

Is Glucokinase Responsible for the Anomeric Specificity of Glycolysis in Pancreatic Islets?*

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At a low concentration of D-glucose (3.3 mM), the phosphorylation rate of this hexose in rat pancreatic islet homogenates incubated at 8 °C is higher with the β - than with the α -anomer, as expected from the anomeric specificity of hexokinase. In the presence of a high concentration of glucose 6-phosphate (3.0 mM), which inhibits hexokinase but not glucokinase, the phosphorylation rates of the two anomers are not significantly different from one another. Nevertheless, in intact islets exposed at 8 °C to the same low concentration of D-glucose, the α -anomer augments, more than the β -anomer, the production of lactic acid and net uptake of ^{45}Ca . At the same concentration (3.3 mM), the α -anomer is also more potent than the β -anomer in enhancing insulin release from perfused pancreases stimulated at 37 °C by L-leucine or by the combination of Ba^{2+} and theophylline. It is concluded that the participation of glucokinase is not essential for the anomeric specificity of glycolysis and insulin release in rat pancreatic islets.

The observation that the higher insulinotropic capacity of the α -anomer, as distinct from the β -anomer, of either D-glucose (1, 2) or D-mannose (3) coincides with a higher rate of glycolysis in rat pancreatic islets exposed to the α -anomer of these hexoses (4, 5) provided crucial support to the fuel concept for insulin release (6, 7). The anomeric preference of glycolysis was first attributed to the α -stereospecificity of both phosphoglucose isomerase (4) and phosphoglucomutase (5, 8), as reviewed elsewhere (9). Alternatively, it was recently proposed that the higher rate of glycolysis found in the islets exposed to α -D-glucose (or α -D-mannose) is attributable to a limited anomeric preference of glucokinase (10-12). There are several reasons to question the validity of the latter concept. For instance, the alleged preference of glucokinase for the α -anomer of D-glucose can hardly account for the lower content of glucose 6-phosphate in islets exposed to α - as distinct from β -D-glucose (4, 13). Moreover, α -D-glucose augments more than β -D-glucose the output of lactic acid from rat erythrocytes in which D-glucose is phosphorylated solely by the intervention of a hexokinase-like enzyme with anomeric preference for β -D-glucose (14). In pancreatic islet homogenates, the rate of glucose phosphorylation, as catalyzed by the hexokinase-like enzyme, is also higher with β - than with α -D-glucose (15).

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The latter findings led us to investigate whether the glycolytic and secretory responses of pancreatic islets would not display α -stereospecificity even when the anomers of D-glucose are used at a low concentration in order to minimize the participation of glucokinase in the phosphorylation of glucose. The results of this study indicate that, at a low concentration (3.3 mM), α -D-glucose indeed increases, more than β -D-glucose, lactic acid production, ^{45}Ca net uptake, and insulin release in rat pancreatic islets.

MATERIALS AND METHODS

Glucose Anomers—Unlabeled α - and β -D-glucose were purchased from Sigma. Crystalline α - and β -D-[U- ^{14}C]glucose were prepared from D-[U- ^{14}C]glucose (3.9 mCi/mmol; New England Nuclear) by a procedure adapted from that described by Miwa *et al.* (16). Briefly, 15 mg of D-[U- ^{14}C]glucose was dissolved in 50 μl of 80% (v/v) acetic acid, heated in boiling water for about 5 min to obtain a clear solution, and cooled at room temperature. To this solution, 15 mg of unlabeled α -D-glucose was added as a seed for crystallization. After 72 h at 4 °C and removal of the remaining solution, the crystals of α -D-[U- ^{14}C]glucose were washed in ethanol and then ether and eventually dried under vacuum at 50 °C for a few hours. Likewise, 15 mg of D-[U- ^{14}C]glucose was dissolved in 150 μl of anhydrous pyridine, heated in boiling water for about 10 min, and cooled at room temperature. About 100 μl of the solvent was evaporated under N_2 flow. Then 15 mg of unlabeled β -D-glucose was added to the remaining solution as a seed for crystallization. After 72 h at 4 °C, the crystals of β -D-[U- ^{14}C]glucose were treated as described above. The anomeric purity of these preparations was assessed by the β -D-glucose oxidase method (17) in a polarographic oxygen analyzer (Model Oxy-5; Gilson Medical Electronics, Middleton, WI) and found identical to that of the pure labeled anomers. Over 60 min of incubation at 8 °C, virtually no change in anomeric purity was observed.

Phosphorylation of Glucose Anomers—Two procedures were used to measure the phosphorylation of glucose anomers in islet homogenates. In the nonradioisotopic procedure, 800 pancreatic islets isolated from fed albino rats were sonicated (3×5 s) in 0.3 ml of a Hepes¹-NaOH buffer (50 mM, pH 7.4) containing 150 mM KCl, 5 mM MgCl_2 , 1 mM EDTA, 2 mM cysteine, and 0.02% (w/v) bovine albumin. Aliquots (10 μl) of this homogenate were mixed with 20 μl of a reaction mixture to yield the following concentrations: 50 mM Hepes-NaOH buffer (pH 7.4), 150 mM KCl, 5 mM MgCl_2 , 5 mM NaF, 1 mM cysteine, 0.5 mM EDTA, and, as required, freshly dissolved 3.3 mM α - or β -D-glucose, 5.0 mM ATP, and/or 0.02 mM glucose 6-phosphate. After 60 min of incubation at 8 °C, the reaction was halted by heating (5 min at 80 °C), and the incubation medium was mixed with 1.2 ml of a Tris-HCl buffer (100 mM, pH 8.1) containing 0.1 mM dithiothreitol, 0.1 mM NADP^+ , 50 milliunits/ml phosphoglucose isomerase (EC 5.3.1.9), and 25 milliunits/ml glucose-6-phosphate dehydrogenase (EC 1.1.1.49). The NADPH formed over 60 min of incubation at 30 °C was measured by direct spectrofluorometry. Standards (1-6 nmol/sample) of glucose 6-phosphate and fructose 6-phosphate were treated in the same manner and yielded identical, linear dose-response relationships. Glucose failed to affect the blank value, which was otherwise measured in the presence of tissue homogenate at the

¹ The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

same concentration of ATP and glucose 6-phosphate, but in the absence of D-glucose. In the isotopic procedure, 800 islets were sonicated in 0.4 ml of the same Hepes-NaOH buffer (see above). Aliquots (15 μ l) of this homogenate were mixed with 25 μ l of a reaction mixture to yield the same final concentrations as above, except for the use of α - or β -D-[U- 14 C]glucose (3.3 mM) and a higher concentration (3.0 mM) of glucose 6-phosphate. After 60 min of incubation at 8 $^{\circ}$ C, the hexose phosphates were separated by anion-exchange chromatography as described elsewhere (18). The blank values were measured in the absence of islet homogenate. In the absence of ATP, but in the presence of the islet homogenate and the labeled anomers, glucose 6-phosphate failed to provoke the appearance of labeled hexose phosphate.

Lactic Acid Production—For measuring lactic acid production, groups of 40 islets each were incubated for 60 min at 8 $^{\circ}$ C in 40 μ l of a Krebs-Ringer phosphate buffer (19) equilibrated against ambient air and containing, as required, freshly dissolved α - or β -D-glucose. After addition of HCl (10 μ l, 100 mM), the tubes containing the islets and medium were placed in liquid N₂ and the islets were disrupted by mechanical vibration (20). After 10 min of heating at 70 $^{\circ}$ C and 1 min of centrifugation at 5000 \times g, aliquots (40 μ l) of the supernatant were examined for their lactic acid content (21).

45 Ca Net Uptake—The net uptake of 45 Ca by the islets was measured as described elsewhere (22), except that the islets were incubated for 60 min at 8 $^{\circ}$ C in a Krebs-Ringer phosphate buffer (19) containing 45 CaCl₂.

Insulin Release—The release of insulin from intact islets incubated for 60 min at 37 $^{\circ}$ C was measured as described elsewhere (23). The method used for measuring insulin release from perfused rat pancreases was also previously described (24). The perfusate was delivered at a flow rate of 2.0 ml/min and consisted of a Krebs-Ringer buffer containing dextran and albumin (24). When Ba²⁺ was used as the secretagogue, a slightly different buffer was used (25) to prevent the precipitation of barium salts. The anomers of D-glucose were dissolved 15 min prior to administration in an iced solution of NaCl (155 mM) placed in a syringe kept on ice and administered at a flow rate of 0.1 ml/min for two periods of 15 min each. In half of the experiments, the administration of α -D-glucose preceded that of β -D-glucose, the reverse order being followed in the other experiments. No increase in insulin release was observed up to the fourth min after introduction of the anomers, the secretory response being computed from this time onwards.

Presentation of Results—All results are expressed as the mean (\pm S.E.) together with the number of individual observations (*n*) and statistical significance of differences, as assessed by Student's *t* test.

RESULTS

Glucose Phosphorylation—At a hexose concentration of 3.3 mM, the rate of glucose phosphorylation by islet homogenates, as measured by a nonradioisotopic procedure, was higher with β - than with α -D-glucose (Table I). Thus, in the absence of glucose 6-phosphate, the α/β ratio in phosphorylation rate averaged 0.705 \pm 0.032 ($p < 0.02$). Glucose 6-phosphate (initial concentration, 0.02 mM) decreased α - and β -D-glucose phosphorylation to 66.1 \pm 5.6 and 72.1 \pm 6.1%, respectively ($p < 0.05$ in both cases), of the paired control value. In the presence of glucose 6-phosphate, the α/β ratio in phosphorylation rate

remained lower than unity, averaging 0.650 \pm 0.042 ($p < 0.02$). In this first series of experiments, there was no indication that endogenous glucose 6-phosphate, which causes a physiological inhibition of hexokinase in the islets (18), would mask in intact islet cells the anomeric difference in glucose phosphorylation as here observed in islet homogenates. In order to further explore this issue, the phosphorylation of glucose was measured by a radioisotopic procedure, allowing the use of a much higher concentration of glucose 6-phosphate. In the absence of glucose 6-phosphate, β -D-[U- 14 C]glucose was again phosphorylated at a higher rate than α -D-[U- 14 C]glucose. The α/β ratio in phosphorylation rate averaged 0.754 \pm 0.033, a value not significantly different ($p > 0.2$) from that found with the nonradioisotopic procedure. Glucose 6-phosphate (initial concentration, 3.0 mM) severely decreased the phosphorylation of the two anomers. In the presence of this high concentration of glucose 6-phosphate, the mean rates of phosphorylation of the two anomers were no longer significantly different from one another ($p > 0.4$). Nevertheless, the data summarized in Table I clearly indicate that the rate of α -D-glucose phosphorylation was not higher than that of the corresponding β -anomer.

Lactic Acid Production—Over a 60-min incubation period at 8 $^{\circ}$ C in the presence of D-glucose (3.3 mM), the production of lactic acid was much lower ($P < 0.001$) in the presence of β -D-glucose (7.77 \pm 0.73 pmol/60 min/islet; $n = 22$) than in the presence of α -D-glucose (12.20 \pm 1.01 pmol/60 min/islet; $n = 21$). After correction for the basal value (3.86 \pm 0.98 pmol/60 min/islet; $n = 16$), the glucose-induced increment in lactic acid production in the presence of the β -anomer averaged no more than 49.6 \pm 11.3% ($n = 22$) of the mean corresponding value found in the same experiments with the α -anomer (100.0 \pm 14.6%, $n = 21$).

45 Ca Net Uptake—The higher rate of glycolysis in islets exposed to the α -anomer of D-glucose coincided with a higher net uptake of 45 Ca. Thus, the net uptake of 45 Ca averaged 401 \pm 24 and 318 \pm 18 fmol/islet ($n = 24$ –26; $P < 0.01$) after 60 min of incubation at 8 $^{\circ}$ C in the presence of α - and β -D-glucose (3.3 mM), respectively. The basal value for 45 Ca uptake amounted to 290 \pm 12 fmol/islet ($n = 22$) and, hence, α -D-glucose significantly augmented 45 Ca net uptake, whereas β -D-glucose failed to do so. Comparable results were obtained in the presence of L-leucine (10.0 mM), which itself augmented 45 Ca net uptake to 516 \pm 13 fmol/islet ($n = 37$). Incidentally, the finding that D-glucose or L-leucine stimulates 45 Ca uptake by islets incubated at 8 $^{\circ}$ C is not surprising since cooling suppresses the secretory but not the cationic response to D-glucose or other secretagogues (25). Moreover, the relationship between 45 Ca uptake at 8 $^{\circ}$ C and insulin release at 37 $^{\circ}$ C (see below) was qualitatively comparable to that previously established when the two variables were measured at 37 $^{\circ}$ C. In both cases, the net uptake of 45 Ca has to exceed a threshold value in order for insulin to be stimulated above basal value (22).

Insulin Release—The secretory response to the low concentration of glucose anomers was tested in isolated perfused rat pancreases. Pilot experiments performed with pancreatic islets incubated for 60 min at 37 $^{\circ}$ C indicated that 3.3 mM D-glucose (in anomeric equilibrium) failed to stimulate insulin release in the absence of another secretagogue but augmented insulin output in the presence of L-leucine (10.0 mM). Thus, under these conditions, the secretory rate averaged 11.6 \pm 2.3 ($n = 16$), 13.6 \pm 1.2 ($n = 44$), 35.8 \pm 2.7 ($n = 44$), and 51.2 \pm 3.4 ($n = 44$) microunits/60 min/islet, respectively, in the absence of exogenous nutrient, the presence of D-glucose (3.3 mM), the presence of L-leucine (10.0 mM), and the presence

TABLE I

Phosphorylation of glucose anomers (3.3 mM) in islet homogenates

Mean values (\pm S.E.) refer to three individual observations (each derived from quadruplicate measurements) and are expressed as per cent of the value found in each experiment for the phosphorylation of β -D-glucose in the absence of glucose 6-phosphate. Such a reference value averaged 34.5 \pm 5.0 pmol/60 min/islet ($n = 6$).

Method	Glucose 6-phosphate mM	Phosphorylation rate	
		α -D-Glucose	β -D-Glucose
		%	
Nonisotopic		70.5 \pm 3.2 (3)	100.0 \pm 4.5 (3)
	0.02	46.5 \pm 3.8 (3)	72.1 \pm 6.1 (3)
Radioactive		75.4 \pm 3.3 (3)	100.0 \pm 1.7 (3)
	3.00	6.0 \pm 1.1 (3)	7.1 \pm 0.6 (3)

of both L-leucine and D-glucose. Likewise, we have previously shown that D-glucose at a concentration of 3.3 mM augments insulin release evoked, in the absence of extracellular Ca^{2+} , by the combination of Ba^{2+} and theophylline (26). Therefore, the secretory response to the anomers of D-glucose was tested in the presence of L-leucine at normal Ca^{2+} concentration (1.0 mM) or in the presence of both Ba^{2+} (2.0 mM) and theophylline (1.4 mM) in the absence of Ca^{2+} .

The results obtained in the presence of L-leucine are illustrated in Fig. 1. As computed between the 44th to 59th and 79th to 94th min of perfusion, the secretory rate averaged 54.6 ± 8.0 and 25.8 ± 3.2 microunits/min in response to α - and β -D-glucose, respectively ($n = 6$ in both cases; $p < 0.01$). After correction for the mean control output found within the same experiment just prior to and immediately after stimulation by D-glucose, the glucose-induced increment in secretory rate was more than twice higher ($p < 0.01$) in response to the α -anomer ($+37.8 \pm 4.9$ microunits/min) than in response to the β -anomer ($+14.1 \pm 2.2$ microunits/min).

Comparable results were obtained when the anomers of D-glucose, always used at a concentration of 3.3 mM, were administered during stimulation of the pancreas by the combination of Ba^{2+} and theophylline in the absence of Ca^{2+} . In this case however, the secretory rate progressively decreased during exposure to these non-nutrient secretagogues, in good agreement with a prior observation (27). Thus, relative to the secretory rate reached just prior to the first administration of D-glucose (*i.e.* 1.36 ± 0.42 milliunits/min, $n = 6$), the output of insulin progressively decreased to 46.3 ± 7.7 , 30.8 ± 4.9 , and $23.4 \pm 3.0\%$, respectively, 23, 30, and 53 min later ($n = 6$

in each case). The anomers of glucose were delivered in a random order over two periods of 15 min each separated by an interval of 15 min. As illustrated in Fig. 2, relative to the output of insulin measured in each experiment just prior to stimulation by D-glucose, the secretory response to this hexose was much higher in the case of the α - than β -anomer. Likewise, if expressed relative to the mean insulin output observed prior to and 30 min after initiation of the secretory response to each anomer, the glucose-induced increment in hormone release was twice higher ($p < 0.01$) with α -D-glucose ($+139.7 \pm 14.6\%$) than with β -D-glucose ($+71.2 \pm 6.6\%$), with a paired α/β ratio averaging (geometric mean) 1.95 ± 0.30 ($n = 6$; $p < 0.01$).

DISCUSSION

The present results unambiguously indicate that, even when used at a low concentration of 3.3 mM, α -D-glucose is a more potent insulin secretagogue than β -D-glucose. In order to document the secretory response to this low concentration of hexose, the experiments were conducted either in the presence of L-leucine at normal Ca^{2+} concentration (1.0 mM) or in the presence of both Ba^{2+} and theophylline but in the absence of Ca^{2+} . The higher rate of insulin release coincided with a higher rate of lactic acid output and a higher net uptake of ^{45}Ca by isolated islets incubated at 8°C . These findings are in good agreement with the concept that the metabolic, cationic, and secretory responses of the islets to nutrient secretagogues are arranged in a cause-to-effect sequence in the process of stimulus-secretion coupling (6).

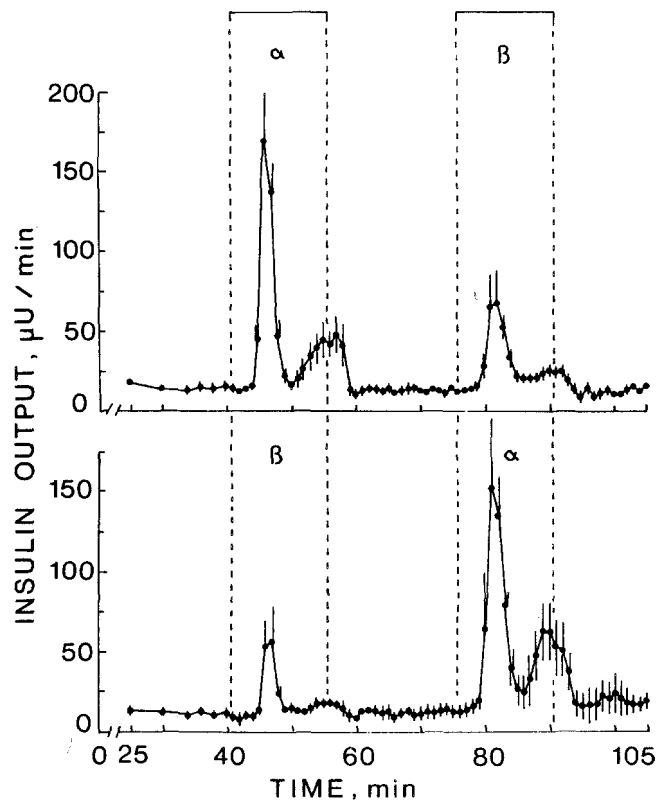


FIG. 1. Insulin output from isolated perfused pancreases exposed throughout perfusion to L-leucine (10.0 mM). During the period indicated by the vertical broken lines, either α - or β -D-glucose (3.3 mM) was administered from a side syringe maintained under ice, no correction being introduced for the dead space of the perfusion system. Mean values (\pm S.E.) refer to three experiments in each case.

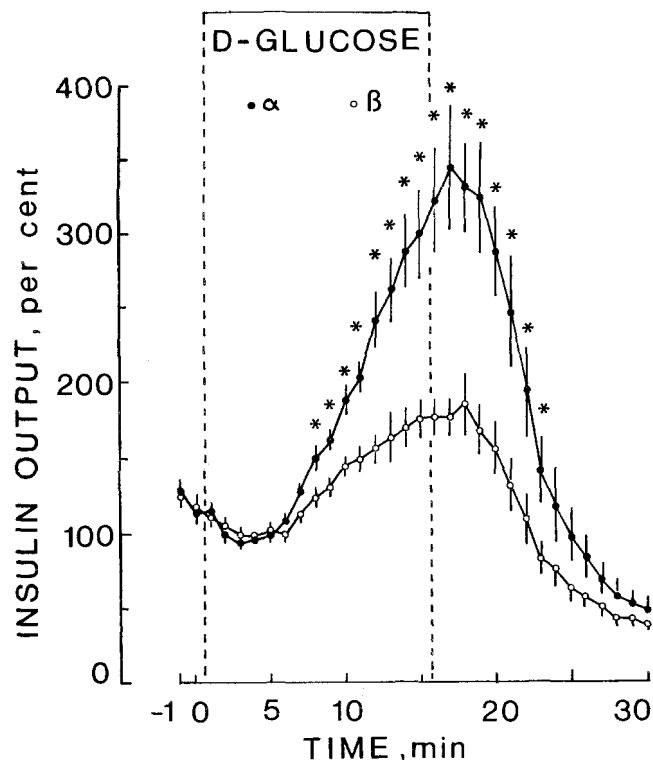


FIG. 2. Insulin output from isolated perfused pancreases exposed throughout perfusion to a medium containing Ba^{2+} (2.0 mM) and theophylline (1.4 mM). Either α - or β -D-glucose (3.3 mM) was administered during the period indicated by the vertical broken lines. The values for insulin output are expressed as per cent of the paired secretory rate found within the same experiment 4 min after introducing the glucose anomer. Mean values (\pm S.E.) refer to six experiments in each case, statistically significant differences ($p < 0.05$ or less) in the presence of each anomer being shown by the asterisks.

Our findings also clearly indicate that the higher rate of glycolysis found in the presence of α -D-glucose cannot be ascribed to any preference for α -D-glucose of the phosphorylating enzymes present in crude islet homogenates. On the contrary, the rate of glucose phosphorylation was higher with the β - than with the α -anomer at least in the absence or in the presence of a low concentration of glucose 6-phosphate. In the presence of a much higher concentration of glucose 6-phosphate, no significant anomeric difference in glucose phosphorylation was detected. This could reflect a balance between the residual activity of hexokinase with its preference for β -D-glucose (14, 15) and a modest activity of glucokinase with its preference for α -D-glucose (10, 12). In this respect, our results are in good agreement with prior observations (28–30) indicating that, in the absence of glucose 6-phosphate, the contribution of glucokinase to the overall rate of glucose phosphorylation by islet homogenates does not exceed 3.1–7.9% at the low glucose concentration (3.3 mM) used in the present experiments.

The situation found in the islets exposed to a low concentration of α - or β -D-glucose is reminiscent of that recently characterized in erythrocytes. Indeed, in erythrocytes, the phosphorylation of D-glucose is catalyzed solely by a hexokinase, which, as in other murine tissues (14, 15), displays a higher maximal velocity with β - as compared to α -D-glucose (or α -D-mannose; see Ref. 31). Yet, the glucose-induced increment in lactic acid output is higher in erythrocytes exposed to α - than to β -D-glucose (14). As described in detail elsewhere (9), these converging findings point to a possible key role for the α -stereospecific enzymes phosphoglucose isomerase and phosphoglucose mutase in the anomeric specificity of hexose metabolism in pancreatic islets and, apparently also, in erythrocytes.

We conclude that the alleged α -stereospecific preference of glucokinase is not an adequate explanation for the higher rates of glycolysis and insulin release observed in islets exposed to the α - as distinct from the β -anomer of D-glucose. In other words, the anomeric specificity of insulin release is not a valid argument in support of the claim that glucokinase represents the key component of the B-cell glucoreceptor system. It should be clearly stated, however, that the present comments are not meant to deny that glucokinase plays a significant, but not exclusive, role in the regulation of glucose metabolism and, hence, insulin release in the pancreatic B-cell. The present work merely stresses the view (32, 33) that steps distal to the site of glucose phosphorylation should not be overlooked in considering such regulatory processes.

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