Glucose Oxidation (14-CO_2 Production) and Insulin Secretion by Pancreatic Islets Isolated from Hyperglycemic and Normoglycemic Rats

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SUMMARY

Pancreatic islets from glucose-infused rats retained a "memory" of the hyperglycemic environment. (a) They oxidized (14-CO_2 production) glucose-U-14-C and glucose-6-14-C at rates three to four times higher than islets from normoglycemic rats. Inhibitors of insulin secretion and synthesis did not affect the rates of 14-CO_2 production. In contrast, ouabain (1 mM) decreased glucose oxidation by nearly 50 per cent. (b) They secreted insulin in vitro at rates similar to those of islets of control rats, in spite of the heavy degranulation of their beta cells. DIABETES *23*:469-73, May, 1974.

Pancreatic islets isolated from rats which had been kept hyperglycemic for twenty-four hours incorporated a much higher proportion of 3-H-leucine into proinsulin and insulin than pancreatic islets from normoglycemic controls.[1] These differences were found at both low and high glucose concentrations in the incubation media. It therefore was of special interest to compare the rate of 14-CO_2 production from D-glucose-14-C by these two groups of pancreatic islets and to study the effect on the rate of 14-CO_2 production of inhibitors of insulin biosynthesis, insulin secretion and of cation membrane transport.

MATERIALS AND METHODS

Materials. Collagenase was obtained from Worthington Biochemical Co. Ltd.; crystallized bovine albumin from Armour Pharmaceutical Co. Ltd., D-glucose-U-14-C, D-glucose-6-14-C, hyamine, PPO and POPOP from Amersham-Searle Corporation; n-sodium octanoate-1-14-C from New England Nuclear Corporation; epinephrine and ouabain from Sigma Chemical Co. Ltd.; puromycin and cycloheximide from Nutritional Biochemical Co. Ltd.; Silastic tubing from Dow Corning Co.; and Intramedic tubing from Clay Adams Co. All other chemicals were obtained from Fisher Scientific Co. and were of the highest purity available.

Animals and glucose infusions. Adult male Wistar rats weighing 380 to 400 gm. were used. The external jugular vein of each rat was cannulated under ether anesthesia. Each cannula consisted of a short piece (3 cm.) of soft Silastic tubing, comprising the intravenous portion of the cannula, telescoped onto Intramedic polyethylene tubing. The polyethylene portion of the cannula was drawn up under the skin to the dorsal area of the neck. Each animal was then positioned in a loose sling, which was attached to a semirigid long wire which slid freely in a longitudinal direction within a plastic trough. The animal thus retained substantial freedom of movement, could rest on the floor of the trough, and had free access to pellets and water. They were allowed to recover from the procedure at least twenty-four hours before infusion. For each experiment, a pair of rats was cannulated and infused simultaneously for twenty-four hours with a Harvard peristaltic pump; one rat received 22 to 25 gm. glucose in 50 to 55 ml. modified Ringer solution and the other an equal amount of the same solution without glucose. Blood glucose levels at killing of the glucose-infused rats ranged between 300 and 450 mg. per 100 ml.

Preparation of pancreatic islets. The islets were isolated by the collagenase method of Lacy and Kostianovsky.[2] After multiple washings of the sediment with cold (4° C.) Hank's solution, intact islets with smooth clean periphery were picked from a Petri

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dish and transferred to another one containing fresh Hank's solution. A binocular dissecting microscope with a calibrated ocular grid was used for this procedure. The Petri dishes were kept cold on thermoelectric plates. The islets were then transferred individually to short 2 cm. long siliconized tubes filled with Krebs-Ringer bicarbonate buffer supplemented with 5 mM Hepes (N-2-Hydroxyethylpiperazine-N-2 ethanesulfonic acid) pH 7.35. Eight to ten islets of the same size distribution were put in each tube. All incubation tubes were previously calibrated so that fluid could be decanted to a 100 μL. mark. Inhibitors of protein synthesis, insulin secretion or ion transport, when used, were added in fresh portions of Krebs-Ringer bicarbonate buffer and permitted to equilibrate with the islets for thirty to forty-five minutes in the cold before incubating.

Output of 14-CO_2. Labeled glucose was added to each tube in 50 μL. Krebs-Ringer bicarbonate buffer containing nonlabeled glucose and bovine albumin. The concentrations were such that the final incubation volume, 0.15 ml., would contain the appropriate amount of substrate and albumin. In the experiments with inhibitors 1 μc. of radioactivity was used per tube, while 0.5 μc. were employed in all others. The albumin concentrations were 20 mg. per cent except in the experiments with octanoate where 40 mg. per cent was used. In each set three tubes were incubated without tissues to allow background radioactivity determination.

A radiospirometer device, modified from that used by Lin and Haist,[3] was used to measure 14-CO_2 evolution. Each incubation tube was inserted into a scintillation vial and the vial covered with a serum stopper (Kontes Glass Co.). The vials were then placed in a Dubnoff metabolic shaking incubator at 37° C. and gassed for approximately five minutes with 95 per cent oxygen, 5 per cent carbon dioxide, saturated with water at 37° C.

At the conclusion of the incubation period (ninety minutes unless otherwise stated), a drop of paraffin oil was placed on the serum stopper. Half a milliliter of hyamine hydroxide was then added to the floor of the vial via a disposable syringe and a no. 23 needle. This was followed by the addition into the incubation tube of 0.3 ml. of a citrate-sodium dihydrogen phosphate buffer, pH 3.1. The vials were left to stand for two hours at room temperature.

At the end of this period the stoppers were removed, and the outside of each incubation tube was washed into its companion vial with 10 ml. of scintillator containing toluene-methanol 4:1, PPO 4 gm. and POPOP 20 mg. per 100 ml. The vials were then placed in a Nuclear Chicago scintillation counter and the radioactivity determined. In experiments with 14-C-labeled $NaHCO_3$, this method was found to release and trap 92 ± 3 per cent of all 14-CO_2 in the incubation tube. Detectable radioactivity in each sample was converted to disintegrations per minute. These values were then converted to picomoles of substrate metabolized to carbon dioxide, using values for the specific activities of each substrate.

Insulin release. Batches of ten islets were preincubated at 4° C. in 1 ml. of Krebs-Ringer buffer containing albumin (1 mg./ml.) and 80 mg. per cent glucose with or without epinephrine or ouabain. After thirty to forty-five minutes these solutions were replaced with warm solutions of buffer containing albumin (3 mg./ml.), 80 or 320 mg. per cent glucose and the same concentrations of inhibitors as in the preincubation period. After a one-hour incubation with intermittent swirling, the medium was separated from the islets by gentle centrifugation and kept frozen at -25° C. until radioimmunoassayed with the back titration and alcohol precipitation method of Wright et al.[4] The immunoassay was calibrated with rat crystalline insulin.

RESULTS

Oxidation of glucose

Formation of 14-CO_2 from uniformly labeled glucose was linear over one and one-half hours in islets from rats preinfused with glucose or with glucose-free buffer (table 1).

Striking differences in the rates of 14-CO_2 formation were found between pancreatic islets from normoglycemic and hyperglycemic rats incubated at both low and high glucose concentrations. As table 2 shows, the stimulated islets showed a fourfold increase in metabolism over the controls: 213 ± 21 picomoles of glucose per hour as compared to 47 ± 13 at 4.5 mM glucose. At a 16.6 mM glucose concentration, the in vivo prestimulated islets metabolized 664 ± 28 picomoles per hour, while a value of 220 ± 15 was obtained from the islets of the buffer-infused rats. The prestimulated pancreatic islets oxidized the same quantity of glucose at a concentration of 4.5 mM as did the non-prestimulated at a concentration of 16.6 mM.

The production of 14-CO_2 from glucose-6-14-C in both groups of islets is also shown in table 2. The

TABLE 1

Production of 14-CO_2 from D-glucose-U-14-C by pancreatic islets from buffer-infused and glucose-infused rats. Means ± S.E. from six incubation tubes from two rats.

	Glucose Concn. (mM)	Incubation time (min.)	14-CO_2 formation (pmoles of glucose per ten islets)
Buffer-infused	4.5	45	50 ± 80
Buffer-infused	4.5	90	130 ± 31
Buffer-infused	16.6	45	210 ± 54
Buffer-infused	16.6	90	395 ± 50
Glucose-infused	4.5	45	164 ± 32
Glucose-infused	4.5	90	327 ± 36
Glucose-infused	16.6	45	450 ± 57
Glucose-infused	16.6	90	1,020 ± 149

relative difference in rates of glucose oxidation (14-CO_2 production) between in vivo prestimulated and control islets was of the same order as in the experiments with D-glucose-U-14-C.

Oxidation of octanoate

The islets from buffer- and glucose-infused rats metabolized octanoate linearly for two hours. In contrast with glucose oxidation, no difference in carbon dioxide production from octanoate was observed between control and stimulated islets. At a concentration of 1 mM octanoate and 4.6 mM glucose, control islets metabolized 45 ± 6.7 picomoles of octanoate per hour, while the stimulated islets metabolized 46 ± 4 picomoles per hour.

Effects of inhibitors of islet function on glucose oxidation

These experiments were conducted at a glucose concentration of 16.6 mM. Epinephrine 5 μM, puromycin 80 μg./ml., cycloheximide 80 μg./ml. and ouabain 20 μM and 1 mM were used. Results are shown in table 3. The same concentration of epinephrine inhibited insulin secretion in both prestimulated and control islets by 70 to 80 per cent (table 5).

Both puromycin and cycloheximide have no effect on glucose oxidation (table 3).

While 20 μM of ouabain had no effect, a concentration of 1 mM inhibited the rates of carbon dioxide production from uniformly labeled glucose by nearly half (table 4). This ouabain concentration inhibited insulin secretion in both stimulated and control islets (table 5).

TABLE 2

Production of 14-CO_2 from D-glucose-U-14-C or D-glucose-6-14-C by pancreatic islets from buffer-infused and glucose-infused rats. Means ± S.E. from ten to twelve incubation tubes from three rats.

	Labeled substrate	Concentration (mM)	14-CO_2 formation (pmoles of glucose/hr. per ten islets)
Buffer-infused	D-glucose-U-14-C	4.5	47 ± 13
Buffer-infused	D-glucose-U-14-C	16.6	220 ± 15
Glucose-infused	D-glucose-U-14-C	4.5	213 ± 21
Glucose-infused	D-glucose-U-14-C	16.6	664 ± 28
Buffer-infused	D-glucose-6-14-C	16.6	114 ± 22
Glucose-infused	D-glucose-6-14-C	16.6	351 ± 50

DISCUSSION

The rates of 14-CO_2 production by pancreatic islets from rats infused with buffer for twenty-four hours fall within the range reported for fed, "cage control" rats or mice by other investigators.[3,5] This indicates that the striking difference in respiratory rates between pancreatic islets of glucose- and buffer-preinfused rats are not due to abnormally low values in our control buffer-infused rats.

TABLE 3

Effect of epinephrine (5 μM), puromycin (80 μg./ml.) and cycloheximide (80 μg./ml.) on 14-CO_2 production from D-glucose-U-14-C by pancreatic islets of buffer-infused and glucose-infused rats. Glucose concentration in the incubation medium 16.6 mM. Means ± S.E. from ten to twelve incubation tubes from three rats.

	Additions	14-CO_2 formation (pmoles of glucose/hr. per ten islets)
Buffer-infused	—	174 ± 19
Buffer-infused	epinephrine	233 ± 21
Glucose-infused	—	571 ± 37
Glucose-infused	epinephrine	624 ± 34
Buffer-infused	—	364 ± 16
Buffer-infused	cycloheximide	346 ± 33
Glucose-infused	—	834 ± 65
Glucose-infused	cycloheximide	941 ± 90
Buffer-infused	—	237 ± 24
Buffer-infused	puromycin	231 ± 19
Glucose-infused	—	604 ± 32
Glucose-infused	puromycin	653 ± 35

This difference may be explained in two ways: The experimental results may reflect a changed metabolic pattern of individual cells which was induced in vivo and which persisted in vitro several hours after the isolation of the pancreatic islets. Because the alpha cells constitute about 25 per cent of the pancreatic islet cells and are functionally inactivated by hyperglycemia, and because there is no new beta-cell formation after twenty-four hours of hyperglycemia,[1] it is reasonable to accept that the metabolic change has occurred in the pre-existing beta cells. Alternately it is possible that pancreatic islet cells of the glucose-

TABLE 4

Effect of ouabain (1 μM) on 14-CO_2 production from D-glucose-U-14-C by pancreatic islets of buffer-infused and glucose-infused rats. Glucose concentration in the incubation medium 16.6 mM. Means ± S.E. from fourteen to sixteen incubation tubes from four rats.

	Additions	14-CO_2 formation (pmoles of glucose/hr. per ten islets)
Buffer-infused	—	495 ± 51
Buffer-infused	Ouabain	220 ± 26
Glucose-infused	—	1,210 ± 140
Glucose-infused	Ouabain	664 ± 62

preinfused rats endure collagenase digestion much better, a greater number of cells retaining a proper metabolic pattern.

Two experimental facts favor the first explanation. Firstly, the islets from glucose-infused rats metabolized n-octanoate at the same rate as the pancreatic islets from the buffer-infused control rats. Secondly, judged by the secretory response of the pancreatic islets to glucose stimulation in vitro, there was no evidence of beta-cell death or membrane leakage. Insulin secretion was well stimulated by high concentration of glucose in the medium and inhibited by epinephrine.

The contribution of the pentose cycle of glucose oxidation is rather small and does not change significantly with glucose concentration.[6,7] As with uniformly 14-C-labeled glucose, 14-CO_2 production from D-glucose-6-14-C by the pancreatic islets of glucose-infused rats was also three times higher than that of normal pancreatic islets. It is highly improbable that some of this 14-CO_2 was released by enzymes of the pentose cycle after re-allocation of the label to position 1 during regeneration of glucose-6-P through recycling of three carbon units. Fructose 1-6 diphosphatase activity is low in the pancreatic islets.[8] It is therefore concluded that decarboxylations at enzymatic steps of the Krebs cycle contribute to the 14-CO_2 arising from oxidation of glucose-6-14-C and that glucose oxidation through the Krebs cycle is very much enhanced in the islets from glucose-infused rats at any glucose concentration in the incubation medium. Islets from glucose-infused rats retained a "memory" of the preceding hyperglycemic environment.

TABLE 5

Rates of insulin secretion of pancreatic islets from glucose-infused and buffer-infused rats

Type of infusion (no. of rats)	Glucose in incubation medium mM	Additions	Insulin secreted μU./hr./ten islets (Mean ± S.E.)
Buffer (10)	4.5	—	567 ± 74
Glucose (10)	4.5	—	710 ± 73
Buffer (10)	17.2	—	1,839 ± 140
Glucose (10)	17.2	—	1,961 ± 131
Buffer (10)	17.2	Epinephrine 5 μM	580 ± 103
Glucose (10)	17.2	Epinephrine 5 μM	450 ± 80
Buffer (10)	17.2	Ouabain 1 mM	1,065 ± 124
Glucose (10)	17.2	Ouabain 1 mM	1,033 ± 102

It can be expected that in beta cells as in many other cells, oxidative glucose metabolism parallels the energy needs of the cell. The difference, then, between in vivo-prestimulated and control islets reflects a higher energy consumption of the beta cells from glucose-infused rats. A series of experiments was therefore designed to test the contribution of major energy sinks to glucose oxidation by means of inhibitors of protein synthesis, insulin secretion and ion transport.

As previously shown for normal islets, puromycin or cycloheximide at doses which inhibit 90 to 95 per cent of proinsulin biosynthesis,[9,10] and epinephrine concentrations which markedly inhibited insulin secretion had no effect on the rates of glucose oxidation by both in vivo-prestimulated and control islets. It should also be noted that prestimulated islets secreted at about the same rate as control islets.

Ouabain at 1 mM concentration decreased glucose oxidation (14-CO_2 production) by 35 to 55 per cent. Recently Matschinsky[11] reported an inhibitory action of ouabain on lactic acid production by rat islets. Assuming that glucose metabolism is tightly coupled to the energy requirements of the beta cell, we have to conclude that other energy sinks must exist which are not related to insulin synthesis, the insulin secretion step sensitive to epinephrine, or to ouabain-sensitive ion transport. It is interesting to speculate that these additional "energy sinks" may also be intracellular or cell membrane ion transport systems.

As glucose utilization in both stimulated and control islets is proportional to glucose concentration, glucose somehow must activate these "energy sinks," unless it uncouples oxidative phosphorylation or switches the glycolytic flux from anaerobic to aerobic glycolysis. The latter alternative can be excluded as it has been shown that lactate production by rat islets parallels glucose consumption and carbon dioxide

production at glucose levels higher than basal.[11] The problem of how glucose exerts these effects is obviously related to the mechanism of "memory" which was found in the pancreatic islets of glucose-infused rats. Insulin secretion rates by both groups of islets (glucose-infused and buffer-infused rats) were very similar at both 4.5 and 17.1 mM glucose concentrations in the incubation medium. This is of particular interest as the pancreatic beta cells of glucose-infused rats are heavily degranulated, and the insulin content of the pancreas is one-fifth to one-seventh of that of control rats.[12]

Assuming that insulin secretion by stimulated beta cells occurs predominantly through reverse pinocytosis,[13] the data would suggest that beta-cell membrane fusion with secretory granules has been profoundly facilitated by the preceding glucose infusion.

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