Stimulation by D-glucose of mitochondrial oxidative events in islet cells

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D-Glucose catabolism was examined in rat pancreatic islets and tumoral insulin-producing cells (RINm5F line) exposed to D-[5-³H]glucose, D-[3,4-¹⁴C]glucose or D-[6-¹⁴C]glucose. In normal islets, a rise in D-glucose concentration from 2.8 to 16.7 mM augmented (i) the transfer of NADH, presumably via the glycerol phosphate shuttle, into the mitochondria, (ii) the decarboxylation of pyruvate and (iii) the oxidation of acetyl residues, even when the flow rates were expressed relative to the total rate of D-glucose utilization. Islet cells are thus organized to favour those mitochondrial oxidative events yielding the major fraction of the energy derived from the catabolism of D-glucose. In tumoral islet cells of the RINm5F line, all three oxidative mitochondrial processes, when expressed relative to the overall rate of D-glucose utilization, occurred at a low rate and failed to increase in response to the rise in D-glucose concentration. These findings emphasize the importance of distal regulatory processes in the control of D-glucose catabolism in normal islet cells, and document the perturbation of such processes in a model of dysfunction for this fuel-sensor organ.

INTRODUCTION

The catabolism of D-glucose in the β -cell represents a critical factor in the control of insulin secretion [1–3]. However, despite numerous publications (for reviews, see [4–6]), fundamental information is still lacking about several aspects of D-glucose catabolism in the endocrine pancreas. First, the flow rate through the glycerol phosphate shuttle remains to be defined. Second, the net generation of pyruvate and lactate from exogenous D-glucose was never directly assessed. Only the glucose-induced increment in unlabelled pyruvate and lactate production above the corresponding basal value was so far measured [7–9]. Since D-glucose interferes with the catabolism of endogenous amino acids [10,11], which represents the major source of pyruvate and lactate in islets deprived of exogenous nutrient [11–13], the glucose-induced increment in unlabelled pyruvate and lactate production does not necessarily correspond to the true rate of conversion of exogenous D-glucose into these organic acids. Third, no information is available on the rate of conversion of pyruvate, derived from exogenous D-glucose, into alanine. Last, the respective contribution of pyruvate decarboxylation and acetyl-CoA oxidation to the overall mitochondrial generation of ¹⁴CO₂ from ¹⁴C-labelled glucose remains to be evaluated. The present study provides this so-far-missing information for both normal pancreatic islets and tumoral insulin-producing cells, the latter being taken as a model of islet cell dysfunction.

MATERIALS AND METHODS

D-[5-³H]Glucose (15.7 Ci/mmol), D-[3,4-¹⁴C]glucose (10.3 mCi/mmol) and D-[6-¹⁴C]glucose (57 mCi/mmol) were all purchased from New England Nuclear (Boston, MA, U.S.A.). They were used at final concentrations

of 17–125 μ Ci/ml for D-[5-³H]glucose, 0.4–6.0 μ Ci/ml for D-[3,4-¹⁴C]glucose and 1.0–10.4 μ Ci/ml for D-[6-¹⁴C]glucose.

Groups of 30 islets prepared by collagenase digestion [14] of pancreases removed from fed albino rats, and groups of 90×10^3 RINm5F cells cultured, harvested and counted as described elsewhere [15], were incubated for 120 min at 37 °C in 30 μ l of bicarbonate-buffered media [14] containing bovine albumin (5 mg/ml) and either 2.8 mM- or 16.7 mM-D-glucose mixed with a tracer amount of labelled hexose.

The methods used to measure the production of ³H₂O [16,17] and ¹⁴CO₂ [18] by the islet cells were described previously.

For the measurement of [¹⁴C]lactate production from D-[3,4-¹⁴C] glucose, the incubation medium containing the islets or cells was mixed with 30 μ l of HClO₄ (5%, v/v), frozen in liquid N₂, homogenized by mechanical vibration [8], heated for 5 min at 70 °C, and centrifuged for 1 min at 5000 g. A sample (50 μ l) of the supernatant extract was neutralized by addition of 25 μ l of Tris (0.2 M)/KOH (1.0 M), and centrifuged for 1 min at 5000 g. Two portions (30 μ l each) of the supernatant solution were then mixed in a tube with an equal volume of a reaction mixture consisting of phosphate buffer (pH 7.3, 100 mM) containing FMN (5.0 μ M) with or without lactate oxidase (EC 1.13.12.4) from *Mycobacterium smegmatis* (0.55 mg/ml; 15 units/mg; Boehringer, Mannheim, Germany). The tubes were placed in sealed counting vials containing Hyamine hydroxide (0.3 ml) and incubated for 45 min at 20 °C. After injection of 0.1 ml of citrate/NaOH buffer (pH 4.9, 400 mM) in the tubes and a further incubation for 60 min at 20 °C, the tubes were removed and the vials examined for their radioactive content by liquid-scintillation counting. The production of [¹⁴C]lactate was judged from the paired difference of readings collected after incubation in the absence and presence of lactate oxidase. These data were

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corrected for the recovery of standards prepared in the incubation medium and treated in the same manner as the sample. These standards contained both unlabelled L-lactate and L-[U- ^{14}C]lactate in a molar ratio close to 5:1, the total concentration of L-lactate covering the range of values encountered in the sample of islets or tumoral cells. Under these conditions, the recovery of L-[U- ^{14}C]lactate, corrected for the fact that lactate oxidase catalyses the production of $^{14}\text{CO}_2$ from only C-1 in the lactate molecule, averaged $15.8 \pm 1.8\%$ ($n = 4$). When media incubated for 120 min at 37°C in the absence of cells were treated in the same manner, no production of [^{14}C]lactate from D-[3,4- ^{14}C]glucose could be detected.

To measure the generation of [^{14}C]pyruvate and ^{14}C -labelled amino acids from either D-[3,4- ^{14}C]glucose or D-[6- ^{14}C]glucose, the incubation medium was first mixed with 0.1 ml of citrate/NaOH buffer (pH 4.9, 400 mM) and further incubated for 60 min at 20°C to measure $^{14}\text{CO}_2$ production [18]. The samples were then frozen and the cells disrupted by mechanical vibration [7]. The homogenate was neutralized by addition of 40 μl of NaOH (0.95 M). Each of three samples (40 μl each) were then mixed with 60 μl of Tris/HCl buffer (pH 8.1, 100 mM) containing either 2-oxoglutarate and L-glutamate (final concn. 12.0 mM; sample A), or 2-oxoglutarate and pig heart glutamate:pyruvate transaminase (EC 2.6.1.2; final concn. 50 $\mu\text{g}/\text{ml}$; Boehringer; sample B) or L-glutamate and glutamate:pyruvate transaminase (same concentrations as above; sample C). After 60 min incubation at 20°C , the reaction was halted by addition of 1.5 ml of iced water, and the amino acids were separated by passing the sample through ion-exchange resin (Dowex 50, H^+ form), as previously described [13]. The paired difference between ^{14}C -labelled amino acids recovered in samples A and B respectively was taken as the amount of [^{14}C]alanine and/or [^{14}C]glutamate present in the medium. The paired difference between ^{14}C -labelled amino acids recovered in samples C and A respectively was taken as the amount of [^{14}C]pyruvate present in the medium. When media first incubated in the absence of islet cells were treated in the same manner, no

significant production of either [^{14}C]pyruvate or ^{14}C -labelled amino acid was observed. No correction for recovery was introduced, since, in this system, the interconversion of relevant amino acids and their corresponding 2-oxo acids is virtually complete [13]. The readings obtained with samples B were also used to estimate the production of ^{14}C -labelled amino acids other than [^{14}C]alanine and [^{14}C]glutamate, such a production being judged from the difference between data obtained in media incubated in the presence or the absence, respectively, of islets (or RINm5F cells).

The results obtained in either islets or RINm5F cells were derived from experiments in which at least two and up to six distinct primary metabolic variables were measured in either the same group of islets or cells or in distinct groups of islets or cells all derived from the same initial preparation. All results are expressed as means ($\pm$ S.E.M.) together with the number of individual observations (n). The S.E.M. for either the sum of or the ratio between mean values was calculated as described elsewhere [13,19]. The statistical significance of differences between mean values was assessed by Student's t test.

RESULTS

Normal pancreatic islets or RINm5F cells were incubated for 120 min at 37°C in the presence of 2.8 mM- or 16.7 mM-D-[5- ^3H]glucose, -D-[3,4- ^{14}C]glucose or -D-[6- ^{14}C]glucose. The primary data for each metabolic variable are listed in Table 1. The results of several calculations based on these primary data are given in Table 2.

In either islets or RINm5F cells, the rate of D-[5- ^3H]glucose conversion into $^3\text{H}_2\text{O}$ increased ($P < 0.001$) as the concentration of the hexose was raised from 2.8 to 16.7 mM. The relative magnitude of such an increment was much greater ($P < 0.001$) in islets than in tumoral cells, the 16.7 mM-/2.8 mM-glucose ratio of $^3\text{H}_2\text{O}$ production averaging respectively 2.82 ± 0.30 (islets) and 1.22 ± 0.04 (RINm5F cells).

The rate of D-[3,4- ^{14}C]glucose utilization, taken as the

Table 1. D-Glucose metabolism in pancreatic islets and RINm5F cells (primary data)

All results are expressed as pmol of D-glucose residue/120 min per islet or per 10^3 RINm5F cells.

Tissue . . .	Pancreatic islets		RINm5F cells	
[D-Glucose] (mM) . . .	2.8	16.7	2.8	16.7
³ H ₂ O production from D-[5- ³ H]glucose	64.4 ± 5.3 (16)	181.8 ± 12.4 (16)	143.2 ± 3.9 (20)	174.0 ± 2.9 (20)
[¹⁴ C]Lactate production from D-[3,4- ¹⁴ C]glucose	52.4 ± 5.7 (12)	137.2 ± 6.1 (12)	110.5 ± 18.0 (12)	152.6 ± 5.6 (12)
[¹⁴ C]Pyruvate production from D-[3,4- ¹⁴ C] or -[6- ¹⁴ C]glucose	4.5 ± 0.5 (24)	10.5 ± 1.0 (24)	16.9 ± 1.1 (24)	22.4 ± 1.0 (24)
¹⁴ CO ₂ production from D-[3,4- ¹⁴ C]glucose	13.9 ± 1.0 (19)	75.2 ± 4.1 (13)	11.9 ± 1.0 (20)	11.8 ± 1.0 (20)
¹⁴ CO ₂ production from D-[6- ¹⁴ C]glucose	6.4 ± 0.4 (19)	40.4 ± 2.2 (19)	4.7 ± 0.3 (18)	3.6 ± 0.3 (16)
[¹⁴ C]Alanine production from D-[3,4- ¹⁴ C]glucose	1.7 ± 0.6 (12)	5.3 ± 1.0 (12)	3.5 ± 0.5 (12)	2.4 ± 0.4 (12)
[¹⁴ C]Alanine and [¹⁴ C]glutamate production from D-[6- ¹⁴ C]glucose	4.0 ± 0.3 (12)	11.9 ± 1.3 (12)	3.0 ± 0.4 (12)	4.1 ± 0.4 (12)

Table 2. D-Glucose metabolism in pancreatic islets and RINm5F cells (primary data)All results are expressed as pmol of D-glucose residue/120 min per islet or per 10³ RINm5F cells.

Tissue . . . [D-Glucose] (mM) . . .	Pancreatic islets		RINm5F cells	
	2.8	16.7	2.8	16.7
D-[3,4- ¹⁴ C]Glucose utilization (pmol/ 120 min per islet or per 10 ³ cells)	72.5 ± 5.8	228.2 ± 7.5	142.8 ± 18.1	189.2 ± 5.8
D-[3,4- ¹⁴ C]Glucose/D-[5- ³ H]glucose utilization ratio (%)	112.6 ± 12.9	125.5 ± 9.5	99.7 ± 12.9	108.7 ± 3.8
[¹⁴ C]Lactate production/D-[3,4- ¹⁴ C]glucose utilization (%)	72.3 ± 2.9	60.1 ± 3.3	77.4 ± 3.1	80.7 ± 1.0
Flow through glycerol phosphate shuttle (pmol/120 min per islet or per 10 ³ cells)	20.1 ± 1.3	91.0 ± 4.3	32.3 ± 1.6	36.6 ± 1.5
Flow through glycerol phosphate shuttle/D-[3,4- ¹⁴ C]glucose utilization (%)	27.7 ± 2.9	39.9 ± 2.3	22.6 ± 3.1	19.3 ± 1.0
[¹⁴ C]Pyruvate production/D-[3,4- ¹⁴ C]glucose utilization (%)	6.2 ± 0.8	4.6 ± 0.5	11.8 ± 1.7	11.8 ± 0.7
[¹⁴ C]Lactate/[¹⁴ C]pyruvate production ratio	11.64 ± 1.81	13.07 ± 1.37	6.54 ± 1.15	6.81 ± 0.39
[¹⁴ C]Pyruvate decarboxylation/ D-[3,4- ¹⁴ C]glucose utilization (%)	19.2 ± 2.0	33.0 ± 2.1	8.3 ± 1.3	6.2 ± 0.6
¹⁴ CO ₂ production from D-[6- ¹⁴ C]glucose/ D-[3,4- ¹⁴ C]glucose utilization (%)	8.8 ± 0.9	17.7 ± 1.1	3.3 ± 0.5	1.9 ± 0.2
¹⁴ CO ₂ production from D-[6- ¹⁴ C]glucose/ [¹⁴ C]pyruvate decarboxylation (%)	46.0 ± 4.4	53.7 ± 4.1	39.5 ± 4.2	30.5 ± 3.6
[¹⁴ C]acetyl-CoA not oxidized (pmol/120 min per islet or per 10 ³ cells)	7.5 ± 1.1	34.8 ± 4.7	7.2 ± 1.1	8.2 ± 1.1
[¹⁴ C]Alanine production/D-[3,4- ¹⁴ C]glucose utilization (%)	2.3 ± 0.9	2.3 ± 0.4	2.5 ± 0.5	1.3 ± 0.2
[¹⁴ C]Glutamate production from D- [6- ¹⁴ C]glucose (pmol/120 min per islet or per 10 ³ cells)	2.3 ± 0.7	6.6 ± 1.6	-0.5 ± 0.7	1.7 ± 0.6
¹⁴ C-labelled amino acids generated from [¹⁴ C]acetyl-CoA (pmol/120 min per islet or per 10 ³ cells)	6.6 ± 1.1	22.3 ± 2.7	0.5 ± 0.8	1.3 ± 0.8
[¹⁴ C]Acetyl-CoA not oxidized and not recovered as ¹⁴ C-labelled amino acid (pmol/120 min per islet or per 10 ³ cells)	0.9 ± 1.6	12.5 ± 5.4	6.7 ± 1.4	6.9 ± 1.4

sum of the production of [¹⁴C]lactate, [¹⁴C]pyruvate, [¹⁴C]alanine and ¹⁴CO₂ (all expressed as glucose residues), increased as a function of the glucose concentration in both islets ($P < 0.001$) and RINm5F cells ($P < 0.025$). The 16.7 mM-/2.8 mM-glucose ratio of D-[3,4-¹⁴C]glucose utilization averaged 3.14 ± 0.27 in islets, compared with ($P < 0.001$) only 1.32 ± 0.17 in tumoral cells. The last two ratios were not significantly different from the corresponding ratios for D-[5-³H]glucose utilization (see above).

The rate of [¹⁴C]lactate production from D-[3,4-¹⁴C]glucose increased as a function of the extracellular concentration of the hexose in both islets ($P < 0.001$) and RINm5F cells ($P < 0.05$). The 16.7 mM-/2.8 mM-glucose ratio of [¹⁴C]lactate production averaged 2.62 ± 0.31 in pancreatic islets, compared with ($P < 0.01$) only 1.38 ± 0.28 in tumoral cells. On pooling of the data obtained at 2.8 mM- and 16.7 mM-D-glucose, the production of [¹⁴C]lactate, relative to the total rate of D-[3,4-¹⁴C]glucose utilization, was lower ($P < 0.001$) in islets ($66.2 \pm 2.2\%$) than in tumoral cells ($79.0 \pm 1.6\%$).

Assuming that the utilization of D-[3,4-¹⁴C]glucose not accounted for by the production of [¹⁴C]lactate corresponds to the flow through the glycerol phosphate shuttle (see the Discussion section), such a flow was 4 times higher in islets exposed to 16.7 mM- rather than

2.8 mM-D-glucose, but was unaffected ($P > 0.05$) by the hexose concentration in RINm5F cells. Even when expressed relative to the total rate of D-[3,4-¹⁴C]glucose utilization, the flow through the glycerol phosphate shuttle remained higher ($P < 0.005$) in the islets exposed to 16.7 mM-D-glucose than in those incubated in the presence of 2.8 mM-D-glucose. Such was not the case in the RINm5F cells. At the low glucose concentration (2.8 mM), the flow through the shuttle, relative to D-[3,4-¹⁴C]glucose utilization, was not significantly different in islets and tumoral cells. However, at the high concentration of D-glucose (16.7 mM), it was twice as high ($P < 0.001$) in islets as in RINm5F cells ($39.9 \pm 2.3\%$ versus $19.3 \pm 1.0\%$).

Virtually identical results were obtained for the production of [¹⁴C]pyruvate in the presence of either D-[3,4-¹⁴C]glucose or D-[6-¹⁴C]glucose. Hence the results obtained with each of these markers were pooled together. The net production of [¹⁴C]pyruvate was higher at 16.7 mM- than 2.8 mM-D-glucose, in both islets and RINm5F cells ($P < 0.001$ in both cases). The 16.7 mM-/2.8 mM-glucose ratio of [¹⁴C]pyruvate production was higher ($P < 0.01$) in islets (2.33 ± 0.34) than in tumoral cells (1.32 ± 0.10). Relative to D-[3,4-¹⁴C]glucose utilization, the production of [¹⁴C]pyruvate was not significantly

different at low and high glucose concentration, in either islets or RINm5F cells. However, the amount of [^{14}C]pyruvate recovered in the tissue and incubation medium, when expressed relative to D-[3,4- ^{14}C]glucose utilization, was about twice as high ($P < 0.001$) in tumoral cells ($11.8 \pm 0.9\%$) as in normal islets ($5.4 \pm 0.5\%$).

The [^{14}C]lactate/[^{14}C]pyruvate production ratio was not affected significantly by the concentration of D-glucose, in either islets or tumoral cells. The ratio was twice as high, however, in islets as in tumoral cells, averaging respectively 12.36 ± 1.13 and 6.68 ± 0.60 ($P < 0.001$; pooled data obtained at 2.8 mM- and 16.7 mM-D-glucose).

The rate of [^{14}C]pyruvate decarboxylation, as judged by the production of $^{14}\text{CO}_2$ from D-[3,4- ^{14}C]glucose, was 5–6 times higher in islets incubated in the presence of 16.7 mM-D-glucose than in those maintained at a low glucose concentration (2.8 mM), but was unaffected by the concentration of the hexose in RINm5F cells. Relative to the total rate of D-[3,4- ^{14}C]glucose utilization, the oxidative decarboxylation of [^{14}C]pyruvate remained higher ($P < 0.001$) in islets exposed to 16.7 mM- rather than 2.8 mM-D-glucose. Such was not the case in RINm5F cells. The ratio of [^{14}C]pyruvate decarboxylation to D-[3,4- ^{14}C]glucose utilization was 2–5 times higher in islets than in tumoral cells, depending on the concentration of D-glucose.

In pancreatic islets the production of $^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose was about 6–7 times higher at high (16.7 mM) than at low (2.8 mM) glucose concentration. In the RINm5F cells, however, the production of $^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose was decreased in response to an increase in D-glucose concentration ($P < 0.02$). By paired comparison, the values recorded in the presence of 16.7 mM-D-glucose averaged $82.5 \pm 3.9\%$ of the corresponding readings collected in the presence of 2.8 mM-D-glucose. When expressed relative to D-[3,4- ^{14}C]glucose utilization, the production of $^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose by the islets was doubled ($P < 0.001$) in response to the increase in D-glucose concentration from 2.8 to 16.7 mM, whereas it was decreased by about 42% ($P < 0.01$) in the RINm5F cells in response to the same change in D-glucose concentration. The production of $^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose relative to D-[3,4- ^{14}C]glucose utilization was almost 3 times higher ($P < 0.001$) in islets than in RINm5F cells in the presence of 2.8 mM-D-glucose, and 9 times higher in the presence of 16.7 mM-D-glucose.

The production of $^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose was much lower than the rate of [^{14}C]pyruvate decarboxylation in islets or cells exposed to D-[3,4- ^{14}C]glucose ($P < 0.001$ in all cases). The ratio between these two metabolic variables was not significantly affected by the concentration of D-glucose, in either islets or RINm5F cells. However, this ratio was significantly higher ($P < 0.001$) in islets ($49.9 \pm 3.1\%$) than in RINm5F cells ($35.0 \pm 2.9\%$), as judged by comparison of the overall mean value obtained in each preparation by pooling the data recorded at low and high concentrations of D-glucose.

The difference between [^{14}C]pyruvate decarboxylation in cells exposed to D-[3,4- ^{14}C]glucose and $^{14}\text{CO}_2$ output by cells exposed to D-[6- ^{14}C]glucose represents theoretically the amount of acetyl-CoA generated from exogenous D-glucose but not oxidized in the tricarboxylic acid cycle (see the Discussion section). The

rise in D-glucose concentration from 2.8 to 16.7 mM increased ($P < 0.001$) the production of such unoxidized acetyl residues from 7.5 ± 1.1 to 34.8 ± 4.7 pmol/120 min per islet, but failed to affect their rate of production in RINm5F cells.

The production of [^{14}C]alanine from D-[3,4- ^{14}C]glucose by pancreatic islets was increased ($P < 0.01$) in response to the rise in D-glucose concentration. Such was not the case in RINm5F cells. Relative to D-[3,4- ^{14}C]glucose utilization, the production of [^{14}C]alanine represented no more than 1–3%, being of comparable magnitude in islets and RINm5F cells. This ratio was unaffected by the concentration of D-glucose in islets, but decreased ($P < 0.05$) in response to the increase in glucose concentration in the tumoral cells.

In pancreatic islets, the generation of [^{14}C]alanine and [^{14}C]glutamate from D-[6- ^{14}C]glucose exceeded ($P < 0.005$ or less) the production of [^{14}C]alanine from D-[3,4- ^{14}C]glucose, this probably reflecting the generation of [^{14}C]glutamate derived from 2-oxoglutarate formed in the tricarboxylic acid cycle. Indeed, the intermediates in the tricarboxylic acid cycle are theoretically labelled with ^{14}C in cells exposed to D-[6- ^{14}C]glucose, but not so in cells exposed to D-[3,4- ^{14}C]glucose. The rise in D-glucose concentration from 2.8 to 16.7 mM increased the production of [^{14}C]glutamate from 2.3 ± 0.7 to 6.6 ± 1.6 pmol/120 min per islet ($P < 0.025$). In the RINm5F cells, a significant ($P < 0.02$) generation of [^{14}C]glutamate could only be detected at high glucose concentration, a situation probably attributable to the fact that the rate of D-[6- ^{14}C]glucose oxidation relative to [^{14}C]alanine production was 3–5 times lower in RINm5F cells than in pancreatic islets. Indeed, when expressed relative to the rate of D-[6- ^{14}C]glucose oxidation, the generation of [^{14}C]glutamate was not significantly different in islets and RINm5F cells, averaging respectively $26.1 \pm 5.9\%$ and $18.3 \pm 11.3\%$ (pooled data obtained at 2.8 mM- and 16.7 mM-D-glucose in both cases).

The data so far presented provide no information on the production of ^{14}C -labelled amino acids other than [^{14}C]alanine and [^{14}C]glutamate. The production of these other ^{14}C -labelled amino acids could be judged, however, from the difference between the control (no islet cells present) and experimental values for the elution of ^{14}C -labelled amino acids after treatment of the islet-cell extract by glutamate:pyruvate transaminase in the presence of exogenous 2-oxoglutarate. As shown in Table 3, even in the presence of D-[3,4- ^{14}C]glucose, the pancreatic islets produced a sizeable amount of ^{14}C -labelled amino acids other than [^{14}C]alanine and, if any, [^{14}C]glutamate. A rise in D-glucose concentration resulted in a significant increase ($P < 0.02$) in the latter production, which probably reflects the sequential conversion of [^{14}C]pyruvate into [^{14}C]oxaloacetate and [^{14}C]aspartate. Our data therefore suggest that D-glucose augments the metabolic flow in the reaction catalysed by pyruvate carboxylase in pancreatic islets. The rise in D-glucose concentration also caused a significant increase ($P < 0.001$) in the generation of ^{14}C -labelled amino acids other than [^{14}C]alanine and [^{14}C]glutamate in pancreatic islets exposed to D-[6- ^{14}C]glucose. The production of ^{14}C -labelled amino acids other than [^{14}C]alanine and [^{14}C]glutamate was close to the limit of detection in RINm5F cells (Table 3).

In Table 3, the difference between the results obtained

Table 3. Production of ^{14}C -labelled amino acids other than [^{14}C]alanine and [^{14}C]glutamate

Values (means $\pm$ S.E.M.) are expressed as pmol of D-glucose residues/120 min per islet or per 10^3 cells, and refer in all cases to 12 individual determinations.

	Tissue . . .	Pancreatic islets		RINm5F cells	
	[D-Glucose] (mM) . . .	2.8	16.7	2.8	16.7
¹⁴ C-labelled amino acid production from D-[3,4- ¹⁴ C]glucose		2.9±0.7	6.0±0.9	0.6±0.2	0.5±0.3
¹⁴ C-labelled amino acid production from D-[6- ¹⁴ C]glucose		7.2±0.6	21.7±2.0	1.6±0.3	0.1±0.5

in the presence of D-[6- ^{14}C]glucose and D-[3,4- ^{14}C]glucose should be representative of the generation of ^{14}C -labelled amino acids (other than [^{14}C]alanine and [^{14}C]glutamate) derived from intermediates in the tricarboxylic acid cycle, which were labelled through the condensation of oxaloacetate and [^{14}C]acetyl-CoA. Therefore, if such ^{14}C -labelled amino acids are added to [^{14}C]glutamate (also derived from D-[6- ^{14}C]glucose), it becomes possible to compare the production of all ^{14}C -labelled amino acids labelled as a result of the condensation of oxaloacetate with [^{14}C]acetyl-CoA with the overall production of unoxidized [^{14}C]acetyl-CoA. The results of such a comparison are illustrated in the last line of Table 2, which refers to the amount of unoxidized [^{14}C]acetyl-CoA which was not recovered as ^{14}C -labelled amino acids (at the exclusion of [^{14}C]alanine) and, hence, possibly channelled into other metabolic pathways, such as the synthesis of fatty acids. In the islets, this metabolic variable was increased in response to the rise in D-glucose concentration ($P < 0.05$). Such was not the case in the RINm5F cells.

DISCUSSION

The present study, which aimed at improving our knowledge of D-glucose catabolism in islet cells, takes advantage of the parallel use of three distinct labelled D-glucose molecules. The production of $^3\text{H}_2\text{O}$ from D-[5- ^3H]glucose is currently assumed to represent the rate of generation of triose phosphates, $^3\text{H}_2\text{O}$ being formed concomitantly with the interconversion of such triose phosphates or, otherwise, when 2-phosphoglycerate is converted into phosphoenolpyruvate. The catabolism of D-[3,4- ^{14}C]glucose leads to the production of pyruvate labelled in the carboxy atom, which is released as $^{14}\text{CO}_2$ during the oxidative decarboxylation of pyruvate. The production of $^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose occurs when acetyl-CoA is oxidized in the tricarboxylic acid cycle. There are at least two potential limitations to these theoretical considerations. First, it could be objected that C-3 (but not C-4) in D-[3,4- ^{14}C]glucose is susceptible to randomization in positions 1, 2 and 3 when glucose 6-phosphate is metabolized in the pentose phosphate pathway [20]. However, from the quantitative standpoint, the randomization process is likely to have only a negligible effect on the estimation of [^{14}C]pyruvate decarboxylation and [^{14}C]lactate production. Indeed, in the two cell types under study here, the flow through the pentose shunt is very low relative to the total generation of triose phosphates, not exceeding 1.4–4.5% in pancreatic islets and 0.5% in the RINm5F cells [21].

Fig. 1. Schematic view of D-glucose utilization (from triose phosphates onwards) in pancreatic islets

The Figure illustrates, from left to right, the respective participation of the glycerol phosphate shuttle and lactate production to the reoxidation of cytosolic NADH, the fate of pyruvate molecules (PYR) not converted into lactate, the oxidation or non-oxidation of acetyl residues, and the possible fate of non-oxidized acetyl residues. Broad columns refer to the situation found in the presence of 16.7 mM-D-glucose, and narrow columns to that in islets incubated in the presence of 2.8 mM-D-glucose. All results are shown relative to the total utilization of D-glucose at the high hexose concentration. Such a total utilization takes into account the apparent generation of ^{14}C -labelled amino acids [e.g. aspartate (ASP)] other than [^{14}C]alanine (ALA) from D-[3,4- ^{14}C]glucose (■).

Second, a minor amount of $^{14}\text{CO}_2$ may be generated from D-[3,4- ^{14}C]glucose in the tricarboxylic acid cycle after the conversion of [^{14}C]pyruvate into oxaloacetate via pyruvate carboxylase, as mentioned above.

With these considerations in mind, the results obtained in pancreatic islets afford three sets of new information (Fig. 1). First, among all metabolic variables measured in this study, the oxidative decarboxylation of [^{14}C]pyruvate derived from D-[3,4- ^{14}C]glucose and the production of $^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose yielded the highest relative increment in response to the rise in glucose concentration. The

16.7 mM-/2.8 mM-glucose ratio was 5.41 ± 0.49 for [^{14}C]pyruvate decarboxylation and 6.31 ± 0.52 for D-[6- ^{14}C]glucose oxidation. Thus the islet cells are apparently well organized to favour, in response to a rise in extracellular D-glucose concentration, those mitochondrial oxidative reactions yielding the major fraction of the total energy generated by the catabolism of this hexose, this coinciding with a marked increase in respiration [22].

Second, our data allow estimation of the flow in the glycerol phosphate shuttle. The activity of the mitochondrial flavin-linked and cytosolic NAD-linked glycerol-3-phosphate dehydrogenases was previously measured in rat islets [23], but the flow rate through the shuttle remained to be assessed. The present data indicate that a rise in D-glucose concentration from 2.8 to 16.7 mM increased 4–5-fold the circulation through the glycerol phosphate shuttle. Even when expressed relative to the rate of D-[3,4- ^{14}C]glucose utilization, such a circulation remained faster at high than at low glucose concentration, increasing from 28 to 40%. Such a change will favour the generation of ATP relative to glycolytic flux. Incidentally, we have here equated the flow through the glycerol phosphate shuttle to the fraction of D-[3,4- ^{14}C]glucose utilization not accounted for by the production of [^{14}C]lactate, assuming that the cytosolic reoxidation of NADH, generated in the glycolytic pathway, is coupled to either the conversion of pyruvate into lactate or the operation of the glycerol phosphate shuttle. It could be objected that there exist other possible modalities for the reoxidation of cytosolic NADH, e.g. the operation of the malate-aspartate shuttle. This alternative possibility is unlikely to be operative, however, in the islet cells. Indeed, in previous studies dealing with the effect of amino-oxycetate on the oxidation of nutrient secretagogues, cytosolic redox state, cationic fluxes and insulin release in isolated islets [24,25], we were led to propose that, in glucose-stimulated islets, the malate-aspartate shuttle may transport electrons from intramitochondrial NADH to the cytosol, rather than in the opposite direction. This proposal remains, however, to be unambiguously documented [26].

Lastly, the present data reveal that the rate of decarboxylation of pyruvate, derived from exogenous D-glucose, represents about twice the rate of oxidation of the resulting acetyl residues. A comparable situation had been previously documented in islets exposed to exogenous [1- ^{14}C]pyruvate, as distinct from either [2- ^{14}C]pyruvate or [3- ^{14}C]pyruvate [27]. It indicates that a significant fraction of the acetyl-CoA derived from exogenous D-glucose escapes full oxidation. This was accounted for, to a large extent, by the incorporation of the C atoms of D-glucose into amino acids other than alanine, e.g. glutamate and aspartate. The remaining fraction of unoxidized ^{14}C -labelled acetyl residues was probably used as a substrate for lipogenesis [27].

When compared with these results obtained in normal pancreatic islets, the situation in the tumoral insulin-producing cells was characterized by several and severe anomalies. First, in fair agreement with previous observations [28–30], the increase in metabolic flux resulting from the rise in D-glucose concentration was much less in tumoral than in normal islet cells. The oxidation of D-[6- ^{14}C]glucose was even lower at 16.7 mM- than at 2.8 mM-D-glucose, as also observed in an independent series of experiments [31]. This coincides

with the fact that O_2 uptake by RINm5F cells is not higher at 16.7 mM- than at 2.8 mM-D-glucose [32]. Second, relative to the total rate of D-[3,4- ^{14}C]glucose utilization, the flow rate through the glycerol phosphate shuttle, the oxidative decarboxylation of [^{14}C]pyruvate and the oxidation of D-[6- ^{14}C]glucose were lower in tumoral than in islet cells. Even when expressed relative to [^{14}C]pyruvate decarboxylation, the oxidation of D-[6- ^{14}C]glucose remained significantly lower in tumoral than in normal islet cells. Lastly, the ratio of [^{14}C]lactate to [^{14}C]pyruvate production was much lower in RINm5F cells than in normal islets, extending a comparable observation made by measuring the output of unlabelled lactate and pyruvate from the tumoral cells [30].

In conclusion, the present data reveal that in normal, but not in tumoral, islet cells a rise in extracellular D-glucose concentration not only increases the total rate of hexose utilization but, relative to total utilization, also favours mitochondrial oxidative processes. This illustrates the participation of regulatory mechanisms, distal to hexose phosphorylation, in the control of D-glucose catabolism in normal islet cells and the perturbation of such mechanisms in a model of β -cell dysfunction.

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