Pentose cycle pathway in normal and tumoral islet cells

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Relative to protein content, the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and the rate of glucose metabolism by the pentose cycle pathway in tumoral insulin-producing cells were similar to or higher than those found in normal rat islets. Hence, the decreased secretory response of tumoral cells to glucose is apparently not attributable to any major anomaly in glucose handling by the hexose monophosphate pathway.

Tumoral insulin-producing cell *Pancreatic islet* *Pentose cycle*

1. INTRODUCTION

At variance with normal pancreatic B-cells, tumoral insulin-producing cells of the RINm5F cell line display a poor secretory response to glucose [1–3]. Our aims were to measure in tumoral cells the metabolism of glucose in the pentose cycle and glycolytic pathway, and to compare the results with those obtained in pancreatic islets exposed to either a low (5.6 mM) or high (16.7 mM) concentration of glucose.

2. MATERIALS AND METHODS

Pancreatic islets were isolated by the collagenase method from fed albino rats [4]. Tumoral insulin-producing cells were cultured, harvested and counted as described [5].

For measuring the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, the tumoral cells were sonicated (3 times 5 s) in Tris-HCl buffer (50 mM, pH 8.0) containing $MgCl_2$ (5 mM), EDTA (1 mM), KCl (100 mM), cysteine (2 mM) and bovine albumin (0.1 mg/ml). The enzymatic activity was measured spectrophotometrically at 22°C in the same buffer and in the presence of glucose 6-phosphate (2 mM) or 6-phosphogluconate (2 mM) and NADP (0.5 mM). The reaction velocity was stable over at least 10 min and proportional to the number of cells ($0.2–1.0 \times 10^6$ cells/cuvette) with a variation coefficient close to 3.6%. The results are expressed as pmol NADPH formed/min per 10^3 cells. In control experiments, we have verified that, under the present experimental conditions, purified enzymes yielded full enzymic activities and that the measurement of glucose-6-phosphate dehydrogenase activity was little affected by the activity of endogenous 6-phosphogluconate dehydrogenase.

The methods used to measure the production of ^3H_2O from D-[5-^{3}H]glucose [6] and oxidation of D-[1-^{14}C]glucose or D-[6-^{14}C]glucose [7] were identical to those described previously, except that all experiments were conducted in a Krebs phosphate buffer [8] equilibrated against ambient air. The protein content of islet cells was measured as in [9].

Mean values (± SE) are expressed together with the number of individual observations (n). The fraction of glucose metabolism that occurs by the pentose cycle was calculated according to Katz and Wood [10].

3. RESULTS

In RINm5F cells, the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase averaged 4.10 ± 0.15 (n = 14) and

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Table 1

Metabolism of glucose in tumoral and normal islet cells

RINm5F cells 2.8 mM D-glucose (pmol/60 min per 10^3 cells)	Pancreatic islets	
	5.6 mM D-glucose (pmol/60 min per islet)	16.7 mM D-glucose
D-[5-^{3}H]Glucose utilization 114.65 ± 4.66 (30)	43.28 ± 1.91 (34)	119.48 ± 8.17 (10)
D-[1-^{14}C]Glucose utilization 3.43 ± 0.19 (50)	4.01 ± 0.29 (20)	11.17 ± 0.76 (20)
D-[6-^{14}C]Glucose oxidation 1.89 ± 0.10 (50)	2.20 ± 0.12 (20)	9.18 ± 0.50 (20)

0.414 ± 0.043 (n = 4) pmol/min per 10^3 cells, respectively.

The rate of D-[5-^{3}H]glucose utilization in tumoral cells incubated at 2.8 mM D-glucose largely exceeded that found in pancreatic islets incubated at 16.7 mM D-glucose. Indeed, if allowance is made for the fact that the protein content averages 139 ± 13 ng/10^3 tumoral cells and 745 ± 88 ng/islet, the rate of D-[5-^{3}H]glucose conversion to ^3H_2O amounted to 825 ± 34 and 160 ± 11 pmol/60 min per μg protein in tumoral cells incubated at 2.8 mM glucose and pancreatic islets incubated at 16.7 mM glucose, respectively.

In tumoral cells, the rate of D-[6-^{14}C]glucose oxidation only represented 1.6% of that of D-[5-^{3}H]glucose utilization, whereas such a ratio averaged 5.1 and 7.7% in pancreatic islets incubated at 5.6 and 16.7 mM D-glucose, respectively. However, if expressed relative to the protein content, the rate of D-[6-^{14}C]glucose oxidation was similar in tumoral cells incubated at 2.8 mM D-glucose (13.6 ± 0.7 pmol/60 min per μg protein) and pancreatic islets incubated at 16.7 mM D-glucose (12.3 ± 0.7 pmol/60 min per μg protein), respectively.

The oxidation of D-[1-^{14}C]glucose exceeded that of D-[6-^{14}C]glucose, even in pancreatic islets incubated at high glucose concentration ($P < 0.025$ by paired comparison). From these data we have calculated both the fraction of the total metabolism of glucose that occurs by the pentose cycle and the absolute flow rate through this pathway. The fraction of glucose metabolism occurring by the cycle averaged 0.46% in tumoral cells incubated at 2.8 mM D-glucose and 1.51% and 0.61% in pancreatic islets incubated at 5.6 and 16.7 mM D-glucose, respectively. The flow rate through the cycle averaged 1.58 pmol/60 min per 10^3 tumoral cells and 1.96–2.18 pmol/60 min per islet (at 5.6–16.7 mM D-glucose). Relative to protein content, these values amounted to 11.36 and 2.64–2.93 pmol/60 min per μg protein in tumoral cells and pancreatic islets, respectively. These data indicate that the relative contribution of the pentose cycle to the total generation of triose phosphates was lower in tumoral than normal islet cells whilst, in absolute terms, the flow rate through the cycle was higher in the tumoral cells than in pancreatic islets.

4. DISCUSSION

In pancreatic islets, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities range from 5.1–27.0 and 3.2–9.2 pmol/min per μg protein [9,11,12], respectively, as compared to 29.4 and 3.0 pmol/min per μg protein in the tumoral insulin-producing cells. In both cell types, the rate of glucose metabolism through the pentose cycle pathway is much lower, averaging no more than 0.19 pmol/min per μg protein in tumoral cells incubated at 2.8 mM D-glucose and 0.04–0.05 pmol/min per μg protein in pancreatic islets incubated at 5.6–16.7 mM glucose. Thus, in terms of either enzymatic activity in cell homogenates or flow rate in intact cells, the tumoral cells are equally or better equipped than normal islet cells for the handling of glucose 6-phosphate in the hexose monophosphate pathway. This finding suggests that the poor secretory responsiveness of

tumoral cells to glucose is not attributable to any obvious anomaly in the metabolism of glucose in the pentose cycle pathway. Incidentally, the present results confirm that the flow through such a cycle is not increased, in normal pancreatic islets, when the glucose concentration is raised from 2.8 to 16.7 mM [11,13]. These observations and the recent demonstration that the metabolism of glucose in the pentose cycle is β-stereospecific in intact islets [14] also suggest that the generation of NADPH in the cycle is not responsible for the stimulant action of glucose upon insulin release.

When expressed relative to the overall rate of glucose utilization, the oxidation of both D-[1-^{14}C]glucose and D-[6-^{14}C]glucose was much lower in tumoral cells than pancreatic islets. However, when expressed relative to protein content, the mitochondrial oxidation of D-[6-^{14}C]glucose was virtually identical in tumoral cells exposed to 2.8 mM D-glucose and pancreatic islets exposed to a much higher concentration of glucose (16.7 mM). In both cell types, these glucose concentrations are known to evoke a close-to-maximal increase in glycolytic flux [14,15]. Hence, the similarity in oxidation rate could suggest that the poor secretory responsiveness to glucose of the tumoral cells is not attributable to a defect in mitochondrial oxidation.

As judged from the present data, the major anomaly in the metabolism of glucose in tumoral cells consists of a much higher rate of glycolysis. We have recently indicated that this coincides with an abnormally low ratio for lactic/pyruvic acid output from these tumoral cells [14]. It is conceivable, therefore, that the poor secretory response to glucose of tumoral cells is attributable to an abnormal cytosolic redox state rather than any quantitative defect in the absolute flow rate through either the pentose cycle, glycolytic pathway or Krebs cycle.

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