 250-mm Zorbax RP C 18 column (DuPont), and quantified amperometrically with a triple-electrode system (ESA, Bedford, NA) by measuring the current produced upon exposure of the column effluent to first oxidizing and then reducing potentials in series. CAs recovery through the alumina-extraction step averaged some 70–80%, as estimated through the use of dihydroxybenzylamine as internal standard [20].

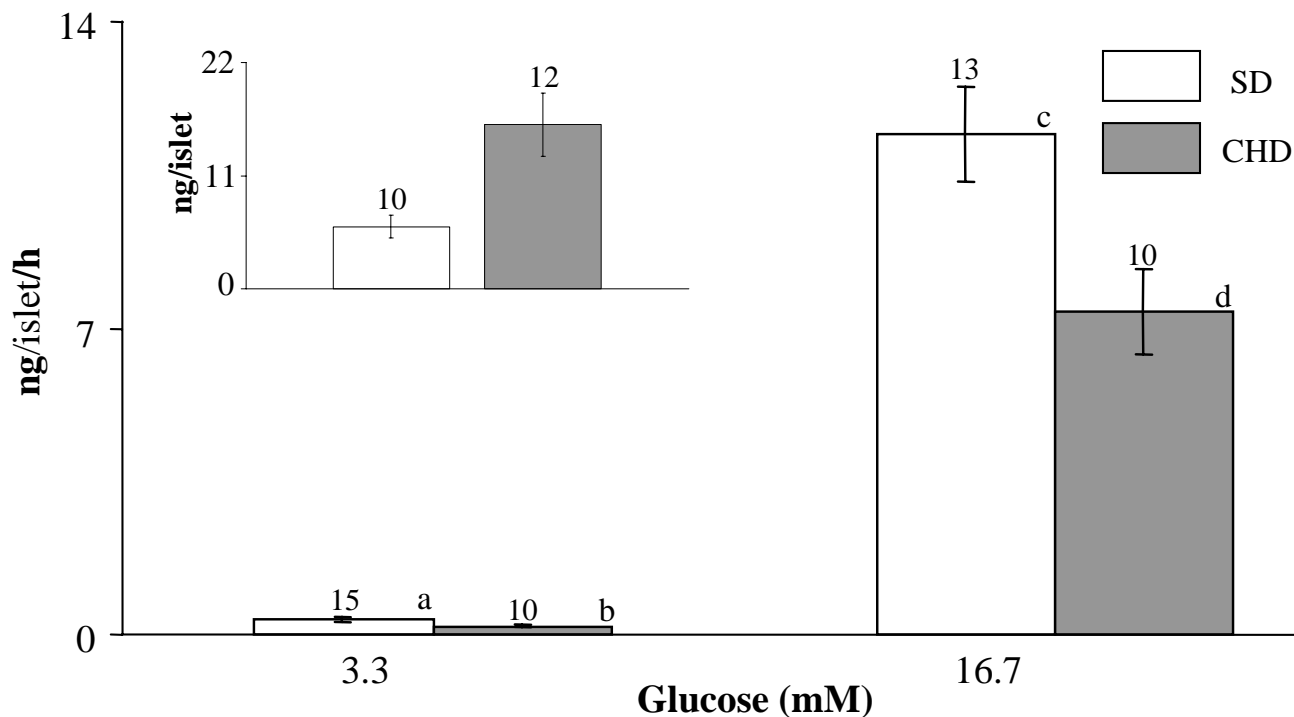
**Data analysis**

For the statistical evaluation of the data, we used both variance analysis and the paired Student's t-test.

**Results**

Although both groups of rats ate comparable amounts of their respective diets, there was an 8% body weight increase in the SD group (214.3 ± 4.1 to 231.7 ± 4.4 g), while CHD animals underwent a 13% weight loss (270.7 ± 7.6 to 233.8 ± 6.3 g). The caloric content of the daily food consumed per animal was significantly lower in CHD rats (58.8 ± 3.5 vs 152 ± 15.2 Cal/d, p < 0.01)

Serum glucose and insulin levels measured at the time of sacrifice were significantly higher and lower, respectively, in the CHD than in the SD group (glucose, 114.5 ± 6.7 (n = 6) vs 80.7 ± 7.25 (n = 6) mg/dl, p < 0.001; insulin, 20.25 ± 2.45 vs 42.5 ± 4.99 µU/ml, p < 0.01).



**Figure 2**

Effect of CHD upon insulin secretion (ng/islet/h) of isolated normal rat islets incubated in the presence of 3.3 or 16.7 mM glucose (SD, open bars; CHD, hatched bars). Each bar represents the mean  $\pm$  SEM, n, above the corresponding bar. P values: a vs c and b vs d,  $p < 0.001$ ; c vs d,  $p < 0.02$ . Inset: Insulin content of nonincubated islets isolated from animals of the indicated group. Each bar represents the mean  $\pm$  SEM, n above the corresponding bar.  $P < 0.005$ .

Islet TH activity of CHD rats was significantly higher than that of SD controls (Fig. 1A,  $p < 0.001$ ). Conversely, no significant differences between these two groups were observed when TH activity was measured in samples of cardiac muscle (data not shown). Similar TH activity values were measured in previous control experiments in islets isolated from normal (N) or solarectomized (S) rats ( $N = 330 \pm 40$  vs  $S = 300 \pm 80$  pmol/mg prot/h).

NE content of islets obtained from CHD rats was significantly lower than that of SD animals (Fig 1B;  $p < 0.05$ ).

Islets isolated from both groups released more insulin in response to 16.7 than 3.3 mM glucose ( $p < 0.001$ ); however, islets from CHD animals released significantly less

insulin in the presence of high glucose than did those from SD rats ( $p < 0.02$ ). By contrast, a similar insulin output by the islets of both groups was observed in response to 3.3 mM glucose (Fig. 2). However, the insulin content of nonincubated islets freshly isolated from CHD rats was significantly higher than that SD rat islets (Fig 2, inset;  $p < 0.05$ ).

**Discussion**

Teitelman *et al.* [21] originally demonstrated the immunocytochemical presence of TH in glucagon- and insulin-secreting cells of the fetal mouse pancreas and its disappearance after birth. Using the same method, however, Iturriza & Thibault [5] detected TH in B-cells of Sprague Dawley rats, two strains of mice, and pigmented guinea

pigs, suggesting that the expression of TH in the mammalian endocrine pancreas was not necessarily a transient phenomenon.

Our study is the first to demonstrate a measurable and modulated TH activity in the endocrine pancreas of normal adult rats. Since similar TH activity values were recorded in islets isolated from S and SD rats, the enzyme activity measured was of islet rather than neural origin. The increase in islet TH activity measured in our CHD islets could be ascribed to the reported stimulatory effect of this type of diet upon the sympathetic activity and on pancreatic – and other tissues as well – NE turnover [22] in man and rats [23]. However, the fact that this diet did not affect TH activity in cardiac muscle would indicate a selective effect upon islet CAs. The possibility that CAs metabolism exhibits a lower response threshold to dietary manipulations in islets than in other peripheral tissues cannot be ruled out.

In CHD animals, the increased islet TH activity was accompanied by a decreased NE content [24]. In other tissues, the limiting step in CA biosynthesis is the conversion of tyrosine to DOPA by TH [25], being this reaction inhibited by the end products of the biosynthetic pathway [26]. Thus, though with caution, we could assume that a similar regulatory mechanism would also operate *in vivo* in normal islets. However, we cannot assure that exposure to the diet for a week would not induce TH synthesis *de novo* [26].

The islets from CHD animals released less insulin than those from SD rats when incubated with 16.7 mM glucose. This lower response was not secondary to a decrease in the storage of the hormone, since the islets from CHD rats contained a higher concentration of insulin than did those from the SD group. These results are consistent with those reporting that monoamines in B cells do not influence insulin storage [27]. The significantly higher blood glucose and lower insulin levels measured in CHD animals would represent the *in vivo* counterpart of the lower capacity of their islets to release insulin in response to glucose *in vitro*. These results are also consistent with our report that a specific TH inhibitor increased significantly the glucose-induced insulin release either in fresh or precultured incubated islets [15], suggesting that the phenomenon could also occur *in vivo*. However, we cannot rule out that the caloric restriction and the protein deficiency in CHD animals could also affect through another mechanism the *in vitro* glucose-induced insulin secretion in this group of rats [28,29].

Altogether, our results are consistent with the following conclusions: a) TH activity in pancreatic islets can be modified, at least by dietary manipulation; b) As in other

tissues, the activity of the enzyme would be inhibited by the end products of the CA biosynthetic pathway, and c) Changes in TH activity of isolated islets are accompanied by significant alterations in the amount of insulin released *in vitro* in response to glucose, a functional association that may also occur *in vivo*. These findings, together with those previously reported [15], would indicate that endogenously-generated islet CAs might well participate in the paracrine control of insulin secretion.

### Competing interests

None declared.

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