Hexose metabolism in pancreatic islets

Inhibition of hexokinase

Marie-Hélène GIROIX,* Abdullah SENER,* Daniel G. PIPELEERS† and Willy J. MALAISSE*

*Laboratory of Experimental Medicine, Université Libre de Bruxelles, and

†Department of Metabolism and Endocrinology, Vrije Universiteit Brussel, Brussels, Belgium

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In islet homogenates, hexokinase-like activity (K_m 0.05 mM; V_{max} 1.5 pmol/min per islet) accounts for the major fraction of glucose phosphorylation. Yet the rate of glycolysis in intact islets incubated at low glucose concentrations (e.g. 1.7 mM) sufficient to saturate hexokinase only represents a minor fraction of the glycolytic rate observed at higher glucose concentrations. This apparent discrepancy between enzymic and metabolic data may be attributable, in part at least, to inhibition of hexokinase in intact islets. Hexokinase, which is present in both islet and purified B-cell homogenates, is indeed inhibited by glucose 6-phosphate (K_i 0.13 mM) and glucose 1,6-bisphosphate (K_i approx. 0.2 mM), but not by fructose 2,6-bisphosphate. In intact islets, the steady-state content of glucose 6-phosphate (0.26–0.79 pmol/islet) and glucose 1,6-bisphosphate (5–48 fmol/islet) increases, in a biphasic manner, at increasing concentrations of extracellular glucose (up to 27.8 mM). From these measurements and the intracellular space of the islets, it was estimated that the rate of glucose phosphorylation as catalysed by hexokinase represents, in intact islets, no more than 12–24% of its value in islet homogenates.

The stimulation of insulin release by glucose is currently thought to result from an increase in the rate of glucose metabolism in the pancreatic B-cell (Sener *et al.*, 1976). Since glucose apparently rapidly equilibrates across the cell membrane (Hellman *et al.*, 1971), the kinetics of glucose phosphorylation at increasing glucose concentrations could play a key role in the control of glucose metabolism. At a physiological glucose concentration (8.3 mM), glucose phosphorylation by islet homogenates is attributable mainly to hexokinase, the relative contribution of glucokinase not exceeding 10–20% (Matschinsky & Ellerman, 1968; Ashcroft & Randle, 1970; Malaisse *et al.*, 1976a). In intact islets, however, glucose utilization at the same sugar concentration is 3 times that observed at a low glucose concentration (1.7 mM) sufficient virtually to saturate hexokinase (Sener *et al.*, 1976). In the present study, we have sought an explanation for this apparent disparity between enzymic and metabolic data. It is proposed that

hexokinase, in intact islets exposed to glucose, is severely inhibited by both glucose 6-phosphate and, to a lesser extent, glucose 1,6-bisphosphate.

Materials and methods

Most experiments were performed with islets isolated by the collagenase method (Lacy & Kostianovsky, 1967) from the pancreas of fed albino rats. In two experiments, however, a group of 2×10^5 purified pancreatic B-cells was prepared as described elsewhere (Van de Winkel *et al.*, 1982).

For the measurement of glucose or mannose phosphorylation, pancreatic islets (or purified B-cells) were sonicated (3 × 5 s; medium power, amplitude 3; MSE Ultrasonic Disintegrator) in 150 mM-KCl, to yield about 400 islets (or 2×10^5 purified B-cells) per 300 μ l. A sample (20 μ l) of these homogenates was mixed with 20 μ l of a reaction mixture consisting of triethanolamine/HCl buffer (100 mM, pH 7.4) containing KCl (150 mM), MgCl₂ (20 mM), cysteine (4 mM), EDTA (2 mM), bovine serum albumin (0.02%, w/v), ATP (10 mM), either D-[U-¹⁴C]glucose or D-

Abbreviations used: Glc-6-P, glucose 6-phosphate; Fru-6-P, fructose 6-phosphate; Glc-1,6-P₂, glucose 1,6-bisphosphate; Fru-2,6-P₂, fructose 2,6-bisphosphate.

[U-¹⁴C]mannose and, as required, Glc-6-*P*, Glc-1,6-*P*₂, Fru-2,6-*P*₂ or mannose 6-phosphate. The specific radioactivity of the hexoses ranged from 2.8 to 348 Ci/mol. The concentration of Glc-6-*P* quoted in the text represents 67% of the true concentration of Glc-6-*P* added to the reaction mixture. This correction was made because of the conversion of Glc-6-*P* into Fru-6-*P*, the concentrations of which rapidly reach equilibrium in islet homogenates (Malaisse *et al.*, 1982). This was confirmed by the finding that Fru-6-*P* inhibited the phosphorylation of glucose (0.25 mM) to virtually the same extent as did Glc-6-*P*, each of these hexose phosphates being used at an initial concentration of 1.0 mM (results not shown). After 30 min incubation at 30°C, the reaction was halted by addition of 2.0 ml of iced water, the diluted reaction medium being then passed through a column (0.5 ml) of Dowex 1-X8 (formate form) for separation of the hexose phosphates by anion-exchange chromatography (Berridge *et al.*, 1983). The column was rinsed with 3 × 2 ml of water, the hexose phosphates then being eluted with 3.0 ml of 1.0 M-ammonium formate/0.1 M-formic acid. The eluate was mixed with 15 ml of scintillation fluid (Aquasol-2; New England Nuclear, Boston, MA, U.S.A.) and its radioactive content determined. Blank values were obtained under identical conditions in the absence of islet homogenate. No more than $1.79 \pm 0.12\%$ ($n = 12$) of the initial radioactivity was found in such control samples. When D-[U-¹⁴C]glucose 6-phosphate (0.3–1.0 mM) was placed on the column, no more than $2.17 \pm 0.41\%$ ($n = 12$) of the initial radioactivity passed through the column, before the elution with the formate/formic acid solution. Unlabelled Glc-6-*P* (0.1–3.0 mM) failed to affect the recovery of D-[U-¹⁴C]glucose 6-phosphate. The above-mentioned blank value was virtually identical with that obtained with the islet homogenate in the absence of ATP and this, whether at low (0.25 mM) or high (10.0 mM) glucose concentration and whether in the absence or presence of unlabelled Glc-6-*P* (initial concn. 1.0 mM). All measurements were performed in triplicate. The reaction velocity was proportional to the number of homogenized islets (12, 25 or 37 islets) present in the reaction mixture and constant with time (15, 30 or 45 min), with coefficient of variations of 7.4 and 7.8% respectively.

For the measurement of the islet content of Glc-6-*P*, groups of 20 islets were incubated for 30 min in 0.1 ml of a bicarbonate-buffered medium (Malaisse *et al.*, 1970). The medium was then removed and replaced by 40 µl of 20 mM-HCl. The tubes containing the islets were placed in liquid N₂, the islets disrupted by mechanical vibration (Malaisse *et al.*, 1978), and the homogenates incubated for 10 min

at 60°C and then centrifuged (20 s, 5000g). Two portions (10 µl each) of the supernatant solution were mixed with a reaction mixture (10 µl) consisting of Tris/HCl buffer (100 mM, pH 8.1), dithiothreitol (0.2 mM), NADP⁺ (0.02 mM), bovine serum albumin (0.02% w/v) and yeast glucose-6-phosphate dehydrogenase (EC 1.1.1.49; 0.02 unit/ml). After 20 min incubation at 20°C and addition of 10 µl of 0.2 M-NaOH, the samples were heated for 10 min at 60°C. The NADPH formed during this first step of the procedure was measured by a cycling procedure (Lowry & Passonneau, 1972). For this purpose, the samples were mixed with 100 µl of Tris/HCl buffer (100 mM; pH 8.1) containing 2-oxoglutarate (5.0 mM), ammonium acetate (10 mM), ox liver glutamate dehydrogenase (EC 1.4.1.3; 9 units/ml), Glc-6-*P* (1.0 mM) and yeast glucose 6-phosphate dehydrogenase (5 units/ml). After 60 min incubation at 37°C, the reaction was halted by heating for 5 min at 100°C. The 6-phosphogluconate formed during the cycling procedure was measured as follows. The samples were mixed with 1.0 ml of Tris/HCl buffer (50 mM; pH 8.1) containing EDTA (0.1 mM), MgCl₂ (5.0 mM), ammonium acetate (30 mM), NADP⁺ (0.1 mM) and yeast 6-phosphogluconate dehydrogenase (EC 1.1.1.44; 6 units/ml). After 30 min incubation at 20°C, the NADPH formed was measured by fluorimetry. Samples devoid of islets (blanks) and Glc-6-*P* standards (containing 0.5–8 pmol of Glc-6-*P*/10 µl of 20 mM-HCl) were treated in the same way. When glucose 6-phosphate dehydrogenase was omitted in the first step of the procedure, the readings were not different from the above-mentioned blank values. Glucose (up to 18 nmol/10 µl of 20 mM-HCl) did not interfere with the assay of Glc-6-*P*.

For measuring the islet content of Glc-1,6-*P*₂, groups of 40 islets were incubated for 30 min in 0.4 ml of the bicarbonate-buffered incubation medium. After removal of the incubation medium and addition of 50 µl of 0.1 M-NaOH, the samples were treated as described elsewhere (Sener *et al.*, 1982).

The apparent space of distribution of ³H₂O, [¹⁴C]urea, [6,6'-(*n*-³H)]sucrose, D-[5-³H]glucose, D-[U-¹⁴C]glucose or D-[U-¹⁴C]mannose was measured over 5 min incubation by a method previously described (Malaisse *et al.*, 1983).

All results, including those mentioned above, are presented as the mean ($\pm$ S.E.M., or limits of individual variations if $n = 2$) together with the numbers of individual observations (n). In view of possible variations in the mean size of islets obtained in separate experiments (Malaisse-Lagae & Malaisse, 1984), the comparison of results (such as those illustrated in Table 3) was usually restricted to data collected within the same experiment(s).

Results

Enzymic data

At increasing concentrations of D-glucose up to 0.5 mM, the rate of glucose phosphorylation by islet homogenates was compatible with the participation of a hexokinase-like enzyme with a K_m for glucose close to 0.05 mM and a V_{max} close to 1.5 pmol/min per islet (Fig. 1). Likewise, at increasing concentrations of D-mannose up to 0.25 mM, the rate of mannose phosphorylation by islet homogenates was compatible with the participation of a single enzyme with a K_m for mannose close to 0.03–0.04 mM (Fig. 1). In a large series of experiments performed at a fixed concentration of hexoses (0.25 mM), the rates of glucose and mannose phosphorylation averaged, respectively, 1.18 ± 0.08 and 0.54 ± 0.04 pmol/min per islet ($n = 32$ and 12). When measured within the same experiments, the rate of mannose phosphorylation averaged $64.9 \pm 2.8\%$ ($n = 6$) of the paired value found with glucose (0.25 mM in both cases).

Glc-6-P caused a dose-related inhibition of glucose phosphorylation as measured in islet homogenates incubated at a low glucose concentration (0.25 mM). The K_i for inhibition by Glc-6-P was close to 0.13 mM (results not shown). The relative magnitude of the inhibitory effect of Glc-6-P on glucose phosphorylation was little affected by the glucose concentration, at least in the range of values used to characterize the kinetics of hexokinase. For instance, at a fixed concentration of Glc-6-P (0.2 mM) and increasing concentrations of glucose (0.05–0.5 mM), the inhibition of glucose

phosphorylation ranged from 60.2 to 69.9%, with a mean value of $65.4 \pm 1.9\%$ ($n = 6$). The phosphorylation of glucose by hexokinase was also inhibited by Glc-1,6- P_2 , whether in the absence or the presence of Glc-6-P (Fig. 2). In the absence of Glc-6-P, the apparent K_i for Glc-1,6- P_2 was close to 0.17 mM. Fru-2,6- P_2 (0.03–0.3 mM) failed to affect the phosphorylation of glucose (0.25 mM), the experimental values averaging $98.5 \pm 1.7\%$ ($n = 3$) of the mean control value.

For mannose phosphorylation, the results concerning the inhibitory action of Glc-6-P and Glc-1,6- P_2 were comparable with those obtained for glucose phosphorylation (Table 1). Incidentally, mannose 6-phosphate (1.0 mM) slightly inhibited phosphorylation of both glucose and mannose, the experimental values in the presence of mannose 6-phosphate averaging, respectively, 77.0 ± 1.9 and $83.1 \pm 1.4\%$ ($n = 3$ in each case) of their paired control value. This modest inhibition could con-

Fig. 1. Double-reciprocal plots for the phosphorylation of D-glucose (●) and D-mannose (○) by islet homogenates. Mean values ($\pm$ S.E.M.) are derived from three or more individual observations.

Fig. 2. Effect of [Glc-1,6- P_2] (logarithmic scale) on the phosphorylation of D-glucose (0.25 mM) in the absence of Glc-6-P (○) and in the presence of either 0.07 mM-Glc-6-P (●) or 0.20 mM-Glc-6-P (▲).

Mean values ($\pm$ S.E.M.) refer to three to five individual observations and are shown as a percentage of their paired control value (no Glc-6-P and no Glc-1,6- P_2).

Table 1. *Glucose and mannose phosphorylation by islet homogenates*

The phosphorylation of D-[U-¹⁴C]glucose and D-[U-¹⁴C]mannose by islet homogenates was measured in the absence or presence of several potential inhibitors.

Inhibitor	Phosphorylation rate (pmol/min per islet)	
	D-Glucose (0.25 mM)	D-Mannose (0.25 mM)
Nil	1.18 ± 0.08 (32)	0.54 ± 0.04 (12)
Glc-6-P (0.7 mM)	0.17 ± 0.01 (6)	0.05 ± 0.01 (3)
Glc-1,6-P ₂ (0.3 mM)	0.42 ± 0.02 (7)	0.18 ± 0.01 (3)
Mannose-6-P (1.0 mM)	0.91 ± 0.02 (3)	0.45 ± 0.01 (3)

Table 2. *Glucose phosphorylation by islet or purified B-cell homogenates*

The phosphorylation of D-[U-¹⁴C]glucose at increasing concentrations of the hexose and in the presence or absence of Glc-6-P was measured in islet and purified B-cell homogenates.

[Glucose] (mM)	[Glc-6-P] (mM)	Glucose phosphorylation	
		Pancreatic islets (pmol/min per islet)	Purified B-cells (pmol/min per 10 ³ cells)
0.5	—	1.18 ± 0.18 (4)	0.23 ± 0.02 (2)
0.5	0.7	0.11 ± 0.03 (4)	0.04 ± 0.01 (2)
5.0	—	1.50 ± 0.05 (4)	0.30 ± 0.01 (2)
20.0	—	2.02 ± 0.16 (4)	0.54 ± 0.02 (2)

ceivably result, in part at least, from the partial conversion of mannose 6-phosphate into Fru-6-P and Glc-6-P (Anjaneyulu *et al.*, 1981).

Homogenates of purified B-cells, like islet homogenates, apparently displayed both hexokinase-like and glucokinase-like activities (Table 2). Thus, on the one hand, the rate of glucose phosphorylation at a low concentration of glucose (0.5 mM) was severely inhibited by Glc-6-P, which does not inhibit glucokinase (Ashcroft & Randle, 1970; Malaisse *et al.*, 1976a). On the other hand, there was a marked increase in phosphorylation rate as the glucose concentration was raised from 5.0 mM, in which case hexokinase is theoretically saturated, to 20.0 mM. Incidentally, the ratio of glucose phosphorylation at high to that at low glucose concentration (20 mM and 0.5 mM respectively) was somewhat higher in purified B-cells (2.36 ± 0.08) than in islets (1.71 ± 0.14), suggesting a higher ratio of glucokinase/hexokinase activity in B-cells than in islets. This difference could be conceivably attributable to hexokinase contributed by non-B islet cells (Gorus *et al.*, 1984).

Metabolic data

Fig. 3 illustrates the biphasic increase in the Glc-6-P and Glc-1,6-P₂ contents of islets incubated for 30 min at increasing concentrations of extracellular glucose. D-Mannose (16.7 mM) also increased the Glc-6-P content from a basal value of 338 ± 36 to 481 ± 41 fmol/islet (*n* = 7 in each case; *P* < 0.025). The mannose-induced increment in Glc-6-P con-

tent above basal value averaged 40% of that evoked by glucose (16.7 mM) within the same experiments. We have previously shown that mannose, like glucose, increases the islet content of hexose 1,6-bisphosphate (Malaisse-Lagae *et al.*, 1982).

To convert the islet content of hexose phosphates into their corresponding cellular concentration, information on the intracellular volume of the islets is required. The data summarized in Table 3 provide such information. In a first series of experiments, the extracellular space as judged from the distribution volume of [³H]sucrose averaged 0.78 ± 0.07 nl/islet. The distribution volume of ³H₂O or [¹⁴C]urea was much larger than that of [³H]sucrose. When compared within the same batches of islets, the [¹⁴C]urea distribution volume averaged 89.5 ± 2.3% (*n* = 9; *P* < 0.005) of the paired value found with ³H₂O. The distribution volume of either D-[U-¹⁴C]glucose or D-[5-³H]-glucose was even smaller than that of [¹⁴C]urea. For instance, the paired difference in the distribution volumes of [¹⁴C]urea and D-[5-³H]glucose averaged 0.50 ± 0.08 nl/islet (*n* = 9). Comparable results were obtained in a second series of experiments. Moreover, in this second series, the intracellular distribution space of D-[U-¹⁴C]glucose was directly compared with that of [¹⁴C]urea, both sets of data being corrected for the paired measurement of the [³H]sucrose space. The intracellular distribution space of D-[U-¹⁴C]glucose was smaller than that of [¹⁴C]urea, with a mean non-paired differ-

Table 3. Distribution volumes of different molecules in pancreatic islets

The intracellular space was calculated after correction for the distribution volume of [6,6'(*n*)-³H]sucrose measured within the same samples. All measurements were made after 5 min incubation.

Expt. no.	Marker molecule	Space	Volume (nl/islet)	<i>n</i>
1	[6,6'(<i>n</i>)- ³ H]Sucrose (1.0 mM) ³ H ₂ O (55.5 M) [¹⁴ C]Urea (1.0 mM)	Extracellular	0.78 ± 0.07	(10)
		Total	2.21 ± 0.09	(19)
		Total	2.10 ± 0.09	(27)
	D-[U- ¹⁴ C]Glucose (16.7 mM) D-[5- ³ H]Glucose (16.7 mM)	Intracellular	1.49 ± 0.08	(9)
		Total	1.51 ± 0.10	(10)
		Total	1.71 ± 0.12	(9)
2	[6,6'(<i>n</i>)- ³ H]Sucrose (1.0 mM) [¹⁴ C]Urea (1.0 mM)	Extracellular	0.79 ± 0.05	(26)
		Total	1.68 ± 0.13	(13)
		Intracellular	0.89 ± 0.08	(13)
	D-[U- ¹⁴ C]Glucose (16.7 mM)	Total	1.22 ± 0.09	(13)
		Intracellular	0.44 ± 0.05	(13)
		Intracellular	0.49 ± 0.08	(9)
3	D-[U- ¹⁴ C]Glucose (16.7 mM)	Intracellular	0.49 ± 0.08	(9)
	D-[U- ¹⁴ C]Mannose (16.7 mM)	Intracellular	0.55 ± 0.06	(10)

Fig. 3. Effect of increasing concentrations of D-glucose on the steady-state contents of Glc-6-P and Glc-1,6-P₂ in intact islets

The numbers of individual observations are shown in parentheses.

ence of 0.45 ± 0.09 nl/islet (degrees of freedom = 24). In the last series of experiments, the intracellular distribution space of D-[U-¹⁴C]mannose was compared with that of D-[U-¹⁴C]glucose, after correction for the paired measurement of the distribution volume of [³H]sucrose. The mean intracellular distribution spaces of the two hexoses were not significantly different from one another (non-paired comparison: *P* > 0.5).

Inhibition of hexokinase

From the data so far presented in this paper, we estimated the rate of glucose phosphorylation in intact islets. This calculation was based on the following assumptions. First, the intracellular concentration of D-glucose was taken as identical with its extracellular concentration. The intracellular concentrations of Glc-6-P and Glc-1,6-P₂ were judged from the data illustrated in Fig. 3, by using a mean intracellular space of 1.19 nl/islet. The rate of glucose phosphorylation, in the absence of inhibitors, was estimated from the plot in Fig. 1. The relative extents of hexokinase inhibition by Glc-6-P and Glc-1,6-P₂ were judged from the data illustrated in Fig. 2. The results of these calculations are illustrated in Fig. 4, which indicates that the relative extent of hexokinase inhibition, in intact islets, increased from 75.6 to 87.7% as the extracellular glucose concentration was raised from 2.8 to 27.8 mM. It is obvious that the inhibition would even be more severe if the assumption was made that hexose phosphates are restricted in the islet cells to a compartment (e.g. the cytoplasmic domain) smaller than the total intracellular space. It should also be underlined that, from the quantitative standpoint, Glc-6-P,

Fig. 4. Calculated values for glucose phosphorylation by hexokinase in islet homogenates (—) and in intact islets (---) at increasing concentrations of D-glucose. For details, see the text.

rather than Glc-1,6- P_2 , acted as the major inhibitor of hexokinase.

Discussion

The present data indicate that D-glucose and D-mannose rapidly accumulate inside islet cells. The intracellular spaces of distribution of these two hexoses, after correction for the extracellular distribution volume of [^3H]sucrose, were not significantly different from one another. Over 5 min incubation, the distribution space of D-glucose was consistently lower than that of [^{14}C]urea, whether corrected or not for extracellular contamination. These findings do not necessarily detract from the current concept that the intracellular concentration (or at least the cytosolic concentration; see Dean, 1973) of D-glucose is virtually identical with its extracellular concentration (Hellman *et al.*, 1971). It was indeed documented that in non-B islet cells, as distinct from B-cells, the intracellular space of distribution of D-[U- ^{14}C]glucose or 3-O-methyl-D-[U- ^{14}C]glucose, after 5 min incubation, represents only 25% of the intracellular [^{14}C]urea space (Gorus *et al.*, 1984). If the assumption is made, therefore, that the cytosolic concentration of D-glucose in the B-cell is indeed virtually identical with its extracellular concentration, the rate of glucose phosphorylation by either hexokinase or glucokinase in intact islets could be inferred from data collected in islet homogenates. The validity of such an inference would be

considerably obscured, however, if the rate of glucose phosphorylation was modulated by other intracellular metabolites.

It is well established and here confirmed that, in islet homogenates (Ashcroft & Randle, 1970; Malaisse *et al.*, 1976a), as in other tissues (Purich *et al.*, 1973), Glc-6- P inhibits glucose phosphorylation as catalysed by hexokinase. From this perspective, the present study affords two novel pieces of information. Firstly, it is shown, in good agreement with the data collected in other tissues (Beitner *et al.*, 1979), that Glc-1,6- P_2 may act synergistically with Glc-6- P in inhibiting islet hexokinase. Such was not the case for Fru-2,6- P_2 . Secondly, the data collected in purified B-cells reveal that hexokinase is indeed present in these insulin-producing cells. This rules out the view that hexokinase would be restricted, in the islets, to non-B cells.

It is known from previous studies that an increase in the extracellular concentration of glucose augments the islet content of Glc-6- P (Ashcroft *et al.*, 1973; Idahl, 1973; Hedekov & Capito, 1974) and Glc-1,6- P_2 (Sener *et al.*, 1982; Malaisse-Lagae *et al.*, 1982). To our knowledge, however, detailed dose-response relationships for the islet contents of these two metabolites, at increasing concentrations of extracellular glucose, have not been established previously. The present data indicate that such relationships display a biphasic appearance, which is reminiscent of the sigmoidal pattern characterizing the rate of glycolysis or glucose oxidation by intact islets (Ashcroft *et al.*, 1970; Hellman *et al.*, 1975; Sener *et al.*, 1976). More precisely, the response to increasing concentrations of extracellular glucose can be described as a dual phenomenon (Malaisse *et al.*, 1976b). An initial increase in the islet content of hexose phosphates (or metabolic fluxes) is seen at low glucose concentrations (<5.6 mM). This reaches a plateau value at very low glucose concentrations (<2.8 mM), a feature compatible with the participation of hexokinase. At higher glucose concentrations (>5.6 mM), however, an additional increase in metabolic variables occurs, this probably reflecting the participation of glucokinase. A somewhat unexpected feature of this dose-response relationship resides in the fact that the islet content of Glc-6- P was far from being negligible in islets incubated in the absence of glucose. In these experiments, the islets were deprived of glucose not solely during the 30 min incubation performed at 37°C but also throughout the isolation procedure conducted at room temperature (about 20°C). In the present system, freshly isolated islets contain no detectable amount of glycogen (Malaisse *et al.*, 1977). Therefore the persistent presence of Glc-6- P in these islets suggests that the catabolism of this

hexose phosphate (e.g. through glycolysis) is abolished in glucose-deprived islets. This, in turn, points to a site distal to Glc-6-*P* availability, e.g. the activation of phosphofructokinase (Giroix *et al.*, 1984), as a potential regulatory mechanism controlling the catabolism of glucose in islet cells.

From the data so far discussed, we have attempted to estimate the extent of hexokinase inhibition in intact islets (see Fig. 4). The residual rate of glucose phosphorylation, at low glucose concentrations (e.g. 2.8 mM), was close to 0.35 pmol/min per islet. This value is in fair agreement with the rate of $^3\text{H}_2\text{O}$ production from D-[5- ^3H]glucose indeed observed, at the same glucose concentration, in intact islets (approx. 0.32 pmol/min per islet; see Sener *et al.*, 1976). A comparable inhibition of hexokinase may occur in islets exposed to D-mannose. Thus D-mannose also augmented the islet content of Glc-6-*P* (the present study) or hexose 1,6-bisphosphate (Malaisse-Lagae *et al.*, 1982).

In conclusion, the information gathered in the present study strongly suggests that hexokinase is severely inhibited in intact islets. It could be argued that the activity of this enzyme may be affected by factors other than the three variables considered, namely the concentrations of glucose, Glc-6-*P*, and Glc-1,6-*P*₂. With this reservation in mind, the data illustrated in Fig. 4 may account, in part at least, for the fact that the rate of glycolysis in intact islets incubated at low glucose concentrations (1.7–2.8 mM), sufficient to saturate hexokinase, is much lower than that observed at higher glucose concentration (e.g. 8.3 mM). This contrasts with the situation found in islet homogenates, in which case the relative contribution of hexokinase to the overall rate of glucose phosphorylation is much higher.

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References

- Anjaneyulu, R., Anjaneyulu, K., Carpinelli, A. R., Sener, A. & Malaisse, W. J. (1981) *Arch. Biochem. Biophys.* **212**, 54–62

- Ashcroft, S. J. H. & Randle, P. J. (1970) *Biochem. J.* **119**, 5–15
- Ashcroft, S. J. H., Hedekov, C. J. & Randle, P. J. (1970) *Biochem. J.* **118**, 143–154
- Ashcroft, S. J. H., Capito, K. & Hedekov, C. J. (1973) *Diabetologia* **9**, 299–302
- Beitner, R., Cohen, T. J., Nordenberg, J. & Haberman, S. (1979) *Biochim. Biophys. Acta* **586**, 266–277
- Berridge, M. J., Dawson, R. M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. (1983) *Biochem. J.* **212**, 473–482
- Dean, P. M. (1973) *Diabetologia* **9**, 115–119
- Giroix, M.-H., Dufrane, S. P., Malaisse-Lagae, F., Sener, A. & Malaisse, W. J. (1984) *Biochem. Biophys. Res. Commun.* **119**, 543–548
- Gorus, F. K., Malaisse, W. J. & Pipeleers, D. G. (1984) *J. Biol. Chem.* **259**, 1196–1200
- Hedekov, C. J. & Capito, K. (1974) *Biochem. J.* **140**, 423–433
- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1971) *Biochim. Biophys. Acta* **241**, 147–154
- Hellman, B., Idahl, L. A., Sehlin, J. & Täljedal, I.-B. (1975) *Diabetologia* **11**, 495–500
- Idahl, L. A. (1973) *Diabetologia* **9**, 403–412
- Lacy, P. E. & Kostianovsky, M. (1967) *Diabetes* **16**, 35–39
- Lowry, O. H. & Passonneau, J. V. (1972) *A Flexible System of Enzymatic Analysis*, pp. 130–136, Academic Press, New York and London
- Malaisse, W. J., Brisson, G. & Malaisse-Lagae, F. (1970) *J. Lab. Clin. Med.* **76**, 895–902
- Malaisse, W. J., Sener, A. & Levy, J. (1976a) *J. Biol. Chem.* **251**, 1731–1737
- Malaisse, W. J., Sener, A., Levy, J. & Herchuelz, A. (1976b) *Acta Diabetol. Lat.* **13**, 202–215
- Malaisse, W. J., Sener, A., Koser, M., Ravazzola, M. & Malaisse-Lagae, F. (1977) *Biochem. J.* **164**, 447–454
- Malaisse, W. J., Hutton, J. C., Kawazu, S. & Sener, A. (1978) *Eur. J. Biochem.* **87**, 121–130
- Malaisse, W. J., Malaisse-Lagae, F. & Sener, A. (1982) *Diabetes* **31**, 90–93
- Malaisse, W. J., Sener, A., Welsh, M., Malaisse-Lagae, F., Hellerström, C. & Christophe, J. (1983) *Biochem. J.* **210**, 921–927
- Malaisse-Lagae, F. & Malaisse, W. J. (1984) in *Methods in Diabetes Research* (Larner, J. & Pohl, S., eds.), John Wiley and Sons, New York, in the press
- Malaisse-Lagae, F., Sener, A. & Malaisse, W. J. (1982) *Biochimie* **64**, 1059–1063
- Matschinsky, F. M. & Ellerman, J. E. (1968) *J. Biol. Chem.* **243**, 2730–2736
- Purich, D. L., Fromm, H. J. & Rudolph, F. (1973) *Adv. Enzymol.* **39**, 249–326
- Sener, A., Levy, J. & Malaisse, W. J. (1976) *Biochem. J.* **156**, 521–525
- Sener, A., Malaisse-Lagae, F. & Malaisse, W. J. (1982) *Biochem. Biophys. Res. Commun.* **104**, 1033–1040
- Van de Winkel, M., Maes, E. & Pipeleers, D. G. (1982) *Biochem. Biophys. Res. Commun.* **107**, 525–532