Impairment of Glycerol Phosphate Shuttle in Islets From Rats With Diabetes Induced by Neonatal Streptozocin

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In islets from adult rats injected with streptozocin during the neonatal period, the oxidative and secretory responses to D-glucose are more severely affected than those evoked by L-leucine. A possible explanation for such a preferential defect was sought by comparing the rate of aerobic glycolysis, taken as the sum of D-[3,4-^{14}C]glucose conversion to labeled CO_2, pyruvate, and amino acid, with the total glycolytic flux, as judged from the conversion of D-[5-^{3}H]glucose to $^{3}H_2O$. A preferential impairment of aerobic relative to total glycolysis was found in islets from diabetic rats incubated at either low or high D-glucose concentration. This coincided in islet mitochondria of diabetic rats with a severe decrease in both the basal (no-Ca^{2+}) generation of $^{3}H_2O$ from L-[2-^{3}H]glycerol-3-phosphate and the Ca^{2+}-induced increment in [^{3}H]glycerophosphate detritiation. The mitochondria of diabetic rats were also less efficient than those of control animals in generating $^{14}CO_2$ from [1-^{14}C]-2-ketoglutarate. The diabetes-induced alteration of 2-ketoglutarate dehydrogenase in islet mitochondria was less marked, however, than that of the FAD-linked glycerophosphate dehydrogenase and was not associated with any change in responsiveness to Ca^{2+}. Sonicated islet mitochondria of diabetic rats displayed normal to slightly elevated glutamate dehydrogenase activity. We propose, therefore, that the preferential impairment of the oxidative and secretory responses of islet cells to D-glucose in this experimental model of diabetes may be at least partly attributable to an altered transfer of reducing equivalents into the mitochondria as mediated by the glycerol phosphate shuttle. *Diabetes* 40:227–32, 1991

The neonatal administration of streptozocin (STZ) leads to a situation in adult rats reminiscent of that found in non-insulin-dependent diabetic subjects and characterized by a preferential impairment of the pancreatic β-cell secretory response to D-glucose as distinct from other nutrient or nonnutrient secretagogues (1–4). Likewise, in islets isolated from diabetic rats, the oxidative response of mitochondria, as judged from the oxidation of D-[6-^{14}C]glucose or L-[U-^{14}C]leucine, is more severely impaired in the case of the hexose than amino acid, despite a close-to-normal rate of glycolysis (5,6). To our knowledge, no explanation is available to account for the preferential alteration of the oxidative response to D-glucose in this experimental model of diabetes. It is apparently not attributable to any obvious anomaly in either the activity or mitochondrial binding of hexokinase isoenzymes (5,6).

In normal islets, a rise in extracellular D-glucose concentration causes a preferential stimulation of mitochondrial oxidative events including the transfer of reducing equivalents into mitochondria (as coupled with aerobic glycolysis and as mediated by the glycerol phosphate shuttle), the oxidative decarboxylation of pyruvate, and the oxidation of acetyl residues in the Krebs cycle (7–9). The major aim of this study is to explore whether the preferential alteration of the oxidative response to D-glucose in the islets of diabetic rats may be attributable, at least in part, to a perturbation in aerobic glycolysis independent of any further anomaly in the mitochondrial oxidation of pyruvate and acetyl residues.

RESEARCH DESIGN AND METHODS

Control rats and animals injected with STZ (10) during the neonatal period were given free access to food (11) up to the time they were killed. The rats were weighed and then decapitated, blood being collected in heparinized tubes. After centrifugation for 10 min at 1000 × *g* at 4°C, the plasma was removed and stored at −20°C. The plasma glucose was measured by the glucose oxidase method (12) and the plasma insulin by radioimmunoassay (13). In each experiment, islets were isolated by the collagenase method (14)

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from the pancreas of two to four rats. The Hank's solution used to inflate the pancreas and isolate the islets contained 16.7 mM D-glucose. Two groups of 12 islets were sonicated (3 × 5-s sonications) in 0.25 ml H_2O for measurement of islet protein by the method of Lowry et al. (15) with bovine serum albumin (BSA) as standard. An aliquot (25 μl) of the islet homogenate was mixed with 1 ml of a phosphate buffer (100 mM, pH 7) containing BSA (1% wt/vol) and stored at −20°C for measurement of the islet insulin content (14).

For measurement of D-[3,4-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization, groups of 15–20 islets were incubated for 120 min at 37°C in 40 μl of a bicarbonate-buffered medium (14) containing BSA (5 mg/ml). The incubation was halted by addition of 20 μl of a citrate-NaOH buffer (0.4 M, pH 4.9) containing KCN (5 mM), rotenone (10 μM), and antimycin A (10 μM). The production of $^{14}CO_2$ and $^{3}H_2O$ was then measured as previously described (16). The acidified medium (60 μl) containing the islets was then stored at −20°C and later examined for its content of ^{14}C-labeled pyruvate and amino acids. For this purpose, the acidified medium was neutralized by addition of 10 μl of NaOH (0.5 M) and mixed with 70 μl of a Tris-HCl buffer (100 mM, pH 8.4) containing L-glutamate (20 mM), pyridoxal phosphate (0.5 mM), dithiothreitol (0.5 mM), and glutamate-alanine transaminase (6.4 U/ml). After a 60-min incubation at 20°C, ^{14}C-labeled amino acids were separated by ion-exchange chromatography (17).

For measurement of mitochondrial variables, groups of 500–700 islets each were washed once and homogenized with Potter-Elvehjem tubes (20 passes) in 0.3 ml of iced HEPES-NaOH buffer (5 mM, pH 7.2) containing 150 mM mannitol, 60 mM sucrose, 15 mM KCl, 3 mM KH_2PO_4, 1 mM $MgCl_2$, and 0.5 mM EGTA. After a 10-min centrifugation at 1000 × *g* and 4°C to remove intact cells, cell debris, and nuclei, the pellet was resuspended in 0.3 ml of the same buffer and again homogenized and centrifuged. The supernatants of these two centrifugations were then pooled together. In this procedure, the activity of glutamate dehydrogenase in the supernatants represented 62.5 ± 3.4 and 57.9 ± 2.7% (n = 3) in control and diabetic rats, respectively, of the total activity measured in the initial crude islet homogenate. Aliquots (≤50 μl) of the postnuclear supernatant were incubated for 10 min at 37°C in a reaction mixture (final vol 75–100 μl) consisting of the same buffer and containing, as required, $CaCl_2$ (0.5 mM), L-[2-^{3}H]glycerol-3-phosphate (0.1 mM), and/or [1-^{14}C]-2-ketoglutarate (50 μM). The incubation was halted by adding 25 μl of a citrate-NaOH buffer (0.4 M, pH 4.9) containing KCN (5 mM), rotenone (10 μM), and antimycin A (10 μM). The $^{3}H_2O$ or $^{14}CO_2$ formed during incubation was then recovered and measured as described elsewhere (16). The results were expressed relative to the protein content of the postnuclear supernatant (383 ± 36 ng/islet, n = 12), which was measured as described above. The concentration of L-[2-^{3}H]glycerol-3-phosphate and [1-^{14}C]-2-ketoglutarate used in these experiments was selected to optimize the demonstration of Ca^{2+}-induced activation of the relevant dehydrogenase. The activity of glutamate dehydrogenase in samples (50 μl) of the postnuclear supernatant, which was sonicated and diluted in a Tris-HCl buffer (100 mM, pH 8) containing 50 mM ammonium acetate, was measured over a 15-min incubation at 37°C after mixing with 50 μl of the same Tris-HCl buffer also containing 1.4 mM 2-ketoglutarate (mixed with a tracer amount of [U-^{14}C]-2-ketoglutarate), 0.6 mM NADPH, and 2 mM ADP. The ^{14}C-labeled L-glutamate formed during incubation was then separated by ion-exchange chromatography (17).

In some experiments, the activity of FAD–glycerophosphate dehydrogenase was measured in crude islet homogenates prepared by sonication of groups of 300 islets each in 0.25 ml of the HEPES-NaOH buffer defined above. Aliquots (30 μl) were then incubated for 20 min at 37°C in a reaction mixture (final vol 90 μl) consisting of the same buffer and containing L-[2-^{3}H]glycerol-3-phosphate (1 mM) and FAD (50 μM). The incubation was halted as described above.

Control experiments for the activity of mitochondrial dehydrogenases were conducted in liver mitochondria, isolated as previously described (18). The protein content of the mitochondrial pellet averaged 18.7 ± 0.8 and 20.5 ± 0.4 μg/mg liver wet wt in control and diabetic rats, respectively. The mitochondrial pellet was resuspended in 8 ml of the HEPES-NaOH buffer (see above), and aliquots (50 μl) of this mitochondrial suspension were examined for the detritiation of L-[2-^{3}H]glycerol-3-phosphate, oxidation of [1-^{14}C]-2-ketoglutarate, or activity of glutamate dehydrogenase in the same manner as described above for pancreatic islets.

All results, including those mentioned above, are expressed as means ± SE, together with the number of observations or degree of freedom and statistical significance of differences as assessed by use of Student's t test. The SE of the sum of or ratio between mean values was calculated as indicated elsewhere (19,20).

RESULTS

The diabetic rats displayed a slightly lower body weight ($P < 0.005$), a higher plasma glucose concentration ($P < 0.001$), and a lower plasma insulin-glucose ratio ($P < 0.001$) than the control animals (Table 1). The insulin

TABLE 1
Metabolic status of control and diabetic rats

	Control	*n*	Diabetic	*n*
Age (wk)	11.1 ± 0.2	29	10.6 ± 0.1	28
Body weight (g)	310 ± 9	29	274 ± 6	28
Plasma glucose concentration (mM)	7.95 ± 0.13	27	9.91 ± 0.25	25
Plasma insulin concentration (μU/ml)	72.3 ± 5.7	23	58.5 ± 4.8	24
Plasma insulin-glucose ratio (U/mol)	9.18 ± 0.72	23	5.94 ± 0.49	24
Islet protein content (ng/islet)	685 ± 86	9	580 ± 93	9
Islet insulin content (μU/islet)	830 ± 111	9	200 ± 26	9

TABLE 2
D-Glucose metabolism in islets from control and diabetic rats

	Metabolic product	Metabolic flow (pmol · islet^{-1} · 120 min^{-1})			
		Control	*n*	Diabetic	*n*
2.8 mM D-glucose					
[5-^{3}H]glucose	^3H_2O	39.40 ± 5.18	10	54.96 ± 5.22	10
[3,4-^{14}C]glucose	Pyruvate + alanine	4.31 ± 0.63	5	7.59 ± 1.23	5
[3,4-^{14}C]glucose	$^{14}CO_2$	18.74 ± 2.44	10	17.03 ± 1.25	10
16.7 mM D-glucose					
[5-^{3}H]glucose	^3H_2O	117.22 ± 5.16	36	100.60 ± 7.48	37
[3,4-^{14}C]glucose	Pyruvate + alanine	12.93 ± 0.84	48	11.10 ± 1.14	46
[3,4-^{14}C]glucose	$^{14}CO_2$	69.42 ± 2.69	54	44.19 ± 2.05	54

content of the islets was severely decreased in the diabetic rats ($P < 0.001$), but the islet protein content was not significantly affected ($P > 0.4$). All metabolic data collected in intact islets were expressed therefore on a per islet basis.

At a low concentration of D-glucose (2.8 mM), the rate of glycolysis, as judged from the generation of ^3H_2O from D-[5-^{3}H]glucose, was not significantly different ($P > 0.05$) in intact islets of control and diabetic rats (Table 2). When tested within the same experiments, a rise in hexose concentration from 2.8 to 16.7 mM tended to increase to a greater relative extent the rate of glycolysis in control (16.7- to 2.8-mM D-glucose ratio 3.36 ± 0.58, df = 18) than diabetic rats (16.7- to 2.8-mM D-glucose ratio 2.73 ± 0.41, df = 18), but such a difference failed to achieve statistical significance. At the low glucose concentration, the rate of aerobic glycolysis, taken as the integrated value for the conversion of D-[3,4-^{14}C]-glucose to $^{14}CO_2$ and ^{14}C-labeled pyruvate and alanine, was virtually identical in control (23.05 ± 3.54 pmol · islet^{-1} · 120 min^{-1}) and diabetic rats (24.62 ± 1.99 pmol · islet^{-1} · 120 min^{-1}). Nevertheless, the paired ratio between D-[3,4-^{14}C]-glucose oxidation and D-[5-^{3}H]glucose utilization was significantly decreased ($P < 0.01$) in the diabetic animals, averaging 33.9 ± 3.7% compared with a control value of 47.6 ± 2.4% (n = 10 in both cases).

In a larger series of experiments conducted in the presence of a higher concentration of D-glucose (16.7 mM), the rate of glycolysis was not significantly lower ($P > 0.05$) in diabetic than control rats (Table 2). However, the rate of aerobic glycolysis was significantly decreased ($P < 0.001$) in the diabetic animals, averaging 55.29 ± 2.46 pmol · islet^{-1} · 120 min^{-1} compared with a control value of 82.35 ± 2.96 pmol · islet^{-1} · 120 min^{-1}. This coincided with a marked decrease ($P < 0.001$) in the oxidation of D-[3,4-^{14}C]glucose (Table 2). Likewise, the paired ratio between D-[3,4-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization was lower ($P < 0.001$) in diabetic (50.3 ± 2.0%, n = 37) than control (66.4 ± 2.1%, n = 36) rats.

When the latter ratios were compared with those recorded in the presence of only 2.8 mM D-glucose, it became obvious that a rise in hexose concentration preferentially stimulates ($P < 0.001$) the oxidation of D-[3,4-^{14}C]glucose relative to total glycolytic rate in both control and diabetic rats (Fig. 1). The relative magnitude of such a preferential stimulation was not significantly different ($P > 0.5$) in the two types of rats. Incidentally, comparable conclusions were reached when the comparison between data obtained at low and high concentrations of D-glucose was restricted to results collected within the same experiments. Pooling all available data, the ratio between D-[3,4-^{14}C]glucose oxidation and D-[5-^{3}H]-glucose utilization in the presence of 16.7 mM D-glucose averaged 144.0 ± 7.7% (df = 89) of the basal value measured in the presence of 2.8 mM D-glucose.

Analysis of the data summarized in Table 2 further indicated that a rise in D-glucose concentration from 2.8 to 16.7 mM increased the absolute rate of aerobic glycolysis to a lesser extent ($P < 0.05$) in diabetic than control rats (16.7- to 2.8-mM ratio 2.25 ± 0.21 vs. control value 3.57 ± 0.56). In fair agreement with a prior observation, the rise in

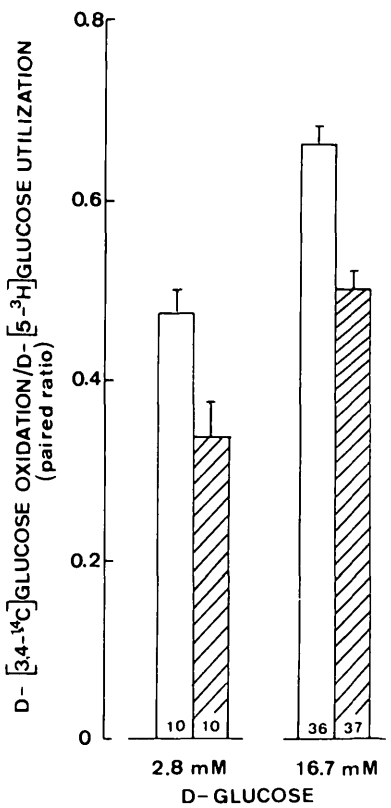

FIG. 1. Mean values ± SE for paired ratio between D-[3,4-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization in islets from control (*open bars*) and diabetic (*hatched bars*) rats incubated in presence of either 2.8 or 16.7 mM D-glucose. Number of individual measurements is shown at bottom of each *bar*.

TABLE 3
Metabolic data collected in intact or sonicated islet mitochondria

Metabolic variable	Control	Diabetic	Paired ratio
L-[2-^{3}H]glycerol-3-phosphate conversion to ^3H_2O (velocity; fmol · min^{-1} · μg^{-1})			
No Ca^{2+}	204 ± 26	66 ± 9	0.31 ± 0.02
Ca^{2+} (8 μM)	372 ± 63	75 ± 15	0.22 ± 0.01
Ca^{2+}–no Ca^{2+} ratio	1.63 ± 0.12	1.16 ± 0.07	0.72 ± 0.04
L-[1-^{14}C]-2-ketoglutarate conversion to $^{14}CO_2$ (velocity; fmol · min^{-1} · μg^{-1})			
No Ca^{2+}	133 ± 17	101 ± 14	0.76 ± 0.05
Ca^{2+} (8 μM)	198 ± 19	158 ± 12	0.81 ± 0.07
Ca^{2+}–no Ca^{2+} ratio	1.52 ± 0.10	1.59 ± 0.09	1.06 ± 0.06
Glutamate dehydrogenase activity in sonicated mitochondria (velocity; pmol · min^{-1} · μg^{-1})	40.2 ± 3.2	45.9 ± 1.0	1.20 ± 0.08

Values are means ± SE and were derived from triplicate measurements collected in 3 separate experiments. The paired ratio between diabetic and control rats was derived from paired comparisons in each experiment.

hexose concentration also increased the absolute rate of anaerobic glycolysis to a lesser extent in diabetic than control rats (6). The 16.7- to 2.8-mM D-glucose ratio averaged 1.49 in diabetic rats compared to 2.13 in control animals. Such a difference failed, however, to achieve statistical significance in this study because of the large summation of the SEs on the three parameters used to calculate the absolute rate of anaerobic glycolysis.

In the postnuclear supernatant containing suspended mitochondria, the generation of ^3H_2O from L-[2-^{3}H]glycerol-3-phosphate (0.1 mM) was 3–5 times lower ($P < 0.001$) in diabetic than control rats (Table 3). Moreover, although Ca^{2+} (8 μM) significantly increased L-[2-^{3}H]glycerol-3-phosphate conversion to ^3H_2O in both control ($P < 0.001$) and diabetic ($P < 0.05$) rats, the relative extent of such an increase was less marked in diabetic than control rats ($P < 0.005$).

The generation of $^{14}CO_2$ from [1-^{14}C]-2-ketoglutarate (50 μM) was also lower ($P < 0.025$ or less) in mitochondria from diabetic than control rats. The oxidation of [1-^{14}C]-2-ketoglutarate was less severely affected, however, than the detritiation of L-[2-^{3}H]glycerol-3-phosphate ($P < 0.001$). Moreover, in the former case, Ca^{2+} (8 μM) increased ($P < 0.001$) the production of $^{14}CO_2$ in mitochondria of control and diabetic rats to the same relative extent ($P > 0.4$).

In sharp contrast with these results, the activity of glutamate dehydrogenase in sonicated mitochondria of diabetic rats was not lower and was even slightly higher ($P < 0.05$) than the paired control value (Table 3). In considering these results, note that the three series of measurements, namely the generation of ^3H_2O from L-[2-^{3}H]glycerol-3-phosphate, the oxidation of [1-^{14}C]-2-ketoglutarate, and the activity of glutamate dehydrogenase, were all conducted in each experiment in aliquots derived from the same postnuclear mitochondrial suspension. In a further series of experiments, the activity of the FAD-linked glycerophosphate dehydrogenase measured in islet crude homogenates in the presence of 1 mM L-[2-^{3}H]glycerol-3-phosphate but absence of Ca^{2+} was again dramatically decreased ($P < 0.001$) from a control value of 822 ± 107 fmol · min^{-1} · islet^{-1} to 106 ± 17 fmol · min^{-1} · islet^{-1} in the diabetic rats ($n = 4$ for both groups).

Control experiments performed in liver of control and diabetic rats indicated the absence of mitochondrial perturbation in hepatic, as distinct from islet, cells (Table 4).

TABLE 4
Enzymatic data collected in intact or sonicated liver mitochondria

Metabolic variable	Control	Diabetic	Paired ratio
L-[2-^{3}H]glycerol-3-phosphate conversion to ^3H_2O (velocity; fmol · min^{-1} · μg^{-1})			
No Ca^{2+}	32.4 ± 2.2	30.0 ± 1.5	0.93 ± 0.09
Ca^{2+} (8 μM)	62.5 ± 3.6	71.2 ± 7.5	1.07 ± 0.15
Ca^{2+}–no Ca^{2+} ratio	2.09 ± 0.25	2.38 ± 0.28	1.11 ± 0.05
L-[1-^{14}C]-2-ketoglutarate conversion to $^{14}CO_2$ (velocity; pmol · min^{-1} · μg^{-1})			
No Ca^{2+}	1.12 ± 0.11	1.02 ± 0.10	0.90 ± 0.06
Ca^{2+} (8 μM)	1.80 ± 0.21	1.77 ± 0.21	0.95 ± 0.07
Ca^{2+}–no Ca^{2+} ratio	1.54 ± 0.06	1.68 ± 0.08	1.10 ± 0.06
Glutamate dehydrogenase activity in sonicated mitochondria (velocity; pmol · min^{-1} · μg^{-1})	431 ± 24	478 ± 27	1.13 ± 0.09

Values are means ± SE and were derived from triplicate measurements collected in 4 separate experiments. The paired ratio between diabetic and control rats was derived from paired comparisons in each experiment.

DISCUSSION

A rise in D-glucose concentration from 2.8 to 16.7 mM increases to a greater extent the rate of aerobic than total glycolysis in pancreatic islets from normal rats (7). The ratio between aerobic and total glycolysis in the presence of 16.7 mM D-glucose averaged 144% of the value found in the presence of only 2.8 mM D-glucose (7). The preferential stimulation of aerobic glycolysis is currently ascribed to the cellular accumulation of Ca^{2+} and subsequent activation of the Ca^{2+}-responsive FAD-linked glycerophosphate dehydrogenase in glucose-stimulated islets (21–23).

In a more recent study, the rate of aerobic glycolysis in islets from normal and diabetic rats was judged indirectly through the difference between D-[5-^{3}H]glucose utilization and the generation of ^{14}C-labeled lactic acid from D-[6-^{14}C] glucose (6). In that study, it was already noticed, in fair agreement with the data in this study, that *1*) the rate of aerobic glycolysis is lower in diabetic than control rats at high but not low D-glucose concentrations, *2*) the relative contribution of aerobic to total glycolysis increases in both types of rats in response to a rise in hexose concentration, and *3*) the relative extent of such an increase is not different in the two types of rats. However, in our previous study, no significant difference between the absolute values for the ratio of aerobic to total glycolysis had been recorded when comparing control with diabetic rats.

In this study, the direct measurement of aerobic glycolysis, taken as the integrated value for the conversion of D-[3,4-^{14}C]glucose to labeled CO_2, pyruvate, and amino acids, revealed that the fractional contribution of aerobic to total glycolysis is significantly impaired in the diabetic rats, whether at a low or high concentration of D-glucose. A rise in D-glucose concentration from 2.8 to 16.7 mM increased the ratio of aerobic to total glycolysis to the same relative extent in both control and diabetic rats. However, this finding does not rule out an impaired response of the FAD-linked glycerophosphate dehydrogenase in terms of the cellular accumulation of Ca^{2+} and subsequent activation of the enzyme in glucose-stimulated islets (24). Indeed, the rise in hexose concentration also provoked a lesser relative increase of anaerobic glycolysis in diabetic than control rats (6). The latter anomaly, which is likely to result from both a difference in hexokinase activity (6) and the primary defect in the glycerol phosphate shuttle, may well mask the impaired response of the mitochondrial dehydrogenase, when such a response is judged from the ratio of aerobic to total glycolysis.

The perturbation of aerobic glycolysis in intact islets of diabetic rats coincided with a severe impairment of the generation of ^3H_2O from L-[-^{3}H]glycerol-3-phosphate in mitochondria. As judged by the latter criterion, the mitochondrial FAD-linked glycerophosphate dehydrogenase was less effeciently activated by a rise in extramitochondrial Ca^{2+} concentration in diabetic than control rats. These two defects are probably interrelated, because the generation of reducing equivalents and ATP may in turn affect the accumulation of Ca^{2+} into islet mitochondria (25).

As a whole, these findings strongly suggest the presence of a severe alteration in the operation of the glycerol phosphate shuttle in the islets of diabetic rats, which is believed to represent the major modality for the transfer of reducing equivalents into the mitochondria as coupled with aerobic glycolysis in the islet cells (9). Such a defect may account, at least in part, for the preferential alteration in the secretory response of the diabetic islets to D-glucose as distinct from other nutrient and nonnutrient secretagogues (1,3,4,6).

Although the enzymatic defects were observed in islets but not in liver cells, these findings raise questions about such items as the specificity of the mitochondrial lesion in β-cells as distinct from non–β-cells and its determinism in terms of either a primary effect of STZ or a secondary change attributable, for instance, to moderate hyperglycemia and/or excessive catabolism.

In further work conducted in the same animals, we observed that the percentage of nonenzymatically glycosylated protein in liver cytosol and the sorbitol and glycogen content of the islets are not significantly increased in the animals injected with STZ during the neonatal period, at variance with the situation found in experimental hyperglycemia due to either glucose infusion or STZ administration to adult rats (26–28). Likewise, our diabetic animals were not suffering from any major catabolic illness, as judged for instance from the β-hydroxybutyrate content of the liver, which failed to be significantly increased in contrast to the situation found in either starved, fully diabetic, or even diazoxide-treated rats. It should be stressed, therefore, that the islet enzymatic perturbation might be due, in this model, to a long-term (29), e.g., genomic (30), effect of STZ.

In conclusion, the results of this study strongly suggest that, in this experimental model of diabetes, the preferential alteration of the β-cell oxidative, and, hence, secretory response to D-glucose, as distinct from other secretagogues, may be attributable, in part at least, to a defect in the activity of the mitochondrial FAD-dependent glycerophosphate dehydrogenase, leading to a decrease in the ratio of aerobic to total glycolysis.

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