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Deficient activity of FAD-linked glycerophosphate dehydrogenase in islets of GK rats

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Summary. In pancreatic islet extracts of rats with hereditary non-insulin-dependent diabetes mellitus (GK rats), the activity of the mitochondrial FAD-linked glycerophosphate dehydrogenase, as measured by either a radioisotopic or colorimetric procedure, only represented 30 to 40% of that found in control rats. This decrease in enzymic activity was not attributable to any sizeable change in either islet DNA content or the relative contribution of insulin-producing beta cells to total islet mass. It contrasted with a normal activity of other mitochondrial dehydrogenases and hexokinase isoenzymes. It coincided, however, with an increased activity of glutamate-pyruvate transaminase, as already observed in adult rats injected with streptozotocin during the neonatal period. The decreased activity of islet FAD-linked glycerophosphate dehydrogenase also contrasted with an increased activity of the same enzyme in the liver of GK, as compared to control rats. In the light of these findings and recent metabolic data collected in intact islets of GK rats, it is proposed that a deficiency of beta-cell FAD-linked glycerophosphate dehydrogenase, the key enzyme of the glycerol phosphate shuttle, may represent a cause of inherited non-insulin-dependent diabetes.

Key words: GK rats, pancreatic islets, liver, FAD-linked glycerophosphate dehydrogenase.

In GK rats, non-insulin-dependent diabetes mellitus represents a hereditary disease [1, 2]. In pancreatic islets prepared from the GK rats, impaired glucose-induced insulin release was reported to coincide with increased glucose cycling or impaired oxidation of the hexose relative to its utilization [3, 4]. It was also recently observed that the ratio between oxidative and total glycolysis is impaired in islets of GK rats [5], a situation comparable to that previously documented in islets of adult rats injected with streptozotocin during the neonatal period [6]. In the latter model of non-insulin-dependent diabetes, this metabolic anomaly is attributable to a deficiency in the activity of the islet mitochondrial FAD-linked glycerophosphate dehydrogenase (m-GDH), the key enzyme of the glycerol phosphate shuttle, and accounts for the preferential alteration of the beta-cell secretory response to D-glucose, as distinct from other nutrient or non-nutrient secretagogues [6–8]. The present report reveals that a comparable enzymatic defect prevails in the islets of GK rats.

Materials and methods

The present study was conducted in 14 control outbred Wistar rats (B&K Universal, Sollentuna, Sweden) and 18 GK rats [3]. An intraperitoneal glucose tolerance test was performed in all rats after an overnight fast. D-glucose (27.8 mmol/l) was injected intraperitoneally at a dose of 2 g/kg body weight and the glycaemia measured with a glucose analyser (Yellow Springs Instruments, Yellow Springs, Ohio, USA).

The rats were later killed whilst in the fed state. At killing, a blood sample was collected for measurement of glycaemia and plasma insulin concentration. The insulin content of the pancreas was determined in 15–25 mg pieces taken from the body of the gland and extracted overnight at 4°C in 2.5 ml of acid ethanol. Insulin was measured by radioimmunoassay using rat insulin as standard and antiporcine antibodies [9].

Islet DNA was measured by a fluorometric procedure [10] in three groups of ten islets each obtained from the same rat and sonicated in 0.1 ml H_2O.

For the measurement of islet enzyme activities, batches of 350 islets, each obtained from two control rats and two to four GK rats, were sonicated (2×10 s) in 350 µl of a Hepes-NaOH buffer (10 mmol/l, pH 7.4) containing 250 mmol/l sucrose, 2.5 mmol/l EDTA, 2.0 mmol/l L-cysteine and 0.2 mg/ml bovine serum albumin. Likewise, in ten control rats and eight GK rats, pieces of liver (0.1 g wet weight) were sonicated in 1.0 ml of the same buffer, except for the absence of albumin. The islet and liver extracts were lyophilized and later reconstituted to their original volume with H_2O. The protein content of the liver extract was measured by the method of Lowry et al. [11], using serum albumin as standard. It averaged 23.5 ± 1.2 and 23.7 ± 2.6 mg/ml in control and GK rats.

The activity of mitochondrial FAD-linked glycerophosphate dehydrogenase (m-GDH), as assessed by both radioisotopic [12] and colorimetric [13] procedures, of ADP-activated glutamate de-

Table 1. Metabolic status of control and GK rats

	Control rats	GK rats	*p*
Body weight (g)	280 ± 8 (14)	276 ± 8 (18)	NS
Blood glucose (mmol/l)	6.5 ± 0.3 (14)	13.9 ± 0.9 (18)	< 0.001
Plasma insulin (μU/ml)	71 ± 8 (12)	131 ± 10 (16)	< 0.001
Pancreatic insulin (mU/mg wet weight)	2.92 ± 0.34 (14)	0.72 ± 0.14 (13)	< 0.001
Islet DNA (ng/islet)	29.8 ± 3.9 (9)	28.6 ± 2.7 (9)	NS

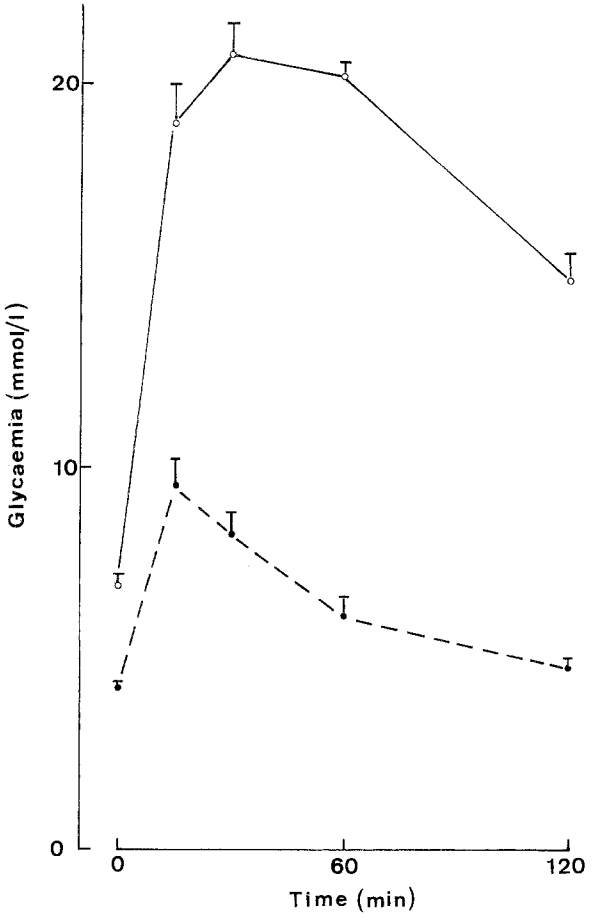

Fig. 1. Glycaemic profile before and after intraperitoneal administration of D-glucose (2 g/kg body weight) in control (--- ● ---) and GK rats (– ○ –). Mean values (± SEM) refer to the 14 control animals and 18 GK rats used in the present study

hydrogenase (GlDH) [14], glutamate-oxalacetate transaminase (GOT) [15], glutamate-pyruvate transaminase (GPT) [15], glutamate decarboxylase [16], 2-ketoglutarate dehydrogenase (α-KGDH) [17], hexokinase and glucokinase [18] were measured over 20–60 min incubation at 37 °C in the absence of Ca^{2+} by methods described in the cited references. The final concentrations of substrates and co-factors amounted to 0.5 mmol/l L-[2-^{3}H]glycerol-3-phosphate and 0.05 mmol/l FAD in the radioisotopic m-GDH assay, 10.0 mmol/l L-glycerol-3-phosphate and 2.0 mmol/l 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium in the colorimetric m-GDH assay, 0.7 mmol/l [5-^{14}C]2-ketoglutarate, 50 mmol/l NH_4^+, 0.3 mmol/l NADPH and 0.5 mmol/l ADP in the GlDH assay, 2.5 mmol/l [5-^{14}C]2-ketoglutarate, 0.9 mmol/l pyridoxal phosphate and either 20 mmol/l L-aspartate in the GOT assay or 20.0 mmol/l L-alanine in the GPT assay, 2.5 mmol/l L-[1-^{14}C]glutamate and 0.5 mmol/l pyridoxal phosphate in the glutamate decarboxylase assay, and 1.0 mmol/l D-[U-^{14}C]glucose and 5.0 mmol/l ATP in the hexokinase assay. The phosphorylation of D-[U-^{14}C]glucose was measured only at a 10.0 mmol/l concentration of the hexose in islet extracts. In liver extracts, the paired difference between measurements conducted at 10.0 mmol/l and 1.0 mmol/l D-glucose was taken as representative of the activity of glucokinase. All enzymic assays were conducted in duplicate or triplicate. The results were corrected for the blank value found in the absence of either tissue homogenate (radioisotopic m-GDH assay, glutamate decarboxylase, α-KGDH, hexokinase and glucokinase assay) or a suitable substrate (i.e. L glycerol-3-phosphate in the colorimetric m-GDH assay, NADPH in the assay of GlDH and exogenous amino acid in the assay of GOT and GPT).

In ten control and eight GK rats, a small piece of the pancreas, taken from the corpus of each gland, was fixed in Bouin's fluid, dehydrated and embedded in paraffin. Sections which were 5 μm thick were mounted on gelatin-coated glass slides and rehydrated just before immunocytochemical staining. The unlabelled antibody immunoperoxidase technique with "double-bridge" was used, as previously described [19, 20]. Consecutive sections were exposed for 48 h at 4 °C to guinea-pig anti-insulin serum, rabbit antiglucagon serum and rabbit antisomatostatin serum (Unipath, Lyon, France) diluted 1/1,000, 1/10,000 and 1/4,000, respectively, in 0.01 mol/l phosphate-buffered saline (pH 7.5) containing 1% (volume/volume) non-immune sheep serum. At the reactive sites, peroxidase was revealed with diaminobenzidin [21].

Statistical analysis

All results, including those mentioned above, are presented as the mean (± SEM), together with the number of individual observations. The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

Results

Metabolic status

The body weight and islet DNA content were comparable in control and GK rats (Table 1). The blood glucose and plasma insulin concentrations were higher and the pancreatic insulin content lower in GK than control rats. The GK rats also displayed obvious intolerance to D-glucose after intraperitoneal injection of D-glucose (2 g/kg body weight) to 18-h fasted animals (Fig. 1).

Enzymic data

In islet homogenates, the activity of m-GDH was significantly lower in GK than control rats, whether in the radioisotopic or colorimetric procedure (Table 2). The paired ratio between colorimetric/radioisotopic measurements was not significantly different ($p > 0.4$) in islets from control and GK rats and, pooling all available data, averaged 76.7 ± 14.0 ($n = 10$). The activity of GLDH, GOT, glutamate decarboxylase and α-KGDH were not significantly different in control and GK rats. Such was also the case for the phosphorylation of D-glucose (10 mmol/l) as catalysed by both hexokinase and glucokinase. The activity of GPT was significantly higher in GK than control rats. Likewise, the paired ratio between GPT/GOT activities averaged 23.2 ± 3.6% in GK rats, as distinct ($p < 0.001$) from only 6.4 ± 1.2% in control rats ($n = 7$ in both cases).

Table 2. Enzymic activities in islets from control and GK rats

Enzyme		Control rats	GK rats	p
m-GDH (radioisotopic)	(fmol · islet^{-1} · min^{-1})	113.8 ± 21.6 (7)	42.0 ± 3.6 (7)	< 0.010
(colorimetric)	(pmol · islet^{-1} · min^{-1})	10.2 ± 0.9 (5)	3.1 ± 0.7 (5)	< 0.001
GlDH	(pmol · islet^{-1} · min^{-1})	6.77 ± 0.18 (7)	6.52 ± 0.16 (4)	NS
GOT	(pmol · islet^{-1} · min^{-1})	7.86 ± 0.70 (7)	7.58 ± 0.50 (7)	NS
GPT	(pmol · islet^{-1} · min^{-1})	0.49 ± 0.07 (7)	1.57 ± 0.41 (7)	< 0.025
Glutamate decarboxylase	(fmol · islet^{-1} · min^{-1})	31.3 ± 6.9 (7)	36.0 ± 8.7 (4)	NS
α-KGDH	(pmol · islet^{-1} · min^{-1})	0.53 ± 0.15 (7)	0.44 ± 0.08 (3)	NS
Hexokinase isoenzymes	(pmol · islet^{-1} · min^{-1})	2.59 ± 0.27 (7)	3.22 ± 0.76 (4)	NS

Values shown are mean ± SEM together with the number of individual observations. m-GDH, FAD-glycerophosphate dehydrogenase; GlDH, glutamate dehydrogenase; GOT, glutamate-oxalacetate transaminase; GPT, glutamate-pyruvate transaminase; α-KGDH, α-Ketoglutarate dehydrogenase

Table 3. Enzymic activities in liver from control and GK rats

Enzyme		Control rats	GK rats	p
m-GDH (radioisotopic)	(fmol · μg^{-1} · min^{-1})	3.95 ± 0.40 (10)[a]	7.43 ± 1.03 (7)	< 0.005
(colorimetric)	(fmol · μg^{-1} · min^{-1})	182 ± 2 (10)	459 ± 54 (7)	< 0.001
GlDH	(pmol · μg^{-1} · min^{-1})	3.80 ± 0.17 (10)	4.01 ± 0.41 (8)	NS
GOT	(pmol · μg^{-1} · min^{-1})	181 ± 5 (10)	207 ± 13 (8)	NS
GPT	(pmol · μg^{-1} · min^{-1})	245 ± 18 (10)	328 ± 18 (8)	< 0.010
α-KGDH	(pmol · μg^{-1} · min^{-1})	0.79 ± 0.10 (9)	1.07 ± 0.12 (7)	NS
Hexokinase	(pmol · μg^{-1} · min^{-1})	0.13 ± 0.02 (10)	0.18 ± 0.02 (7)	NS
Glucokinase	(pmol · μg^{-1} · min^{-1})	0.35 ± 0.02 (10)	0.42 ± 0.06 (8)	NS

[a] Mean values (± SEM) are expressed per μg liver protein and refer to the number of animals examined in each case. m-GDH, FAD-glycerophosphate dehydrogenase; GlDH, glutamate dehydrogenase; GOT, glutamate-oxalacetate transaminase; GPT, glutamate-pyruvate transaminase; α-KGDH, α-Ketoglutarate dehydrogenase

In the liver, the activity of m-GDH was higher in GK than control rats, whether in the radioisotopic or colorimetric procedure (Table 3). The paired ratio between colorimetric/radioisotopic measurements did not differ significantly in the two types of animals, yielding an overall mean value of 53.3 ± 5.1 (n = 17). No significant difference between control and GK rats was observed for the activity of GlDH, GOT, α-KGDH, hexokinase and glucokinase in liver extracts. The activity of GPT was higher, however, in the liver of GK than control rats.

Histological data (Fig. 2)

In the ten pancreatic samples from control rats, 67 small islets (diameter < 100 μm), 21 medium-sized islets (diameter: 100 to 200 μm) and 8 large islets (diameter > 200 μm) were examined. In the eight samples from GK rats, 39 small, 10 medium-sized and 10 large islets were scrutinized. The small islets were heavily and homogenously stained with anti-insulin serum in both control and GK rats (Fig. 2, I). Such was also the case for medium-sized and large islets from control rats (Fig. 2, A and C). In the pancreas of GK rats, however, some medium-sized and virtually all large islets displayed heterogeneity in the staining of distinct cells located in the core of the islets (Fig. 2, E and G), suggesting variability in their insulin content. Some heavily labelled cells neighboured lightly-labelled or even virtually unlabelled cells. Most of these degranulated cells were identified as beta-cells using less diluted anti-insulin serum. The degranulation might conceivably reflect increased secretory activity in the hyperglycaemic GK rats.

In all islets, glucagon- and somatostatin-producing cells were found at the periphery of the islets (Fig. 2, B, D, F and H). This location, as well as the thickness of such a mantle of non-beta cells, were comparable in control and GK rats.

Taken as a whole, the present histological data indicate that there is no sizeable change in the contribution of beta cells to total islet mass, even in medium-sized or large islets, such as those collected for enzymic determination.

Discussion

The present results reveal that the activity of m-GDH is decreased in islets from GK rats. This decrease cannot be attributed to a different size of the islets collected for the enzymatic measurements. Thus, the DNA content per islet was not different in control and GK rats. Moreover, the activity of several other enzymes, when expressed per islet, failed to be decreased in the GK rats. The decrease in islet m-GDH activity could also not be attributed to any sizeable decrease in the contribution of beta cells to total islet mass. Moreover, no decrease in the activity of glutamate decarboxylase was detected in the islets of GK rats,

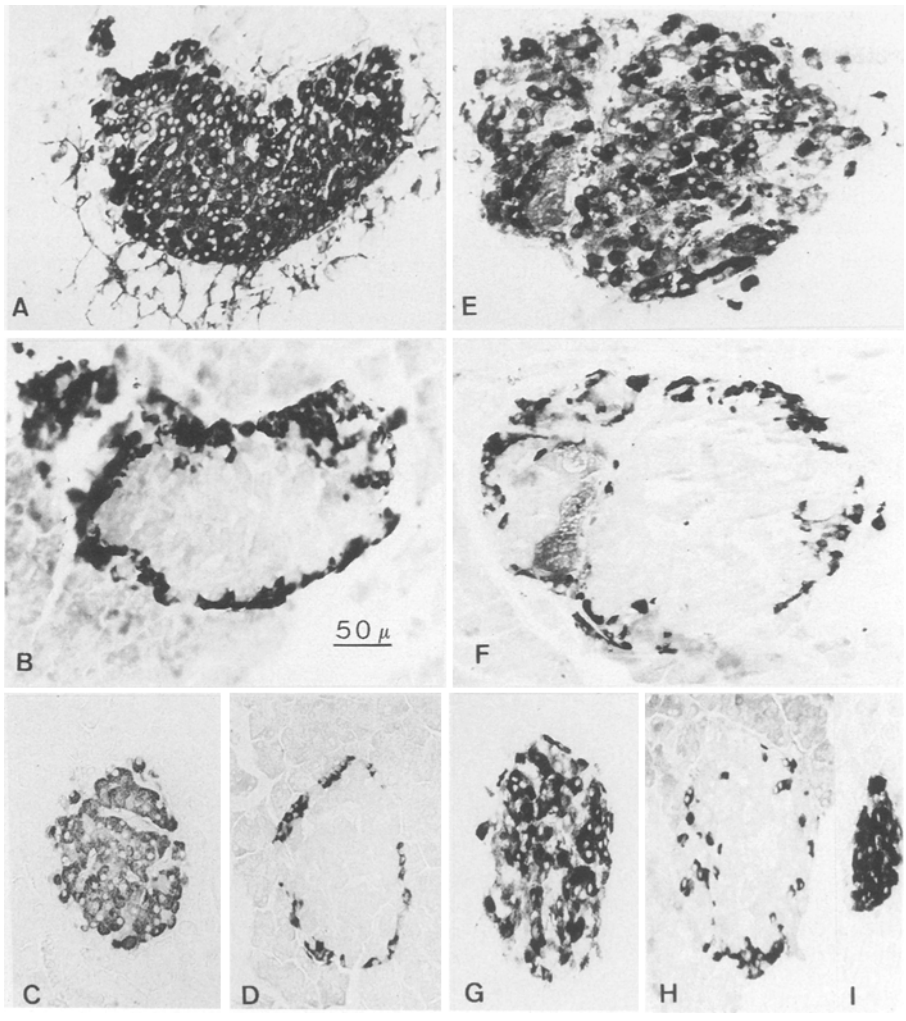

Fig. 2 A–I. Sections of pancreas from control rats (**A–D**) and GK rats (**E–I**) immunostained for insulin (**A**, **C**, **E**, **G**, **I**) or glucagon (**B**, **D**, **F**, **H**). Serial sections of either large islets (**A** and **B**, **E** and **F**) or medium-sized islets (**C** and **D**, **G** and **H**), as well as a section in a small islet (**I**) are all reproduced at the same magnification (Bar = 50 μm as shown in **B**)

although both m-GDH and glutamate decarboxylase activities are virtually restricted to beta cells, as distinct from non-beta-islet cells [22, 23].

The deficiency in islet m-GDH activity was documented by both a radioisotopic technique and colorimetric assay. The enzymic activity was much higher in the latter than former procedure, as expected from both the difference in concentration of L-glycerol-3-phosphate and the intrinsic properties of m-GDH [13]. The velocity of the reaction catalysed by semi-purified m-GDH is indeed much higher when iodonitrotetrazolium is used as electron acceptor (colorimetric assay) rather than FAD (radioisotopic assay). In the GK rats, the decreased activity of islet m-GDH contrasted with an abnormally high activity of the same enzyme in liver extracts. Once again, the latter anomaly was documented by both the radioisotopic and colorimetric procedures. These findings indicate that, in GK rats, a deficient activity of m-GDH, as observed in pancreatic islets, does not represent a generalized feature found in all cell types. Incidentally, since the protein content of each islet is close to 0.8 μg, the present data confirm that, in the liver of normal rats, the activity of m-GDH only represents 2 to 3 % of that found in pancreatic islets.

Interestingly, in the GK rats, the islet defect in m-GDH coincided with an abnormally high activity of GPT and, hence, elevated GPT/GOT ratio. Identical enzymic findings were recently identified in islets from adult rats injected with streptozotocin during the neonatal period [24]. These associated enzymatic perturbations might be attributable, in the GK and streptozotocin-injected rats, respectively, to a hereditary genetic anomaly and acquired genomic defect.

No significant difference between control and GK rats was observed in terms of the activity of GlDH and α-KGDH, whether in islet or liver extracts. This indicates that the reduced activity of m-GDH in the islets of GK rats cannot be attributed to an overall decrease in all mitochondrial dehydrogenases.

No decrease in the activity of glucokinase was observed in the liver of GK rats. This coincided with a high concentration of plasma insulin. In the islets, the phosphorylation of D-glucose as catalysed by both hexokinase and glucokinase also failed to be lower in GK than control rats. This coincides with a close-to-normal, or even increased, rate of D-[5-^{3}H]glucose conversion to ^{3}HOH in intact islets of GK rats, at least when expressed relative to protein or DNA content [3–5]. These findings suggest that the defect of glucose-stimulated insulin release in these animals [3, 4, 25] is not attributable to any major anomaly in the rate of D-glucose phosphorylation.

It should be emphasized that the decreased activity of m-GDH in the islets of GK rats is not secondary to chronic

hyperglycaemia. Thus, no decrease in m-GDH activity is observed in islets from normal rats exposed for prolonged periods to high concentrations of D-glucose, whether in vitro [26] or in vivo [13].

In conclusion, the present report provides the first example of a deficiency of m-GDH activity in pancreatic islets of animals with inherited non-insulin-dependent diabetes. The enzymatic defect coincides with an altered ratio between oxidative and total glycolysis in intact islets from GK rats [5]. Since m-GDH and its activation by cytosolic Ca^{2+} plays a crucial role in the glucose-sensing device of the pancreatic beta cell [27], the enzymatic defect and its metabolic consequences might well account for the alteration of glucose-stimulated insulin release in the GK rats. It is tempting to speculate that a comparable situation could conceivably prevail in patients with non-insulin-dependent diabetes.

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References

1. Goto Y, Kakizaki M, Masaki N (1975) Spontaneous diabetes produced by selective breeding of normal Wistar rats. Proc Jpn Acad 51: 80–85
2. Goto Y, Suzuki K-I, Sasaki M, Ono T, Abe S (1988) GK rat as a model of nonobese, noninsulin-dependent diabetes: selective breeding over 35 generations. In: Shafrir E, Renold AE (eds) Frontiers in diabetes research. Lessons from animal diabetes II. John Libbey, London, pp 301–303
3. Östenson C-G, Khan A, Abdel-Halim SM et al. (1993) Abnormal insulin secretion and glucose metabolism in pancreatic islets from spontaneously diabetic GK rats. Diabetologia 36: 3–8
4. Giroix M-H, Vesco L, Portha B (1992) Functional and metabolic perturbations in isolated pancreatic islets from the GK rat, a genetic model of non-insulin-dependent diabetes. Diabetologia 35 [Suppl 1]: A77 (Abstract)
5. Sener A, Giroix M-H, Portha B, Malaisse WJ (1992) Preferential alteration of oxidative relative to total glycolysis in islets of rats with inherited or acquired non-insulin-dependent diabetes. Diabetologia 35 [Suppl 1]: A78 (Abstract)
6. Giroix M-H, Rasschaert J, Bailbe D et al. (1991) Impairment of the glycerol phosphate shuttle in islets from rats with diabetes induced by neonatal streptozotocin. Diabetes 40: 227–232
7. Giroix M-H, Rasschaert J, Sener A et al. (1992) Study of hexose transport, glycerol phosphate shuttle and Krebs cycle in islets of adult rats injected with streptozotocin during the neonatal period. Mol Cell Endocrinol 83: 95–104
8. Giroix M-H, Baetens D, Rasschaert J et al. (1992) Enzymic and metabolic anomalies in islets of diabetic rats: relationship to B-cell mass. Endocrinology 130: 2634–2640
9. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ (1965) Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25: 1375–1384
10. Hinegardner RT (1971) An improved fluorometric assay for DNA. Anal Biochem 39: 197–201
11. Lowry OH, Rosebrough NJ, Farr AL, Randall PJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
12. Rasschaert J, Malaisse WJ (1991) Hexose metabolism in pancreatic islets. Glucose-induced and Ca^{2+}-dependent activation of FAD-glycerophosphate dehydrogenase. Biochem J 278: 335–340
13. Sener A, Malaisse-Lagae F, Malaisse WJ (1992) Pancreatic islet FAD-linked glycerophosphate dehydrogenase activity in a model of B cell glucotoxicity. Med Sci Res 20: 701–703
14. Sener A, Malaisse WJ (1990) A sensitive radioisotopic method for the measurement of NAD(P)H: its application to the assay of metabolites and enzymatic activities. Anal Biochem 186: 236–242
15. Perales M-A, Sener A, Malaisse WJ (1992) Radioisotopic assay of aspartate and alanine aminotransferase. Clin Biochem 25: 105–107
16. Malaisse-Lagae F, Giroix M-H, Malaisse WJ (1992) Streptozotocin-induced impairment of glutamate decarboxylase activity in pancreatic islet Beta-cells. Med Sci Res 20: 489–490
17. Sener A, Rasschaert J, Malaisse WJ (1990) Hexose metabolism in pancreatic islets. Participation of Ca^{2+}-sensitive 2-ketoglutarate dehydrogenase in the regulation of mitochondrial function. Biochim Biophys Acta 1019: 42–50
18. Giroix M-H, Sener A, Pipeleers DG, Malaisse WJ (1984) Hexose metabolism in pancreatic islets. Inhibition of hexokinase. Biochem J 223: 447–453
19. Petrusz P, Ordronneau P, Finley CW (1980) Criteria of reliability for light microscopic immunocytochemical staining. Histochem J 12: 333–342
20. Vacca LL, Abrahams SS, Nafichi E (1980) A modified peroxidase-antiperoxidase procedure for improved localization of tissue antigens: localization of substance P in rat spinal cord. J Histochem Cytochem 28: 297–307
21. Sternberger LA (1979) Immunocytochemistry. Wiley, New York
22. Rasschaert J, Ling Z, Malaisse WJ (1993) Effect of streptozotocin and nicotinamide upon FAD-glycerophosphate dehydrogenase activity and insulin release in purified pancreatic beta-cells. Mol Cell Biochem 120: 135–140
23. Sorensen RL, Garry DG, Brelje TC (1991) Structural and functional considerations of GABA in islets of Langerhans. Diabetes 40: 1365–1374
24. Sener A, Giroix M-H, Malaisse-Lagae F et al. (1993) Metabolic response to non-glucidic nutrient secretagogues and enzymic activities in pancreatic islets of adult rats after neonatal streptozotocin administration. Biochem Med Metab Biol (in press)
25. Portha B, Serradas P, Bailbe D, Suzuki K-I, Goto Y, Giroix M-H (1991) β-cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. Diabetes 40: 486–491
26. Rasschaert J, Eizirik DL, Malaisse WJ (1992) Long term in vitro effects of streptozotocin, interleukin-1, and high glucose concentration on the activity of mitochondrial dehydrogenases and the secretion of insulin in pancreatic islets. Endocrinology 130: 3522–3528
27. Malaisse WJ (1992) Glucose-sensing by the pancreatic B-cell: the mitochondrial part. Int J Biochem 24: 693–701

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