Glucose Cycling in Islets From Healthy and Diabetic Rats

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Pancreatic islets from healthy (control) and neonatally streptozocin-induced diabetic (STZ-D) rats, a model for non-insulin-dependent diabetes mellitus, were incubated with ^3H_2O and 5.5 or 16.7 mM glucose. At 5.5 mM glucose, no detectable [^{3}H]glucose was formed. At 16.7 mM, 2.2 patom · islet^{-1} · h^{-1} of ^{3}H was incorporated into glucose by the control islets and 5.4 patom · islet^{-1} · h^{-1} by STZ-D islets. About 75% of the ^{3}H was bound to carbon-2 of the glucose. Glucose utilization was 35.3 pmol · islet^{-1} · h^{-1} by the control and 19.0 pmol · islet^{-1} · h^{-1} by the STZ-D islets. Therefore, 4.5% of the glucose-6-phosphate formed by the control islets and 15.7% by the STZ-D islets was dephosphorylated. This presumably occurred in the β-cells of the islets catalyzed by glucose-6-phosphatase. An increased glucose cycling, i.e., glucose → glucose-6-phosphate → glucose, in islets of STZ-D rats may contribute to the decreased insulin secretion found in these animals. *Diabetes* 39:456–59, 1990

Glucose-6-phosphatase activity has been found in the β-cells of pancreatic islets from mice, rats, and guinea pigs (1–3), except for one report of its absence in the islets from rats (4). The enzyme activity can be induced in vivo with cortisone (1). We recently showed that glucose cycling (GC), i.e., glucose → glucose-6-phosphate → glucose, operates in the isolated islets of *ob/ob* mice. About 40% of the glucose that was phosphorylated by the islets was dephosphorylated (5). As in liver, increased GC in the islets may play a role in the regulation of glucose-6-phosphate flux and thus affect glucose-induced insulin release from islets. We have investigated the extent of GC in isolated islets of normal and neonatally streptozocin-induced diabetic (STZ-D) rats.

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RESEARCH DESIGN AND METHODS

The procedure for inducing diabetes was similar to that previously described (6,7). Lactating Sprague-Dawley rats with their offspring were obtained (ALAB, Stockholm) on the day after delivery. On that day, the pups were injected with 90 mg/kg body wt i.p. STZ dissolved immediately before use in 0.01 M citrate buffer at pH 4.5. Pups from control litters received citrate buffer alone. Blood samples were taken from the tails 48 h after STZ injection, and pups with blood glucose concentrations ≥10 mM were included in the study. After 4 wk, the rats were weaned and maintained on a standard pellet diet (ALAB).

Healthy (control) and STZ-D rats of both sexes between 6 and 10 wk of age were used. They were fed until they were killed by decapitation. The islets were isolated by collagenase digestion of pancreases (8). The control rats weighed 243 ± 12 g (n = 20), and the diabetic rats weighed 243 ± 13 g (n = 20). Blood glucose concentrations at death were 6.8 ± 0.3 mM (n = 18) in the control and 9.5 ± 0.4 mM (n = 19, $P < 0.001$) in the STZ-D rats.

Incubation with ^3H_2O. Islets were incubated in Krebs bicarbonate buffer, pH 7.4, with 0.5% bovine albumin (Sigma, St. Louis, MO), 5.5 or 16.7 mM glucose, and ^3H_2O (5 mCi/100 μl of medium). The ^3H_2O (100 mCi/ml) was purchased from Du Pont-NEN (Boston, MA). In each experiment with 5.5 or 16.7 mM glucose, 100 islets were incubated in 100 μl of medium in one vial, and 100 μl of medium was incubated in a second vial but without islets. The contents of each vial was gassed with 95% O_2/5% CO_2, and the vial was sealed. After 3 h of incubation at 37°C, 100 μl of 10% perchloric acid was injected into the vial. Then 2.5 mg glucose was added, the supernatant was neutralized with KOH, and potassium perchlorate was removed by centrifugation for 10 min at 3000 × g. The supernatant was passed through a mixed-bed ion-exchange resin (MB-3; BDH, Poole, UK), and the effluent was freeze dried. The residue was dissolved

in water and evaporated. Solution in water and evaporation was repeated twice more to ensure the removal of ^3H_2O.

On high-performance liquid chromatography (HPLC; HPX-87P system, Bio-Rad, Richmond, CA), each residue from the incubate with islets gave a single peak of radioactivity with the mobility of glucose. An average of 75% (range 50–86%) of the glucose added to the medium was recovered in the residue after deionization, and an average of 88.4% (range 85–98%) of that added to the HPLC column was recovered in the peak. Residues from the incubates without islets gave no peak. The disintegrations per minute in the radioactive peak multiplied by 2.6 or 2.8, i.e., 2.5 mg of glucose added at the end of the incubation plus 0.1 mg of glucose in the medium at the beginning of incubation at 5.5 mM and 0.3 mg at 16.7 mM, and divided by the milligrams of glucose in the peak gave the disintegrations per minute of ^3H from ^3H_2O incorporated into glucose. Because there are 1.1×10^{10} dpm in 5 mCi of ^3H and 1.1×10^{10} patom of hydrogen in 100 μl of water, the disintegrations per minute in the glucose equals the picoatoms of ^3H incorporated into glucose. Glucose was determined with a glucose oxidase method (model 23A glucose analyzer, YSI, Yellow Springs, OH).

[2-^{14}C]glucose (Amersham, Amersham, UK) was added to [^{3}H]glucose isolated by HPLC so that the ^{14}C in disintegrations per minute added was about the same as the disintegrations per minute of ^3H, giving a ratio of ~1.0. Unlabeled glucose (100 mg) was added. A portion of that glucose was oxidized to CO_2 and H_2O with a model 306 TriCarb sample oxidizer (Packard, Downers Grove, IL). The $^{14}CO_2$ and the ^3H_2O were assayed for radioactivity in disintegrations per minute giving the $^3H/^{14}C$ ratio in the glucose. Recoveries of radioactivity from ^3H and ^{14}C standards that were oxidized were 92 and 101%, respectively, and the crossovers between ^3H and ^{14}C were <0.1%.

The remainder of the glucose to which the [2-^{14}C]glucose had been added was converted to its glucosazone. The $^3H/^{14}C$ in an aliquot of the glucosazone was determined by also oxidizing it to CO_2 and H_2O and assaying these for radioactivity. The glucosazone should retain all the hydrogens bound to the carbons of the glucose except the one bound to carbon-2. We have previously demonstrated, by preparing osazones from [1-^{3}H]- and [3-^{3}H]glucose as well as [2-^{3}H]glucose, that only the hydrogen bound to carbon-2 is removed in the formation of the osazone (5). Subtracting the $^3H/^{14}C$ ratio in the osazone divided by the ratio in the glucose from 1.0 and multiplying the result by the picoatom of hydrogen incorporation into glucose and dividing by 300, i.e., 3 h × 100 islets, gave the picoatoms per islet per hour of ^3H incorporation in carbon-2 of the glucose.

Incubation with [5-^{3}H]glucose. Islets were incubated under the same conditions as with ^3H_2O, but the glucose was labeled with [5-^{3}H]glucose (Amersham) with specific activity of 1.8 and 0.6 mCi/mmol at 5.5 and 16.7 mM glucose, respectively, and the incubation was done in triplicate, each with 15 islets. The utilization of glucose was measured from the yield of ^3H_2O (9).

Measurement of insulin secretion. Batches of three islets were incubated in triplicate for 1 h at 37°C in 300 μl of Krebs bicarbonate buffer, pH 7.4, with 2 mg/ml bovine albumin and 5.5 or 16.7 mM glucose. The incubation was preceded by a preincubation for 30 min at 3.3 mM glucose. After incubation, an aliquot of the medium was stored at −70°C until the radioimmunoassay of insulin was performed (10). Results are expressed as means ± SE.

RESULTS

In islets from control and STZ-D rats incubated with ^3H_2O at 5.5 mM glucose, no incorporation of ^3H into glucose was detectable. Glucose utilization was 16.24 ± 2.14 pmol · islet^{-1} · h^{-1} by islets from the control rats and 8.31 ± 0.50 pmol · islet^{-1} · h^{-1} by islets from the STZ-D rats. Results for the incubations at 16.7 mM glucose are presented in Table 1. Incorporation of ^3H into glucose by the STZ-D islets was more than twice that by the control islets. About 75% of the incorporated ^3H was bound to carbon-2 of the glucose by both control and STZ-D islets. Thus, the incorporation of ^3H into carbon-2 of glucose was also more than twice as much by the STZ-D as control islets. About 25% of ^3H incorporated into carbons other than carbon-2 of glucose was not localized further. Via the pentose cycle and transaldolase exchange, ^3H can be bound to the other carbons of glucose-6-phosphate (5,11,12).

Glucose utilization by the control islets was about twice that by the STZ-D islets. Therefore, 15.73% of the phosphorylated glucose in the STZ-D islets and 4.49% in control islets was dephosphorylated. Insulin secretion by control islets was almost 5-fold enhanced when comparing the secretion at 16.7 mM glucose with that at 5.5 mM glucose (Table 2). In STZ-D islets, the corresponding increase was 4.4-fold. The rate of insulin secretion on a per-islet basis by STZ-D islets at 5.5 and 16.7 mM glucose was 52 and 46%, respectively, that of control islets (Table 2).

DISCUSSION

The only known pathway by which ^3H could bind to carbon-2 of glucose is GC (5). At 16.7 mM glucose, 2.2 ± 0.2 pmol · islet^{-1} · h^{-1} of glucose was formed from glucose-6-phosphate by control islets and 5.4 ± 0.5 pmol · islet^{-1} · h^{-1} by STZ-D islets. From these quantities and the amounts of glucose utilized, the percentage of phosphorylated glucose that was dephosphorylated was 4.5% in control and 15.7% in STZ-D islets. Thus, GC is almost four times more active in STZ-D than in control islets.

Our method for measurement of GC is based on the assumption that there is a complete isotopic equilibration of the hexose-6-phosphates relative to the hydrolysis of glucose-6-phosphate, so that for every tritiated hydrogen bound

TABLE 1
Incorporation of ^3H in glucose, glucose utilization, and calculated glucose cycling (GC) in rat islets incubated at 16.7 mM glucose

Source of islets	Control	Diabetic
A: ^3H incorporated into glucose (patom · islet^{-1} · h^{-1}; n = 8)	2.24 ± 0.22	5.35 ± 0.77
B: fraction of incorporated ^3H bound to carbon-2 of glucose (n = 6)	0.74 ± 0.04	0.77 ± 0.05
C: ^3H bound to carbon-2 of glucose (A × B)	1.66 ± 0.21	3.55 ± 0.52
D: glucose utilization (pmol · islet^{-1} · h^{-1}; n = 6)	35.30 ± 0.48	19.02 ± 1.95
E: percent GC [100 C/(C + D)]	4.49	15.73

TABLE 2
Glucose-induced insulin secretion in isolated rat islets

Glucose concentration in medium (mM)	Control	STZ-D
5.5	9.9 ± 1.5	5.1 ± 0.9
16.7	48.9 ± 8.2*	22.5 ± 3.0*

Insulin secretion ($\mu U \cdot islet^{-1} \cdot h^{-1}$) was measured in islets incubated for 1 h in Krebs bicarbonate buffer containing 5.5 or 16.7 mM glucose. Values are means ± SE of triplicate incubations of islets from 6 healthy and 6 streptozocin-induced diabetic (STZ-D) rats.
*$P < 0.001$ vs. 5.5 mM glucose.

at carbon-2 of glucose, one molecule of glucose is dephosphorylated (5). To the extent that the tritiation is incomplete, the extent of dephosphorylation will be underestimated. In the liver of rats (13,14) and humans (15), the detritiation at the hexose isomerase level is not complete, resulting in an underestimation of GC by 10–25%. In the liver, the extent of tritiation seems to parallel the extent of detritiation at carbon-2 in the conversion of fructose-6-phosphate to glucose-6-phosphate. Thus, when hepatocytes are incubated with ^3H_2O and unlabeled lactate and pyruvate (16) or when ^3H_2O is injected into intact rats (13,17), the amount of ^3H bound to carbon-2 of glucose from glycogen is ~0.9 atoms of hydrogen per molecule of glucose.

The STZ-D rat is an experimental model showing partial similarity to non-insulin-dependent diabetes mellitus (NIDDM) in humans. The rat is nonobese and nonketotic with mild hyperglycemia (6,7). In vivo and in the perfused pancreas, the insulin response to glucose is markedly impaired, whereas the response to arginine and nonnutrient secretagogues is normal or even exaggerated (6,7,18). Insulin release from isolated STZ-D islets, stimulated by 16.7 mM glucose, is 40–50% of that from control islets (19–21). About 50% of the cells in STZ-D islets and ~70% in control islets are β-cells, whereas the mass of non–β-cells has been reported to be the same in control and STZ-D islets (22). The DNA content of STZ-D islets is <80% (20,21), and STZ-D islet volume has been estimated to be ~40% that of control islets (23).

In control islets, measurements of glucose metabolism mainly reflect events in the β-cells, where the rate of glucose metabolism determines the extent of insulin secretion (24). The ATP/ADP ratio probably represents the main regulator of glucose-induced insulin release by closing ATP-sensitive K^+ channels, which then leads to β-cell membrane depolarization, Ca^{2+} influx, and insulin secretion (25). Because most of the K^+ channels have to be closed to initiate secretion, even small alterations in the steady-state level of ATP may alter insulin release.

Does the impaired insulin response to glucose in STZ-D islets reflect a defect in glucose metabolism in the β-cells? In a recent study by Portha et al. (21), STZ-D islets had normal low- and high-K_m glucose-phosphorylating activity but a 50% reduced glucose utilization rate per islet compared with control islets. Recalculation on a per-islet DNA basis still indicated that glucose utilization was significantly lower in freshly isolated STZ-D islets than in control islets (21). The most impressive finding by Portha et al. was a marked decrease in rates of basal and glucose-stimulated respiration and glucose oxidation in STZ-D islets. Therefore, they concluded that the unresponsiveness to glucose of STZ-D islets may be attributed mainly to a defect in glucose metabolism resulting from alterations in the β-cell mitochondrial function (21). In contrast, Colella et al. (26) did not find glucose utilization to be significantly lower in STZ-D than in control islets.

Like Portha et al. (21), we found a 50% decrease in glucose utilization, per islet, in STZ-D versus control islets. In our experiments, the islet capacity to release insulin was also decreased by 50% in STZ-D animals, suggesting that glucose is mainly metabolized in the preserved β-cells in STZ-D islets. If non–β-cells have no glucose-6-phosphatase but utilize glucose, the increase in GC in the islets of STZ-D rats was underestimated in our study.

Because one molecule of ATP is consumed for every glucose molecule passing through GC, an increased GC in STZ-D islets would decrease the islet ATP pool. Hence, in addition to a mitochondrial defect, an increased flux of glucose-6-phosphate through glucose-6-phosphatase may also contribute to the decreased glucose-induced insulin response of STZ-D islets. It is not clear whether increased GC is due to a direct effect of STZ on the β-cell or is inherent in the diabetic state. The augmented GC in islets from hyperglycemic *ob/ob* mice compared with their lean littermates, however, favors a metabolic rather than toxic effect (unpublished observations).

GC was demonstrated in control and STZ-D islets incubated at 16.7 mM glucose but was undetectable at 5.5 mM glucose. This contrasts with our recent report of an extensive GC in fasted *ob/ob* mouse islets at 5.5 mM glucose (5). This difference is presumably at least partly due to significantly higher glucose-6-phosphatase activity in *ob/ob* mouse islets than in rat islets (1). Fasting is also known to increase glucose-6-phosphatase activity in liver and islets (27–29). Thus, with more enzyme, the *ob/ob* islets may dephosphorylate more glucose-6-phosphate at 5.5 mM than the rat islets at 16.7 mM glucose.

The rates of glucose utilization in our control and STZ-D islets are 2–3 times lower than in the reviewed studies (21,26). The glucose-induced insulin responses in our animal models are also 2–3 times lower than in previous reports (19,21). This discrepancy may be due to the use of rats of different age-groups and/or selection of islets of different size ranges.

An increased GC in liver occurs in the early stage of NIDDM, indicating increased activity of glucose-6-phosphatase (30). Thus, glucose-6-phosphatase activity may participate in the development of the two early defects characterizing NIDDM, i.e., impaired insulin release and increased hepatic glucose production (31).

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