The Effects of Streptozotocin on Glucose Oxidation by Isolated Islets of Langerhans (35195)

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Streptozotocin, the *N*-nitrosourea derivative of glucosamine (1) exhibits antitumor and antibacterial activities (2–4). It also produces permanent diabetes in dogs, monkeys, and rodents (2, 5–7), probably by causing irreversible damage to the beta cells (6, 8). In the following study, an early effect of streptozotocin on the metabolism of islets was determined by measuring U-^{14}C-glucose oxidation by islets isolated from rats after they had received a diabetogenic dose of streptozotocin.

Methods. Rats (Upj:TUC(SD)spf), weighing 125–160 g were fasted overnight, anesthetized with ether, and injected iv through the tail vein with saline adjusted to pH 4.4 with 0.05 *M* citric acid either free of or containing streptozotocin (65 mg/kg of body wt). One control and one treated rat were used for each experiment. Fifteen or 60 min after treatment, rats were again anesthetized with ether, the pancreas was removed and islets were isolated using the Lacy and Kostianovsky modification (10) of the method described by Moskalewski (11). The pancreas was minced in Hanks' solution (9) and transferred to a covered glass dish containing 100 mg of collagenase (Worthington Biochemical Lot No. CLS9CB) in 5 ml of Hanks' solution. The dish was placed on a magnetic stirrer and incubated at 37° for about 30 min, after which the mixture was poured into a 25-ml conical beaker. After the tissue was allowed to settle for about 1 min, the supernatant was removed. The tissue was washed six times with fresh Hanks' solution and then transferred to a petri dish. Islets were transferred with a microliter pipette to a second petri dish containing Krebs–Ringer bicarbonate buffer (13). Two groups of 50 islets were placed into center wells of Warburg flasks or into cups suspended by a rubber stopper fitted on a scintillation vial.

Excess buffer was removed from islets and 0.2 ml of incubation medium was added to each flask. The medium consisted of Krebs–Ringer bicarbonate buffer containing 2 mg/ml of total glucose (glucose + U-^{14}C-glucose; sp act 12 μCi/mg) and 2 mg/ml of crystalline bovine plasma albumin (Armour catalog No. B70307). Flasks were placed in a Dubnoff Shaker, gassed for 5 min with a mixture of 95% O_2 and 5% CO_2, and then incubated with shaking for 90 min at 37.5°. The metabolic activity of the islets was stopped and CO_2 was liberated by adding 0.1 ml of *N* HCl to the incubation mixture. The CO_2 was collected for 1 hr at 37.5° in 0.1 ml of NaOH (saturated NaOH solution diluted 1:10 with H_2O) in the side arm of the Warburg flask or the bottom of the scintillation vial. Two ml of H_2O and 10 ml of Bray's solution (12) were added to NaOH containing CO_2 and each sample was counted for 2 min in a Packard Tri Carb liquid scintillation spectrometer.

Results. Islets removed from rats 15 min after treatment with a diabetogenic dose of streptozotocin produced about 30% as much $^{14}CO_2$ from U-^{14}C-glucose as islets isolated from rats 15 min after treatment with saline (Table I). Carbon dioxide production by islets removed 1 hr after streptozotocin injection was reduced to approximately 5% of the control value (Table I).

Discussion. The reduced capacity of islets to oxidize glucose is the earliest effect observed after streptozotocin treatment and occurs before the described microscopic changes (6). Although it is not known how streptozotocin inhibits the oxidation of glucose, glucose utilization could be depressed if streptozotocin inhibited enzymes involved in the metabolism of glucose.

TABLE I. $^{14}CO_2$ Production from U-^{14}C-Glucose by Islets from Control and Streptozotocin-Treated Rats.

Time islets isolated after treatment (min)	dpm $^{14}CO_2$/Islet			
	Control	No. of observations	Streptozotocin	No. of observations
15	64.7 ± 13.7	10	18.5 ± 2.9 $p < 0.01$	10
60	60.0 ± 20.6	6	2.7 ± 0.45 $p < 0.02$	6

The inhibition of glucose oxidation by islets following streptozotocin injection is similar to the inhibition of glucose oxidation following D-mannoheptulose treatment (16, 17, 18). Since mannoheptulose prevents the phosphorylation of glucose by competitively inhibiting the enzyme ATP-D-glucose-6-phosphotransferase in isolated islet homogenates (14, 15), it would be expected that CO_2 production from glucose would be blocked. Therefore, the effects of streptozotocin could be a result of the inhibition of one or more enzymes involved in glucose oxidation.

Enzymes might also be inactivated if their coenzymes were unavailable. Schein and Loftus have shown that a single injection of streptozotocin reduces mouse liver pyridine nucleotides (19). The decrease in liver pyridine nucleotides and the diabetogenic action by streptozotocin can be prevented by nicotinamide treatment (19–21). This suggests that streptozotocin prevents the beta cell from maintaining adequate levels of pyridine nucleotides. Since these nucleotides are coenzymes involved in several steps in the metabolism of glucose, their reduction could produce a decrease in the utilization of glucose.

It is not clear if the streptozotocin inhibition of glucose oxidation causes the diabetogenic activity of streptozotocin or is the result of some other detrimental effect on the beta cell. Studies showing that nicotinamide reverses the diabetogenic effect of streptozotocin up to 2 hr after streptozotocin treatment (22) and that mannoheptulose, which inhibits the oxidation of glucose, apparently does not damage the beta cells permanently indicate that the diabetogenic action of streptozotocin may not be due to the early inhibition of glucose oxidation. Additional studies are needed to determine how streptozotocin inhibits glucose oxidation and whether this inhibition is related to the diabetogenic effect of streptozotocin.

Summary. Glucose oxidation by islets removed from rats 15 min after they had been treated with streptozotocin was reduced to about 30% of the control value. Glucose oxidation by islets removed 1 hr after streptozotocin treatment was reduced to about 5% of the control value.

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