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FAD-linked glycerophosphate dehydrogenase deficiency in pancreatic islets of mice with hereditary diabetes

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The mitochondrial enzyme FAD-linked glycerophosphate dehydrogenase plays a key role in the glucose-sensing device of the insulin-producing pancreatic B-cell. Its activity was found to be decreased in islet, but not liver, homogenates of BL / Ks-*db*/*db* mice, in which diabetes mellitus represents an inherited disease. The decreased activity of FAD-linked glycerophosphate dehydrogenase contrasted with a normal activity of glutamate dehydrogenase and 2-ketoglutarate dehydrogenase in the islets of *db*/*db* mice. It is proposed that a site-specific defect of FAD-linked glycerophosphate dehydrogenase in the pancreatic B-cell might represent a far-from-uncommon causal or contributing factor in the pathogenesis of non-insulin-dependent diabetes mellitus.

Pancreatic islet; FAD-linked glycerophosphate dehydrogenase; *db*/*db* mouse

1. INTRODUCTION

The mitochondrial enzyme FAD-linked glycerophosphate dehydrogenase (m-GDH) plays a key role in the glucose-sensing device of the pancreatic B-cells [1,2]. It catalyzes the rate-limiting reaction in the glycerol phosphate shuttle and its activation by Ca^{2+} accounts for the preferential stimulation of oxidative glycolysis in pancreatic islets exposed to increasing concentrations of D-glucose [3,4]. As a result, the generation of ATP, which couples metabolic to cationic events in the process of glucose-stimulated insulin release, is markedly increased through both accelerated circulation in the glycerol phosphate shuttle and enhanced oxidation of glucose-derived pyruvate.

In adult rats which were injected with streptozotocin during the neonatal period, the activity of m-GDH is severely decreased in islet, but not liver, homogenates [5]. This coincides, in intact islets, with an impaired circulation in the glycerol phosphate shuttle and a decreased contribution of both oxidative glycolysis and pyruvate oxidation to total D-glucose utilization [5–7]. Such metabolic anomalies are thought to account for the preferential alteration of the B-cell secretory response to D-glucose, as distinct from other nutrient or non-nutrient secretagogues, found in this animal model of non-insulin-dependent diabetes [8–11]. The present report reveals that the activity of m-GDH is also decreased in islets of diabetic mice, in which diabetes mellitus represents an inherited, rather than drug-induced, disease.

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2. MATERIALS AND METHODS

Diabetes and control mice from the Diabetes Forschungsinstitut at the University of Düsseldorf (Germany) had free access to food and water up to the time of sacrifice. Diabetic mice were either C57BL / KsJ-*db*/*db* or C57BL / KsJ-+*db*/+*db* mice, i.e. diabetic mice in which the misty gene (*m*) is maintained in repulsion with diabetes (*db*). Diabetic mice of both lines are here referred to as *db*/*db*, whereas +/+, *db*?/+, and +*db*?/*m*+ mice are referred to as controls.

The method used to measure plasma glucose and insulin concentrations [12,13], to isolate islets [14], to assess the activity of m-GDH by either a radioisotopic [1] or colorimetric [15] procedure and to measure the activity of glutamate dehydrogenase [16], 2-ketoglutarate dehydrogenase [17], glutamate-pyruvate transaminase [18] and glutamate-oxalacetate transaminase [18] were previously described in the cited references. Both islets (2 islets/μl) and liver (100 mg wet wt/ml) were homogenized in a HEPES-NaOH buffer (20 mM, pH 7.4) containing 250 mM sucrose and 2 mM EGTA. After removal of an aliquot of the tissue extract for protein determination [19], the homogenate was mixed with an equal volume of a solution containing 4 mM L-cysteine and 0.04% (w/v) bovine serum albumin. All enzymatic assays were conducted in the absence of Ca^{2+}, the final concentrations of substrates and co-factors amounting to 0.5 mM L-[2-^{3}H]glycerol-3-phosphate and 0.05 mM FAD in the radioisotopic assay of m-GDH, 10.0 mM L-glycerol-3-phosphate and 2.0 mM 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride in the colorimetric assay of m-GDH, 0.7 mM [U-^{14}C]2-ketoglutarate, 50 mM ammonium acetate, 0.3 mM NADPH and 1.0 mM ADP in the assay of glutamate dehydrogenase, 0.5 mM [1-^{14}C]2-ketoglutarate, 1.0 mM NAD^+, 0.5 mM coenzyme A and 0.2 mM cocarboxylase in the assay of 2-ketoglutarate dehydrogenase, 2.5 mM [U-^{14}C]2-ketoglutarate, 0.7 mM pyridoxal phosphate and either 24 mM L-alanine or L-aspartate in the assay of glutamate-pyruvate and glutamate-oxalacetate transaminases. All results are presented as the mean value (± S.E.M.) together with the number of individual observations (*n*).

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3. RESULTS

As shown in Table I, pancreatic islets were isolated from 64 control mice (30 males and 34 females), 8.4 ± 0.1 week-old (range 5 to 10 weeks) and 43 diabetic mice (20 males and 23 females). Except for one batch of islets (D4) obtained from 4 older diabetic animals (18 weeks), the mean age of the diabetic mice (8.5 ± 0.4 weeks; n = 39) was virtually identical to that of the control animals. Fifteen batches of 150 to 360 islets (mean: 256 ± 17 islets) were obtained, each from 4 to 12 mice of comparable age and identical genetic background. The plasma glucose and insulin concentrations were measured in pooled samples obtained from several or all mice used to prepare each batch of islets. The plasma glucose concentration averaged 31.3 ± 2.1 mM (n = 12) in diabetic mice, as compared ($P < 0.001$) to 9.8 ± 0.2 mM (n = 14) in control animals. The plasma insulin concentration averaged 215 ± 32 μU/ml (n = 12) in diabetic animals, as distinct ($P < 0.001$) from 37 ± 5 μU/ml (n = 14) in control mice. At comparable age, the diabetic mice were also heavier ($P < 0.001$) than control animals, with mean values of 39.2 ± 1.8 g (n = 24) and 23.1 ± 0.4 g (n = 58), respectively.

The results of enzymic measurements in islets and liver homogenates are summarized in Table II. The islets from *db/db* mice displayed a lower activity of m-GDH than those from control animals. The protein content and the activities of both glutamate dehydrogenase and 2-ketoglutarate dehydrogenase were not significantly different in islets from control and diabetes mice. No decrease in m-GDH activity was found in the liver of the *db/db* mice, as assessed by either a radioisotopic or colorimetric procedure. As expected from the intrinsic properties of the enzyme [15], the reaction velocity was higher in the colorimetric than radioisotopic assay. The paired ratio between the measurements made by colorimetric and radioisotopic procedures was not significantly different in control and diabetic mice, averaging respectively 54.8 ± 2.3 (n = 27) and 55.0 ± 3.4 (n = 24). In liver homogenates, the sole significant differences consisted in somewhat higher activity of glutamate-pyruvate transaminase, glutamate-oxalacetate transaminase and 2-ketoglutarate dehydrogenase in *db/db* than control mice.

4. DISCUSSION

The process of glucose-induced insulin release from incubated pieces of pancreatic tissue is impaired in 7–9 week-old *db/db* mice [20]. The enhancing action of theophylline upon glucose-stimulated insulin secretion is preserved, however, in these diabetic animals [20]. The present findings suggest that the preferential alteration of the B-cell secretory response to D-glucose could be caused, or at least favoured, by a decrease of m-GDH activity in the islets from *db/db* mice.

In pure B-cells prepared from normal rats, the activity of m-GDH, when expressed relative to DNA content, is about 10 times higher than in islet non-B cells [21]. The decreased activity of this enzyme in the islets of *db/db* mice is unlikely, however, to be substantially attributable to a decreased contribution of B-cells relative to total islet mass. Previous studies on both B-cell replication and volume density of the different cell types in the islets indeed indicate that a reduction in the number of islet B-cells occurs only in older *db/db* mice [22–24]. Furthermore, even in *db/db* mice as old as 7–8

Table I

Genetic and metabolic status of mice used for the preparation of islet batches from control (C_1 to C_7) or diabetic (D_1 to D_8) animals

Sample (no.)	Mice (n)	0/0	Genetic status	Age (weeks)	Body wt (g)	Plasma glucose (mM)	Plasma insulin (μU/ml)	Islets (n)
C1	6	3/3	*db?/+*	5–6	N.D.[b]	10.2	35	150
C2	6	4/2	*+/+*	8–9	21.8 ± 0.9	9.7–9.9	26–26	200
C3	12	3/9	*+db?/m+*	7–10	22.3 ± 0.8	8.3–9.1–9.8	43–55–58	300
C4	10	0/10	*db/+*	9	20.8 ± 0.4	9.1–9.5	20–23	350
C5	10	10/0	*db/+*	9	25.5 ± 0.3	10.6–11.4	41–77	240
C6	10	0/10	*db/+*	9	21.7 ± 0.6	9.2–9.2	16–30	360
C7	10	10/0	*db/+*	9	25.9 ± 0.7	9.8–10.8	36–68	320
D1	6	2/4	*+db/+db*	6–7	N.D.	27.1	377	300
D2	5	2/3	*+db/+db*	8	N.D.	22.6	110	230
D3	4	1/3	*+db/+db*	13	N.D.	29.4	142	300
D4	4	2/2	*db/db*	18	N.D.	29.6	178	300
D5	6	6/0	*+db/+db*	5–11[a]	44.3 ± 2.3	35.1–41.7	139–270	230
D6	6	0/6	*+db/+db*	5–11[a]	39.2 ± 5.4	31.3–31.6	83–179	200
D7	6	6/0	*+db/+db*	5–11[a]	41.0 ± 1.8	40.0–42.8	150–192	200
D8	6	1/5	*+db/+db*	5–11[a]	32.3 ± 3.1	21.9–21.9	367–393	160

[a] Mean age: 8–9 weeks.
[b] N.D., not determined.

Table II

Protein content and enzymic activities in islet and liver homogenates

Mice	Control	Diabetes	P
Pancreatic islets	0.63 ± 0.13 (7)	0.68 ± 0.07 (8)	N.S.
Protein (μg/islet)	143.1 ± 18.0 (7)	60.5 ± 13.8 (8)	<0.005
m-GDH (fmol/min per μg)[a]	31.6 ± 4.2 (7)	27.3 ± 1.2 (8)	N.S.
Glutamate dehydrogenase (pmol/min per μg)	1.37 ± 0.24 (7)	1.30 ± 0.37 (8)	N.S.
2-Ketoglutarate dehydrogenase (pmol/min per μg)			
Liver	198.6 ± 17.6 (28)	198.3 ± 4.1 (24)	N.S.
Protein (mg/g wet weight)	7.64 ± 0.50 (28)	7.26 ± 0.87 (24)	N.S.
m-GDH (fmol/min per μg)[a]	422 ± 34 (27)	379 ± 43 (24)	N.S.
m-GDH (fmol/min per μg)[b]	23.6 ± 0.5 (27)	23.8 ± 0.5 (24)	N.S.
Glutamate dehydrogenase (pmol/min per μg)	0.20 ± 0.01 (28)	0.37 ± 0.03 (24)	<0.001
2-Ketoglutarate dehydrogenase (pmol/min per μg)	134.8 ± 5.5 (28)	165.1 ± 5.4 (23)	<0.001
Glutamate-pyruvate transaminase (pmol/min per μg)	130.9 ± 7.2 (28)	162.6 ± 7.3 (23)	<0.005
Glutamate-oxalacetate transaminase (pmol/min per μg)			

The activity of FAD-linked glycerophosphate dehydrogenase (m-GDH) was measured by either a radioisotopic procedure[a] or colorimetric technique[b]. *P* values are not provided when there was no significant difference (N.S.) between control and diabetic mice.

months, differential cell counting of the endocrine islet cells demonstrates that the frequency of B-cells is barely decreased in the diabetic animals, averaging 72.9 ± 3.3% as compared to 80.6 ± 1.2% in the control mice [25]. Likewise, the decreased activity of m-GDH in pancreatic islets of *db/db* mice is unlikely to represent the consequence of hyperglycemia. No decrease in the activity of this enzyme is found in islets exposed for a prolonged period to high concentrations of D-glucose, whether in vitro or in vivo [15,16]. The islet enzymic defect cannot be blamed on a generalized decrease in the activity of all mitochondrial dehydrogenases, as documented by our measurement of glutamate dehydrogenase and 2-ketoglutarate dehydrogenase in islet homogenates.

It is quite conceivable, therefore, that the deficiency of islet m-GDH represents a primary factor contributing to the alteration of B-cell function in this model of hereditary diabetes. A comparable situation was recently documented in another animal model of inherited non-insulin-dependent diabetes, namely in GK rats [27,28]. In both cases, the decrease in m-GDH activity was restricted to B-cells and not observed in liver homogenates. It is proposed, therefore, that a site-specific deficiency of m-GDH might represent a far-from-uncommon causal or contributive factor in the pathogenesis of non-insulin-dependent diabetes mellitus.

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