IDENTIFICATION OF THE α-STEREOSPECIFIC GLUCOSENSOR IN THE PANCREATIC B-CELL

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Received 23 February 1976

1. Introduction

The α-anomer of D-glucose is better able than the β-anomer to stimulate the secretion of insulin [1–5], increase the concentration of cyclic AMP [6], provoke the efflux of phosphate ions [7] and suppress the release of glucagon in pancreatic islets [3–5]. The α-stereospecific system responsible for this situation is unknown. It could be a membrane-associated gluco-receptor [1–7], a carrier for glucose transport across the cell membrane [8] or an enzyme involved in the early steps of glucose metabolism [9]. The latter hypothesis was examined in the present study, to be reported in full detail elsewhere.

2. Materials and methods

The methods used for the isolation [10] and homogenization [11] of rat islets, and the measurement of sorbitol accumulation [12], glycolytic intermediates content [13], glucose oxidation [14], lactate output [15], ^{45}Ca efflux [16], ^{45}Ca net uptake [17], and insulin release [18] in the islets were previously described. The method used for the measurement of [1-^{14}C]glucose phosphorylation by islet homogenates was also reported elsewhere [11]. For the direct measurement of α- and β-D-glucose phosphorylation by the islet homogenate, the unlabeled anomers were exposed for 6 min to [γ-^{32}P]ATP (1.0 mM, 25 Ci/mol), glucose-[6-^{32}P]phosphate being then isolated from the medium by anion exchange chromatography [19]. The affinity of the islet glucose-6-phosphate dehydrogenase and phosphoglucose isomerase towards α- and β-glucose-6-phosphate was tested by a fluorometric method allowing for the continuous monitoring of the reaction rate [12], the hexose-phosphates being extemporaneously generated from their respective glucose anomer (11 μM) in the presence of ATP and yeast hexokinase (28 mU/ml). In the case of glucose-6-phosphate dehydrogenase, the formation of 6-phosphogluconate by the islet homogenate (0.15 ml) was judged from the appearance of NADPH in a medium (2.0 ml) buffered to pH 7.5 with triethanolamine·HCl (50 mM) and containing $MgCl_2$ (10 mM), EDTA (0.5 mM), albumin (0.01%, w/v), ATP (2.5 mM) and NADP (1.0 mM). In the case of phosphoglucoisomerase, the formation of fructose-6-phosphate by the islet homogenate (0.1 ml) was assessed by the appearance of NADH in the same buffer (2.0 ml) containing $MgCl_2$ (6 mM), EDTA (1.0 mM), Na_2HAsO_4 (3 mM), cysteine (2 mM), albumin (0.01%, w/v), ATP (0.5 mM), NAD (0.5 mM) and the auxiliary enzymes (fructose-6-phosphate kinase 3.0 U, aldolase 0.45 U, triosephosphate isomerase 1.0 U, and glyceraldehyde-3-phosphate dehydrogenase 4.0 U).

3. Results

No significant difference could be detected in the rate of α- and β-D-glucose phosphorylation by the islet enzymes. Indeed, over 5 min incubation in the presence of ATP (0.1 mM) and the islet homogenate, both anomers (9.0 mM) inhibited to the same degree the formation of [1-^{14}C]glucose-6-phosphate from [1-^{14}C]glucose (1.0 mM) in anomeric equilibrium (table 1, line 1). Moreover, when the islet homogenate was exposed for 6 min to [γ-^{32}P]ATP, the

Table 1
The effect of glucose anomers on different metabolic parameters in pancreatic islets are invariably expressed in per cent of the mean value found with β-D-glucose

Line	Metabolic parameter	α-D-glucose[a]	β-D-glucose[a]	P
1	Inhibition of [1-^{14}C]glucose phosphorylation	104.9 ± 4.3 (17)	100.0 ± 4.6 (17)	N.S.
2	Rate of glucose-[6-^{32}P]phosphate formation	93.1 ± 11.0 (4)	100.0 ± 3.8 (4)	N.S.
3	Rate of conversion of G6P to 6-P-gluconate	63.3 ± 3.8 (6)	100.0 ± 10.2 (6)	< 0.01
4	Sorbitol accumulation	40.3 ± 3.5 (5)	100.0 ± 14.3 (5)	< 0.005
5	Glucose-6-phosphate content	65.9 ± 6.9 (10)	100.0 ± 5.9 (11)	< 0.01
6	F6P + F1,6-diP + triose-P content	136.6 ± 9.3 (11)	100.0 ± 7.4 (11)	< 0.01
7	Rate of conversion of G6P to F6P	143.6 ± 13.8 (6)	100.0 ± 5.3 (6)	< 0.02
8	Inhibition of $^{14}CO_2$ production from [U-^{14}C]glucose	166.1 ± 20.1 (19)	100.0 ± 7.5 (22)	< 0.005
9	Lactate output	136.6 ± 11.6 (23)	100.0 ± 8.0 (23)	< 0.02
10	Inhibition of ^{45}Ca efflux	180.9 ± 11.9 (9)	100.0 ± 11.6 (6)	< 0.005
11	Increment in ^{45}Ca net uptake	161.3 ± 13.1 (5)	100.0 ± 0.0 (5)	< 0.02
12	Insulin release	190.5 ± 27.0 (12)	100.0 ± 11.2 (12)	< 0.005

[a] Mean values (± SEM) are shown together with the number of individual determinations (in parentheses) and the statistical significance (N.S., not significant) of differences between α- and β-D-glucose (*P*).

same amount of glucose-[6-^{32}P]phosphate accumulated whether in the presence of α- or β-D-glucose (10.0 mM) (table 1, line 2).

The first indication of a difference in the metabolism of the 2 anomers was obtained by examining the affinity of the islet glucose-6-phosphate dehydrogenase towards α- and β-glucose-6-phosphate. The hexose-phosphate was extemporaneously generated from each anomer in the presence of yeast hexokinase and ATP. When the generation of glucose-6-phosphate was the rate-limiting factor, the formation of 6-phosphogluconate by the islet homogenate occurred later and at a slower rate in the presence of α- as distinct from β-D-glucose (table 1, line 3). This result indicates that, in the islets, like in other tissues [20], the enzyme glucose-6-phosphate dehydrogenase is stereospecific for β-D-glucose-6-phosphate. Also more sorbitol accumulated in intact islets exposed for 5 min to freshly dissolved β- as distinct from α-D-glucose (16.7 mM) (table 1, line 4). This behaviour, which could theoretically be due to a stereospecific affinity of the islet aldose reductase, might well result from the preferential orientation of β-glucose-6-phosphate to the pentose pathway, more NADPH being then available for the conversion of glucose to sorbitol (fig.1).

Since neither the phosphorylation of glucose, nor its conversion to either 6-phosphogluconate or sorbitol offered a satisfactory explanation for the more marked insulinotropic action of α-D-glucose, we turned our attention to the possible participation of glycolysis in

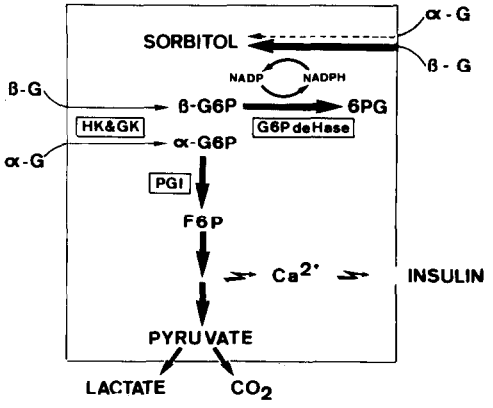

Fig.1. Schematic view for the metabolism of D-glucose anomers (αG, βG) in pancreatic islets. The thick arrows illustrate the preferential utilization of substrates (G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate; F6P, fructose-6-phosphate) by different enzymes (HK, hexokinase; GK, glucokinase; PGI, phosphoglucose isomerase; G6PdeHase, glucose-6-phosphate dehydrogenase). Also depicted is the postulated link between glycolysis, calcium handling and insulin release.

such a process. After 5 min incubation in the presence of glucose (7.2 mM), the concentration of glucose-6-phosphate was lower and that of further glycolytic intermediates higher in the islets exposed to α- as distinct from β-D-glucose (table 1, lines 5 and 6). This suggests that the phosphoglucose isomerase of the islets, like that of other tissues [20,21], is stereospecific for α-D-glucose-6-phosphate. We were able to confirm such a view, using a model comparable to that defined above for the study of the islet glucose-6-phosphate dehydrogenase. When the availability of glucose-6-phosphate was the rate-limiting factor (namely at low glucose and yeast hexokinase levels), the rate of fructose-6-phosphate formation by the islet homogenate was almost 50% higher with α- than β-D-glucose-6-phosphate (table 1, line 7).

We obtained two independent indications that, in the intact B-cell like in the islet homogenate, the rate of glycolysis was higher in the case of α-D-glucose. First, in islets exposed for 6 min to [U-^{14}C]glucose (2.2 mM) in anomeric equilibrium, much less radioactivity was recovered as $^{14}CO_2$ when unlabeled α- as distinct from β-D-glucose (7.8 mM) was also present in the incubation medium (table 1, line 8). This indicates that the α-anomer is better able to dilute the metabolic pool from which $^{14}CO_2$ is eventually derived, under conditions where more than 90% of the total $^{14}CO_2$ production is accounted for by glycolysis [22]. Second, the output of lactate from islets incubated for 5 min with freshly dissolved α-D-glucose (7.4 mM) was significantly higher than that found in islets exposed to β-D-glucose (table 1, line 9).

In islets prelabeled with ^{45}Ca, the inhibitory effect of glucose (5.8 mM) upon ^{45}Ca efflux [16] was more pronounced in the case of α-D-glucose. Indeed, relative to the value for ^{45}Ca efflux found prior to the introduction of glucose, the rate of fall in effluent radioactivity averaged 6.6 ± 0.4 and 3.6 ± 0.4%·min^{-1} over the 5 min period following the addition to the perifusate of α- and β-D-glucose, respectively (table 1, line 10). Consistent with the latter finding, the glucose-induced increment in ^{45}Ca net uptake, above the basal value found in the absence of glucose, was significantly higher (paired comparison) in the presence of α- as distinct from β-D-glucose (8.4 mM) (table 1, line 11). Over 6 min incubation, the α-anomer (8.1 mM) also provoked a higher release of insulin (table 1, line 12).

4. Discussion

We have recently obtained evidence to indicate that, in the process of glucose-induced insulin release, glycolysis controls the rate of Ca^{2+} transport across the B-cell membrane [23] and that, in turn, Ca^{2+} accumulation in the B-cell triggers insulin release [24]. The present data indicate that the more marked insulinotropic action of α- as distinct from β-D-glucose is associated with a higher glycolytic flux, itself attributable to the stereospecificity of the islet phosphoglucose isomerase (fig.1). It is likely, therefore, that such a metabolic behaviour accounts for the preferential secretory response of pancreatic endocrine cells towards α-D-glucose.

Acknowledgements

We are grateful to Mr D. Martins, and Mrs M. Mahy, J. Schoonheydt and M. Urbain for technical assistance and to Mrs B. Noël for secretarial help. This work was supported in part by grant 3.4527.75 from the Fonds de la Recherche Scientifique Médicale (Brussels, Belgium).

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