

## Glucokinase is not the pancreatic B-cell glucoreceptor

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**Summary.** A series of recent experimental findings are reviewed to indicate that glucokinase does not represent the pancreatic B-cell glucoreceptor. (1) Whether in liver, pancreatic islet or insulin-producing tumoral cell homogenates, glucokinase fails to yield a higher reaction velocity with  $\alpha$ - than  $\beta$ -D-glucose. (2) At a high glucose concentration (40 mmol/l), when the phosphorylation of glucose by glucokinase is indeed higher with  $\beta$ - than  $\alpha$ -D-glucose, no preference for  $\beta$ -D-glucose is observed in intact islets, as judged from the utilization of D-[5-<sup>3</sup>H]glucose, production of lactic acid, oxidation of D-[U-<sup>14</sup>C]glucose, net uptake of <sup>45</sup>Ca or release of insulin. (3) The glucose 6-phosphate content of intact islets is higher in the presence of  $\beta$ - than  $\alpha$ -D-glucose. (4) At a low glucose concentration (3.3 mmol/l), when the participation of glucokinase to hexose phosphorylation is minimal,  $\alpha$ -D-glucose is still better metabolized and stimulates both <sup>45</sup>Ca net uptake and insulin release more efficiently than  $\beta$ -D-glucose, despite the fact that

hexokinase yields a higher reaction velocity with  $\beta$ - than  $\alpha$ -D-glucose. (5) In intact islets,  $\beta$ -D-glucose is used preferentially to  $\alpha$ -D-glucose in the pentose cycle pathway as judged from the oxidation of  $\alpha$ - or  $\beta$ -D-[1-<sup>14</sup>C]glucose relative to that of  $\alpha$ - or  $\beta$ -D-[6-<sup>14</sup>C]glucose. (6) In islets removed from fasted rats, the rate of glycolysis is more severely decreased than expected from the repression of glucokinase. (7) The metabolism of glucose in tumoral insulin-producing cells differs, in several respects, from that in normal pancreatic islets, although the pattern of hexokinase and glucokinase activities is similar in these two types of cells. All these observations point to the participation of regulatory sites distal to glucose phosphorylation in the control of glucose metabolism in islet cells.

**Key words:** Pancreatic islets, insulin-producing tumoral cells, glucose anomers, glucokinase, hexokinase.

In the 1972 Minkowski Lecture [1], a model was presented for the stimulation of insulin release by nutrient secretagogues and its modulation by polypeptide and adrenergic hormones. The model aimed at illustrating how changes in either the fluxes and intracellular distribution of calcium or generation of cyclic AMP may provide a multifactorial regulation for a single process of release, as mediated by the microtubular-microfilamentous effector system. In addition, it was briefly proposed, at variance with a current view at that time [2, 3], "that glucose has to be transported and metabolized in the B cell in order to stimulate insulin secretion". In further work, my colleagues and I concentrated mainly on the validity of the latter proposal. We eventually reached the conclusion that the insulinotropic action of nutrient secretagogues indeed reflects their capacity to augment oxidative fluxes in the islet cells, this being defined as the fuel hypothesis [4] or fuel concept [5] for insulin release.

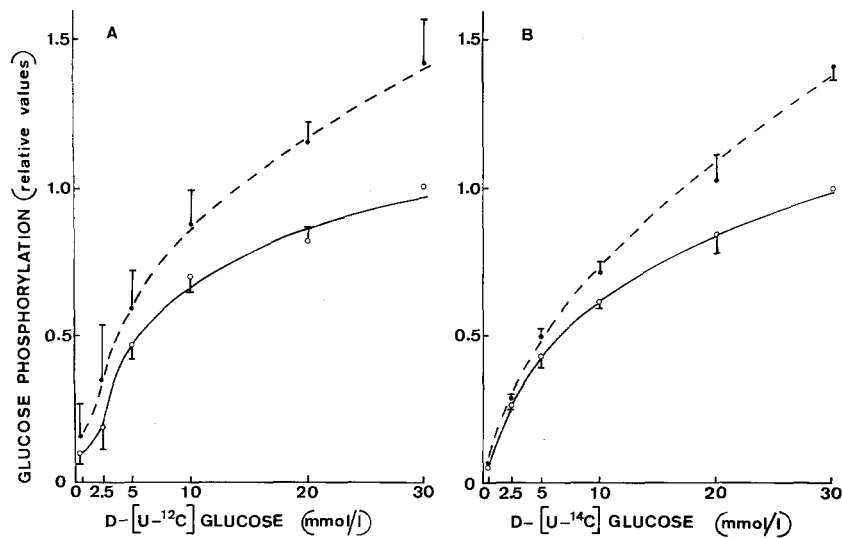
One of the essential arguments in support of this fuel concept consisted of the finding that the higher insulinotropic capacity of the  $\alpha$ - than  $\beta$ -anomer of either D-glucose or D-mannose coincides with a higher rate of

glycolysis in pancreatic islets exposed to the  $\alpha$ - as distinct from  $\beta$ -anomer of these two hexoses [6, 7]. We attributed such a metabolic difference to the anomeric specificity of phosphoglucose isomerase and phosphoglucomutase, as reviewed in detail elsewhere [8]. Recently, however, Matschinsky et al. proposed that the anomeric specificity of hexose metabolism in islet cells is attributable to a limited preference of glucokinase for the  $\alpha$ -anomer of either D-glucose or D-mannose [9, 10]. The latter claim was considered in the framework of the concept that glucokinase would act as a glucoreceptor in the pancreatic B-cell [11, 12].

In the present report, we will review recent experimental findings which clearly indicate that glucokinase is not the enzyme responsible for the anomeric specificity of glucose metabolism in islet cells.

### Is glucokinase an $\alpha$ -stereospecific enzyme?

In considering the possible role of glucokinase in the anomeric specificity of glucose metabolism in pancreatic islets, the first point to be discussed is whether gluco-



**Fig. 1.** Comparison of  $\alpha$ -D-glucose ( $\circ$ — $\circ$ ) and  $\beta$ -D-glucose ( $\bullet$ — $\bullet$ ) phosphorylation by liver homogenates at 7°C. For the sake of comparison the results obtained by (A) a non-isotopic or (B) radioactive procedure are expressed relative to the mean value found within the same experiments in the presence of  $\alpha$ -D-glucose (30 mmol/l). Such a reference value averaged  $6.48 \pm 1.34$  (left) and  $4.67 \pm 0.52$  (right) pmol/60 min per  $\mu$ g wet wt. ( $n = 4-6$ ). Mean  $\pm$  SEM values are derived from three to six separate experiments

**Table 1.** Metabolic and functional variables in islet homogenates or intact islets incubated at 7°C in the absence or presence of  $\alpha$ - or  $\beta$ -D-glucose (40 mmol/l)

| Metabolic variables   | No glucose            | $\alpha$ -D-glucose   | $\beta$ -D-glucose    |
|---|-----------------------|-----------------------|-----------------------|
| <i>D</i> -[ $U$ - $^{14}C$ ]glucose phosphorylation (pmol/60 min per islet) |                       |                       |                       |
| No glucose 6-phosphate  |                       | $23.8 \pm 1.7$ (8)    | $43.2 \pm 2.7$ (8)    |
| Glucose 6-phosphate (3.0 mmol/l)  |                       | $4.4 \pm 1.0$ (8)     | $8.2 \pm 1.2$ (8)     |
| <i>D</i> -[ $5$ - $^3H$ ]glucose utilization (pmol/60 min per islet)        |                       | $10.52 \pm 1.25$ (22) | $10.16 \pm 1.23$ (22) |
| Lactate production (pmol/60 min per islet)                                  |                       |                       |                       |
|   | $10.02 \pm 1.16$ (28) | $15.87 \pm 1.86$ (27) | $14.19 \pm 1.37$ (28) |
| <i>D</i> -[ $U$ - $^{14}C$ ]glucose oxidation (fmol/60 min per islet)       |                       | $219 \pm 20$ (19)     | $164 \pm 21$ (19)     |
| $^{45}Ca$ net uptake (fmol/60 min per islet)                                |                       |                       |                       |
|   | $354 \pm 24$ (42)     | $962 \pm 41$ (42)     | $864 \pm 46$ (42)     |

Mean values ( $\pm$  SEM) are shown together with the number of individual observations (in parentheses)

kinase is indeed an  $\alpha$ -stereospecific enzyme. Previous studies do not provide an unambiguous answer to this question. Salas et al. [13] first reported that, at a high concentration (50 mmol/l),  $\alpha$ - and  $\beta$ -D-glucose are phosphorylated at the same rate by rabbit liver glucokinase. Miwa et al. [14], however, proposed that glucokinase displayed a higher affinity for  $\alpha$ - than  $\beta$ -D-glucose, without any anomeric difference in maximal velocity. Meglasson and Matschinsky [9] reported that the maximal velocity is 15–20% lower, but the affinity is twice as high for  $\alpha$ - than  $\beta$ -D-glucose. As a result of these two differences, the rate of glucose phosphorylation was somewhat higher with  $\alpha$ - than  $\beta$ -D-glucose in a restricted range of concentration between 2 and 11 mmol/l, with the opposite situation at higher glucose concentrations (40–80 mmol/l). Using both a non-radioisotopic and a radioisotopic procedure for the assay of glucokinase activity in rat liver homogenates, we observed that the apparent  $K_m$  may be slightly lower for  $\alpha$ - than  $\beta$ -D-glucose, but that the reaction velocity was always lower in the presence of the  $\alpha$ - rather than  $\beta$ -anomer, whatever the concentration of glucose (Fig. 1). Comparable results were obtained when the

glucokinase activity was examined in either pancreatic islet or insulin-producing tumoral cell homogenates [15].

Our findings with liver or islet cell glucokinase differ from those reported by Meglasson and Matschinsky [9] in one respect, namely the absence of a higher reaction velocity with  $\alpha$ -D-glucose at low concentrations of the hexose. Although the reason for these conflicting findings is not evident, we do not feel that glucokinase should be considered as an  $\alpha$ -stereospecific enzyme. Nevertheless, since the enzymatic data collected in different laboratories are conflicting, further experiments were designed to assess the possible role of glucokinase in the anomeric specificity of the metabolic and functional response in intact islet cells.

#### Lack of $\beta$ -stereospecificity of glucose metabolism in intact islets incubated at a high glucose concentration

As already mentioned, at high glucose concentrations (e.g. 40 mmol/l), the reaction catalyzed by glucokinase displays a higher velocity with  $\beta$ - than  $\alpha$ -D-glucose, as

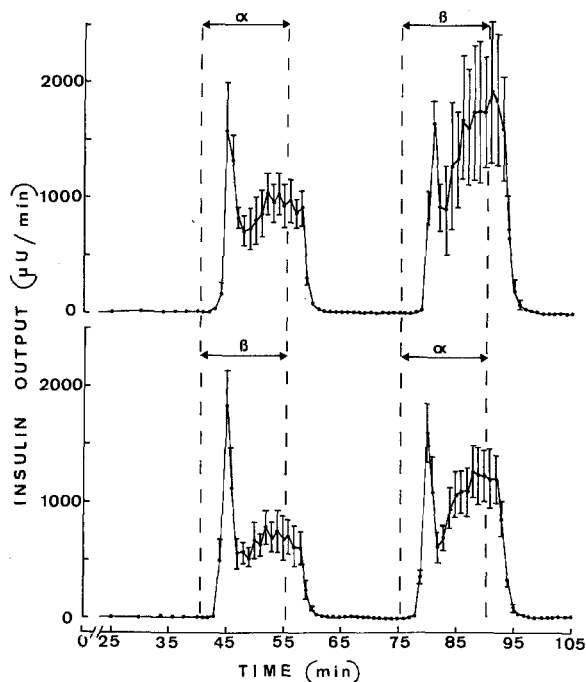


Fig. 2. Time course for insulin release from isolated perfused pancreases exposed for 15 min to either  $\alpha$ - or  $\beta$ -D-glucose (40 mmol/l) delivered from a side syringe maintained under ice. The dotted vertical lines indicate the period during which glucose was administered, no correction for dead space being introduced. Mean  $\pm$  SEM values refer to three experiments in each case

judged from the data reported by Meglasson and Matschinsky [9] and Sener et al. [15]. Therefore, the rate of glycolysis in islets exposed to an elevated concentration of D-glucose would be higher with  $\beta$ - than  $\alpha$ -D-glucose, if glucokinase were indeed to act as the key regulator of glucose metabolism in pancreatic islet cells. We therefore measured the phosphorylation of D-[U- $^{14}$ C]glucose in islet homogenates, as well as the metabolism of D-[5- $^3$ H]glucose and D-[U- $^{14}$ C]glucose, production of lactic acid, and net uptake of  $^{45}$ Ca in intact islets exposed at 7°C to 40 mmol/l  $\alpha$ - or  $\beta$ -D-glucose [16].

The rate of glucose phosphorylation by islet homogenates incubated at 7°C in the presence of D-glucose (40 mmol/l) was higher ( $p < 0.001$ ) in the case of  $\beta$ - than  $\alpha$ -D-[U- $^{14}$ C]glucose (Table 1). Even when glucose 6-phosphate was added to the reaction mixture in a high concentration (initial concentration of glucose 6-phosphate: 3.0 mmol/l) in order to inhibit the low- $K_m$  hexokinase, the rate of phosphorylation of  $\beta$ -D-[U- $^{14}$ C]glucose remained significantly higher than that of the corresponding  $\alpha$ -anomer ( $p < 0.05$ ). However, in intact islets no significant anomeric differences were observed for the various metabolic and functional variables. The mean values for either D-[5- $^3$ H]glucose utilization, lactate production and D-[U- $^{14}$ C]glucose oxidation tended, if anything, to be higher with  $\alpha$ - than  $\beta$ -D-glucose. Such was also the case for the net uptake of  $^{45}$ Ca. When insulin release was measured at 37°C in isolated perfused pancreases, the secretory response to  $\alpha$ -

D-glucose averaged  $103.1 \pm 15.0\%$  ( $n = 6$ ) of the paired response to  $\beta$ -D-glucose (Fig. 2). In the latter experiments,  $\alpha$ -D-glucose was given first in three cases,  $\beta$ -D-glucose being administered first in the three other cases. In all cases, the secretory rate was higher during the second than the first stimulation, with a paired ratio of  $141.8 \pm 13.5\%$  ( $p < 0.05$ ;  $n = 6$ ). This probably reflects, in part at least [17], a phenomenon of B-cell memory [18].

These results afford direct evidence against glucokinase being the B-cell stereospecific receptor. Thus, at the high glucose concentration (40 mmol/l), the rate of phosphorylation by islet homogenates was higher with  $\beta$ - than  $\alpha$ -D-glucose. In the absence of glucose 6-phosphate, this situation could reflect, in part at least, the participation of hexokinase, which displays a higher maximal velocity with  $\beta$ - than  $\alpha$ -D-glucose (see below). However, in the presence of glucose 6-phosphate, the residual rate of phosphorylation is mainly attributable to glucokinase and, nevertheless, remained higher with  $\beta$ - than  $\alpha$ -D-glucose. The latter finding confirms that the rate of  $\beta$ -D-glucose phosphorylation, as catalyzed by glucokinase, exceeds that of  $\alpha$ -D-glucose, at least at a high concentration of hexose. Despite such an anomeric difference, the metabolic and functional response of intact islets failed to be higher in the presence of  $\beta$ - than  $\alpha$ -D-glucose. On the contrary, for all metabolic and functional variables, the trend was, if anything, in favour of the  $\alpha$ -anomer. The present work indicates therefore that, at high glucose concentration (40 mmol/l), the rate of glycolysis in intact islets cells does not reflect the anomeric behaviour of glucokinase. This observation suggests, therefore, that regulatory sites distal to glucose phosphorylation participate in the metabolism of glucose anomers in islet cells.

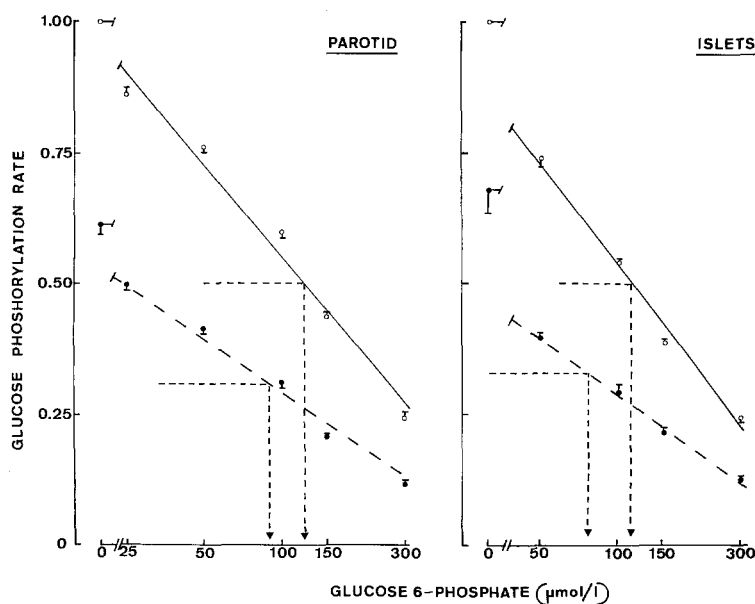
#### Anomeric specificity of hexokinase

In homogenates of rat islets or insulin-producing tumoral cells, hexokinase accounts for a major fraction of the total rate of glucose phosphorylation measured in the absence of exogenous glucose 6-phosphate [19]. Although hexokinase is largely inhibited in intact islet cells [20], we investigated whether hexokinase could participate in the anomeric specificity of glucose metabolism in intact islets. For such a purpose, we characterized the anomeric behaviour of hexokinase in islets and other tissues. The results are summarized in Table 2 and indicate that in all mammalian tissues so far investigated (even in liver, [15]), the maximal velocity is significantly higher with  $\beta$ - than  $\alpha$ -D-glucose, but the  $K_m$  value lower for  $\alpha$ - than  $\beta$ -D-glucose. This was also the case for the anomers of D-mannose, whether in pancreatic islet or parotid gland homogenates. Thus, in terms of both maximal velocity and affinity, anomeric behaviour of mammalian hexokinase represents a mirror image of that of yeast hexokinase [21–23]. This phylogenetic evolution is compatible with the view that anomeric speci-

**Table 2.** Anomeric specificity of hexokinase

| Tissue                          | Hexose    | $V\alpha/V\beta$<br>(ratio) | $K_m\alpha$       | $K_m\beta$       |
|---------------------------------|-----------|-----------------------------|-------------------|------------------|
|                                 |           |                             | $\mu\text{mol/l}$ |                  |
| Rat pancreatic islets           | D-glucose | $0.702 \pm 0.027$           | $30.5 \pm 1.7$    | $78.5 \pm 4.5$   |
| Rat pancreatic islets           | D-mannose | $0.514 \pm 0.067$           | $24.7 \pm 1.5$    | $62.9 \pm 0.5$   |
| Insulin-producing tumoral cells | D-glucose | $0.690 \pm 0.021$           | $18.0 \pm 2.3$    | $33.0 \pm 2.1$   |
| Rat parotid gland               | D-glucose | $0.673 \pm 0.029$           | $16.7 \pm 2.2$    | $34.5 \pm 4.8$   |
| Rat parotid gland               | D-mannose | $0.626 \pm 0.054$           | $28.2 \pm 1.8$    | $55.6 \pm 2.5$   |
| Rat erythrocytes                | D-glucose | $0.659 \pm 0.027$           | $20.4 \pm 1.4$    | $43.6 \pm 2.4$   |
| Lymphocytic leukemia cells      | D-glucose | $0.725 \pm 0.013$           | $38.0 \pm 1.1$    | $122.5 \pm 33.9$ |
| Breast cancer cells             | D-glucose | $0.815 \pm 0.004$           | $35.7 \pm 3.1$    | $88.6 \pm 2.0$   |

Mean values ( $\pm$ SEM) for the  $\alpha/\beta$  ratio in maximal velocity ( $V\alpha/V\beta$ ) and the  $K_m$  values for  $\alpha$ - and  $\beta$ -D-glucose or mannose ( $K_m\alpha$ ,  $K_m\beta$ ) are derived from measurements performed at 7 °C



**Fig. 3.** Effect of increasing concentrations of glucose 6-phosphate (logarithmic scale), in anomeric equilibrium, upon the phosphorylation of  $\alpha$ -D-[U- $^{14}$ C]glucose ( $\bullet$ — $\bullet$ ) and  $\beta$ -D-[U- $^{14}$ C]glucose ( $\circ$ — $\circ$ ) both used at an 0.5 mmol/l concentration, in parotid gland and pancreatic islet homogenates incubated at 7 °C. All results are expressed relative to the mean control value found within the same experiments in the presence of  $\beta$ -D-[U- $^{14}$ C]glucose and absence of glucose 6-phosphate. Such a reference value averaged  $0.56 \pm 0.03$  pmol/min per  $\mu\text{g}$  parotid (wet weight) and  $0.74 \pm 0.04$  pmol/min per islet. Mean ( $\pm$ SEM) values refer to three to eight individual determinations. Also shown are the concentrations of glucose 6-phosphate yielding a 50% decrease in reaction velocity as judged from the regression lines

ficity of enzymes reflects adaptation to a defined chemical environment, itself dictated by thermodynamic constraints [8, 24].

Since glucose 6-phosphate acts as a major inhibitor of hexokinase in pancreatic islets, we investigated also whether glucose 6-phosphate, in anomeric equilibrium, affects preferentially  $\alpha$ - or  $\beta$ -D-[U- $^{14}$ C]glucose phosphorylation in islet or parotid homogenates. The apparent  $K_i$  for glucose 6-phosphate was lower in the presence of  $\alpha$ - than  $\beta$ -D-glucose (Fig. 3). Thus, even in respect to inhibition by glucose 6-phosphate, the rate of glucose phosphorylation by hexokinase would be expected to be higher with  $\beta$ - than  $\alpha$ -D-glucose.

At this point, it should be noted that, in two independent reports it was observed that the concentration of glucose 6-phosphate in intact islets was higher in the presence of  $\beta$ - than  $\alpha$ -D-glucose when the hexose was used at concentrations in the range of 6.0–7.2 mmol [6, 25]. This finding, already reported 10 years ago, clearly argues against the view that the alleged  $\alpha$ -stereospecificity of glucokinase would represent the major determinant of the anomeric difference in the rate of glycolysis

in intact islet cells. It may help to explain, however, that the anomeric preference of hexokinase for  $\beta$ -D-glucose is masked in intact cells [26] as a result of a higher glucose-6-phosphate content in cells exposed to  $\beta$ - as distinct from  $\alpha$ -D-glucose.

#### $\alpha$ -Stereospecificity of metabolism in intact islets exposed to a low concentration of glucose

The fact that hexokinase displays a higher maximal velocity with  $\beta$ - than  $\alpha$ -D-glucose led us to scrutinize the anomeric stereospecificity of glucose metabolism in intact islets exposed to a low concentration (3.3 mmol/l) of glucose anomers [27]. This low concentration was selected to prevent or, at least, minimize the contribution of glucokinase to the phosphorylation of glucose. The results of this study are summarized in Table 3.

In the islet homogenates, the rate of D-[U- $^{14}$ C]glucose or D-[U- $^{12}$ C]glucose phosphorylation was higher with  $\beta$ - than  $\alpha$ -D-glucose. Even in the presence of a very high concentration of glucose 6-phosphate (initial

**Table 3.** Metabolic and functional variables in islet homogenates or intact islets incubated at 7 °C in the absence or presence of  $\alpha$ - or  $\beta$ -D-glucose (3.3 mmol/l)

| Metabolic variable  | No glucose       | $\alpha$ -D-glucose | $\beta$ -D-glucose |
|---|------------------|---------------------|--------------------|
| <i>D</i> -[ <sup>12</sup> C]glucose phosphorylation (pmol/60 min per islet) |                  |                     |                    |
| No glucose 6-phosphate  |                  | 22.0 ± 1.0 (3)      | 31.2 ± 1.4 (3)     |
| Glucose 6-phosphate (0.02 mmol/l)   |                  | 14.5 ± 1.2 (3)      | 22.5 ± 1.9 (3)     |
| <i>D</i> -[ <sup>14</sup> C]glucose phosphorylation (pmol/60 min per islet) |                  |                     |                    |
| No glucose 6-phosphate  |                  | 28.5 ± 1.2 (3)      | 37.8 ± 0.6 (3)     |
| Glucose 6-phosphate (3.0 mmol/l)  |                  | 2.3 ± 0.4 (3)       | 2.7 ± 0.2 (3)      |
| Lactic acid production (pmol/60 min per islet)                              |                  |                     |                    |
|   | 3.86 ± 0.98 (16) | 12.20 ± 1.01 (21)   | 7.77 ± 0.73 (22)   |
| <i>D</i> -[ <sup>14</sup> C]glucose oxidation (fmol/60 min per islet)       |                  |                     |                    |
|   |                  | 146 ± 11 (28)       | 96 ± 8 (30)        |
| <sup>45</sup> Ca net uptake (fmol/60 min per islet)                         |                  |                     |                    |
|   | 290 ± 12 (22)    | 401 ± 24 (24)       | 318 ± 18 (26)      |

Mean values ( $\pm$  SEM) are shown together with the number of individual observations (in parentheses)

**Table 4.** Metabolism of  $\alpha$ - and  $\beta$ -D-glucose (5.6 mmol/l) in islets incubated at 7 °C

| Metabolic variables   | $\alpha$ -D-glucose | $\beta$ -D-glucose  |
|---|---------------------|---------------------|
| <i>D</i> -[ <sup>5-3</sup> H]glucose utilization (pmol/60 min per islet)                        | 7.03 ± 0.59 (21)    | 4.12 ± 0.36 (21)    |
| <i>D</i> -[ <sup>14</sup> C]glucose oxidation (fmol/60 min per islet)                           | 289.7 ± 22.9 (21)   | 350.9 ± 21.9 (19)   |
| <i>D</i> -[ <sup>6-14</sup> C]glucose oxidation (fmol/60 min per islet)                         | 94.3 ± 12.3 (29)    | 47.9 ± 6.3 (29)     |
| <i>D</i> -[ <sup>14</sup> C]glucose/<br><i>D</i> -[ <sup>6-14</sup> C]glucose oxidation (ratio) | 3.07 ± 0.47 (21/29) | 7.32 ± 1.07 (19/29) |

Mean values ( $\pm$  SEM) are shown together with the number of individual observations (in parentheses)

concentration of glucose-6-phosphate: 3.0 mmol/l), no inversion of the  $\alpha$ -stereospecific anomeric preference could be detected. Yet, in intact islets the rate of glycolysis was much higher with  $\alpha$ - than  $\beta$ -D-glucose, as judged from either the glucose-induced increment in lactate production or oxidation of *D*-[<sup>14</sup>C]-glucose. Likewise, the net uptake of <sup>45</sup>Ca was significantly higher in islets incubated at 7 °C with  $\alpha$ - than  $\beta$ -D-glucose, and this coincided with a higher secretory response in isolated perfused pancreas exposed at 37 °C to the same low concentration (3.3 mmol/l) of  $\alpha$ - as distinct from  $\beta$ -D-glucose (Fig. 3). Incidentally, in order to characterize the secretory response to this low concentration of glucose, the experiments were conducted in the presence of L-leucine (10 mmol/l) administered throughout the perfusion. After correction for the mean control output found within each experiment just prior to and immediately after stimulation by D-glucose, the glucose-induced increment in insulin secretion was much higher

( $p < 0.001$ ) in response to the  $\alpha$ -anomer (+37.8 ± 4.9  $\mu$ U/min) than in response to the  $\beta$ -anomer (+14.1 ± 2.2  $\mu$ U/min). Comparable results were obtained when the secretory response to the two anomers was tested in pancreases perfused in the absence of Ca<sup>2+</sup>, but in the presence of both Ba<sup>2+</sup> (2 mmol/l) and theophylline (1.4 mmol/l).

These findings clearly indicate that the metabolic, ionic and secretory responses of islet cells display  $\alpha$ -anomeric specificity even when glucose is used at a low concentration (3.3 mmol/l) selected to minimize the contribution of glucokinase to hexose phosphorylation. Thus, from prior observations [19, 28, 29], it was calculated that the contribution of glucokinase to the overall rate of glucose phosphorylation by islet homogenates did not exceed 3.1–7.9% at the low glucose concentration (3.3 mmol/l).

The situation found in the islets exposed to a low concentration of  $\alpha$ - or  $\beta$ -D-glucose is reminiscent of that recently characterized in rat erythrocytes [26]. Indeed, in erythrocytes the phosphorylation of D-glucose is catalyzed solely by a hexokinase which, like in other murine tissues, displays a higher maximal velocity with  $\beta$ - than  $\alpha$ -D-glucose (Table 2). Yet, the glucose-induced increment in lactic acid output is higher in erythrocytes exposed to  $\alpha$ - than  $\beta$ -D-glucose [26]. Thus, the presence or participation of glucokinase is not a prerequisite for the  $\alpha$ -stereospecificity of glycolysis. Instead, data obtained in either islets exposed to a low glucose concentration or erythrocytes point to a key role for  $\alpha$ -stereospecific enzymes, such as phosphoglucose isomerase and phosphoglucomutase, in the anomeric specificity of glycolysis, as reviewed elsewhere [8].

#### Preferential utilization of $\beta$ -D-glucose 6-phosphate in the pentose cycle

The observations so far reviewed all point to the existence of regulatory sites distal to glucose phosphorylation in the control of D-glucose metabolism in islet

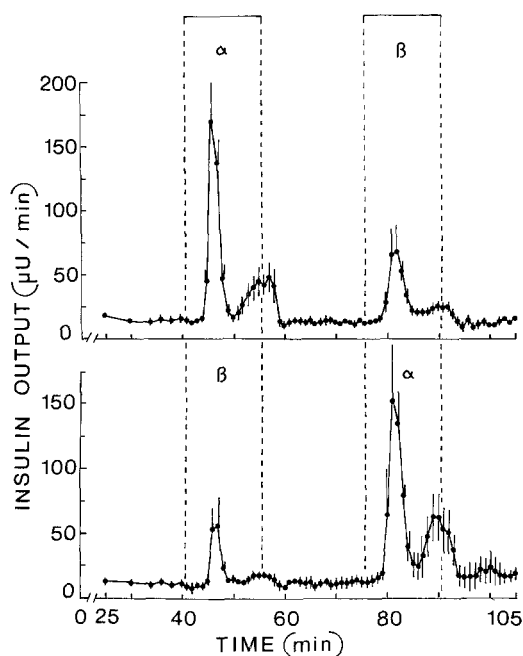


Fig. 4. Insulin output from isolated perfused pancreases exposed throughout perfusion to L-leucine (10 mmol/l). During the period indicated by the vertical dotted lines, either  $\alpha$ - or  $\beta$ -D-glucose (3.3 mmol/l) was administered from a side syringe maintained under ice, no correction being introduced for the dead space of the perfusion system. Mean ( $\pm$  SEM) values refer to three experiments in each case

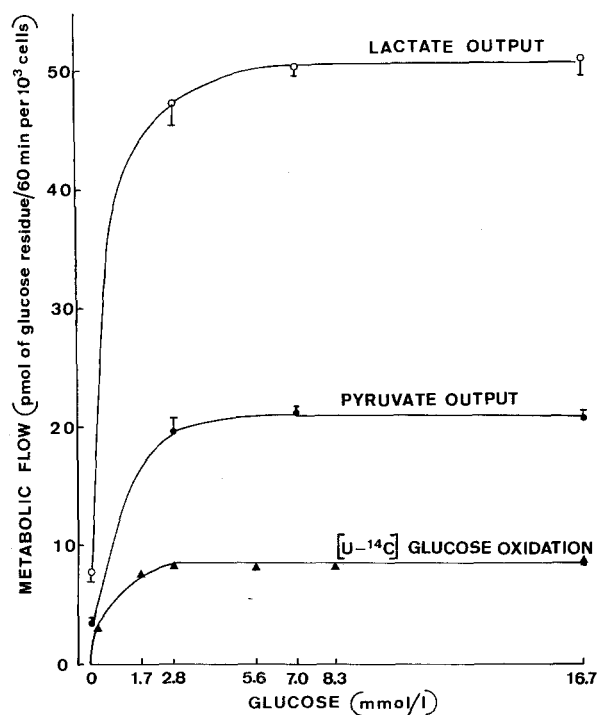


Fig. 5. Output of lactic and pyruvic acids and oxidation of D-[U- $^{14}$ C]glucose in RINm5F cells incubated at increasing concentrations of D-glucose. Mean values ( $\pm$  SEM, whenever it exceeds the size of the mean symbol) refer to 28–44 (lactate output), 16–32 (pyruvate output) and 8–28 (glucose oxidation) individual measurements

cells. For instance, we have documented previously the  $\alpha$ -stereospecificity of phosphoglucose isomerase in islet homogenates [6] and underlined its relevance to the anomeric difference in the rate of glycolysis [8]. At variance with phosphoglucose isomerase, the first enzyme in the pentose cycle, namely glucose-6-phosphate dehydrogenase, was shown to represent a  $\beta$ -stereospecific enzyme in islet homogenates [6]. If, as postulated by Matschinsky and his colleagues, glucokinase were to be responsible for the anomeric specificity of glucose metabolism in pancreatic islets and were to display preference for  $\alpha$ -D-glucose at close-to-physiological glucose concentrations, all metabolic fluxes beyond glucose 6-phosphate generation would be expected to be higher in islets exposed to  $\alpha$ - rather than  $\beta$ -D-glucose. These considerations led us to measure the flow rate through the pentose cycle in islets exposed at 7°C to the anomers of D-glucose at a concentration of 5.6 mmol/l. In doing so, we took advantage of the isolation of purified  $\alpha$ - and  $\beta$ -D-glucose labelled with  $^{14}$ C in positions 1 and 6, respectively.

The rate of  $\beta$ -D-[1- $^{14}$ C]glucose oxidation was slightly but not significantly higher than that of  $\alpha$ -D-[1- $^{14}$ C]glucose oxidation ( $p < 0.07$ ; Table 4). However, the  $\alpha$ -anomer of D-[6- $^{14}$ C]glucose was oxidized at a higher rate than the corresponding  $\beta$ -anomer ( $p < 0.005$ ). The overall rate of glucose utilization, as judged from the production of  $^3\text{H}_2\text{O}$  by islets exposed to D-[5- $^3\text{H}$ ]glucose, was also higher in presence of the  $\alpha$ - than  $\beta$ -anomer ( $p < 0.001$ ). The oxidation of [1- $^{14}$ C]glucose largely exceeded that of [6- $^{14}$ C]glucose, whether in the presence of  $\alpha$ - or  $\beta$ -D-glucose. The ratio of D-[1- $^{14}$ C]glucose/D-[6- $^{14}$ C]glucose oxidation was much lower in the case of  $\alpha$ - than  $\beta$ -D-glucose ( $p < 0.001$ ). Likewise, when the fraction of the total metabolism of glucose that occurs by the pentose cycle pathway was calculated according to Katz and Wood [30], the  $\beta/\alpha$  ratio amounted to 2.73. Even when corrected for glucose utilization, the flow rate through the pentose pathway remained higher with  $\beta$ - than  $\alpha$ -D-glucose with a  $\beta/\alpha$  ratio close to 1.60. Incidentally, at the same glucose concentration and at the same temperature,  $\alpha$ -D-glucose stimulated more than  $\beta$ -D-glucose the net uptake of  $^{45}\text{Ca}$  ( $p < 0.005$ ), which averaged  $727 \pm 45$  and  $549 \pm 39$  fmol/islet at the 60th min of incubation ( $n = 22$  in both cases).

These metabolic findings have several implications. First, they confirm that  $\alpha$ -D-glucose is metabolized more efficiently than  $\beta$ -D-glucose in islet cells exposed to a close-to-physiological concentration of the hexose. Second, since  $^{45}\text{Ca}$  net uptake and insulin release are higher in islets exposed to  $\alpha$ - than  $\beta$ -D-glucose at these glucose concentrations [6], the present results suggest that the generation of NADPH in the pentose cycle pathway does not represent a major or, at least, the sole determinant of the functional response of islet cells to glucose. Third, our data provide the first demonstration that, in intact cells,  $\beta$ -D-glucose 6-phosphate is utilized

preferentially to  $\alpha$ -D-glucose 6-phosphate in the pentose cycle, as expected from the anomeric specificity of glucose 6-phosphate dehydrogenase. Our data demonstrate, therefore, the existence of an anomerically specific regulatory site distal to glucose 6-phosphate generation. This implies that the rate of anomerization of glucose 6-phosphate is not sufficiently rapid to impede expression of the anomeric specificity of enzymes involved in the catabolism of this hexose phosphate. In other words, the present findings again illustrate that the metabolism of glucose anomers in islet cells is not regulated solely at the glucokinase level.

### Physiological and pathological dissociation between glucokinase activity and glycolysis in islet cells

The evidence so far reviewed convincingly indicates that glucokinase is not the enzyme responsible for the  $\alpha$ -stereospecificity of glycolysis in islet cells. There are other arguments, however, to deny the alleged role of glucokinase as the B-cell glucoreceptor. Thus, there are at least two situations characterized by a dissociation between changes in glucokinase activity and glycolytic rate, respectively, in islet cells. The first situation refers to the influence of fasting upon islet function, whilst the second concerns the metabolism of glucose in tumoral insulin-producing cells.

In the first of these instances, it was shown that the rate of glycolysis in islets removed from fasted rats is much more severely impaired [31] than expected from the decrease in glucokinase activity [19, 32]. This situation coincided with a failure of glucose to increase the glucose 1,6-bisphosphate content of islets removed from fasted rats [31]. The data were interpreted, therefore, in support of the view that phosphofructokinase needs to be activated by suitable hexose bisphosphates, in order for the rate of phosphorylation of fructose 6-phosphate to keep pace with its generation rate [33]. In the same perspective, we have recently indicated that the glucose-induced changes in the content of fructose 2,6-bisphosphate in intact islets or purified B cells are sufficiently marked and sufficiently rapid to indeed participate in the control of phosphofructokinase activity in response to an increase in glucose concentration [34].

In the second instance, namely in insulin-producing tumoral cells, we were unable to detect any gross difference in the relative activities of hexokinase and glucokinase when compared with the situation found in normal islets cells [35]. This is in agreement with data obtained by Meglasson and Matschinsky [36], but at variance with those reported by Halban et al. [37]. Despite the similarity in the pattern of glucose phosphorylation found at increasing concentrations of glucose in tumoral cell and pancreatic islet homogenates, respectively, several anomalies of glucose metabolism were present in the RINm5F cells. For instance, the rate of glucose

metabolism reached a close-to-maximal value at a glucose concentration of 2.8 mmol/l (Fig. 5). Moreover, the rate of glucose oxidation relative to that of glucose utilization was much lower in the tumoral cells than in normal pancreatic islets [38]. Lastly, the lactate/pyruvate ratio was lower in the tumoral cells than that measured in pancreatic islets, whether at low or high glucose concentration [31]. The latter two anomalies clearly indicate that the mitochondrial oxidation of pyruvate and its cytosolic conversion to lactate represent regulatory sites in glucose metabolism susceptible to perturbation in pathological situations, independently of any change in glucokinase activity.

### Conclusion

The anomeric specificity of hexose utilization in pancreatic islets, and the perturbation of glucose catabolism in either islets removed from fasted rats or tumoral insulin-producing cells illustrate the relevance of regulatory steps distal to glucose phosphorylation in the control of glucose metabolism in islet cells. From these converging observations and when considering the original definition given to the B-cell glucoreceptor [3], we conclude that it represents not solely an unfair verbal pirouette but also, and more importantly, a misleading concept to equate glucokinase with such a receptor.

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