Review Articles

The Glycolytic Cascade in Pancreatic Islets

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It is currently thought that the process of glucose-induced insulin release is secondary to the increase in glycolytic and oxidative fluxes evoked in the islet cells by a rise in the extracellular concentration of glucose [1–3]. The aim of the present report is to review recent acquisitions on the regulation of glycolysis in pancreatic islets, with emphasis on the role of hexose-bisphosphates, especially fructose-2,6- P_2 , in such a process.

Fructose-2,6- P_2 was recently identified in hepatocytes as a potent activator of phosphofructokinase [4–6]. In the liver, fructose-2,6- P_2 also inhibits the activity of fructose-1,6- P_2 bisphosphatase [7]. A salient feature of the effect of fructose-2,6- P_2 upon phosphofructokinase activity consists in the fact that the activator enhances the reaction velocity at non-saturating concentrations of fructose-6-P, but fails to augment the maximal velocity recorded at a saturating concentration of fructose-6-P [8]. In the liver, the activator is formed from fructose-6-P and ATP in a reaction catalyzed by a novel enzyme, fructose-6-P,2-kinase [9–11]. Thus, fructose-6-P not only acts as a substrate in the reaction catalyzed by phosphofructokinase, but also represents the precursor in the synthesis of fructose-2,6- P_2 (Fig. 1). We have recently observed that a comparable situation prevails in pancreatic islets [8, 12–14].

Activation of Phosphofructokinase by Hexose-Bisphosphates

The velocity of the reaction catalyzed by phosphofructokinase is influenced inter alia by the concentrations of fructose-6-P and ATP. In order to characterize the activation of phosphofructokinase by hexose-bisphosphates, a suitable experimental design includes the use of a low concentration of fructose-6-P (0.25 mmol/l) and a high concentration of ATP (10 mmol/l), so that the reaction occurs at a low rate in the absence of activator [9, 12]. Figure 2 illustrates the effect of increasing concentrations of fructose-2,6- P_2 [kindly given by Drs. E. Van Schaftingen and H.-G. Hers; 15] upon the activity of purified muscle phosphofructokinase under these experimental conditions. The relationship illustrated in Figure 2 can be used to estimate the apparent content in

fructose-2,6- P_2 of biological samples, e.g. islet homogenates.

Figure 2 also documents that fructose-2,6- P_2 is not the sole hexose-bisphosphate capable of activating phosphofructokinase [16]. Glucose-1,6- P_2 mimics the effect of fructose-2,6- P_2 . The maximal increments in reaction velocity evoked by these two hexose-bisphosphates are not significantly different from one another [14]. However, the concentration of glucose-1,6- P_2 required to achieve a given degree of activation is about 100 times higher than that of fructose-2,6- P_2 . This difference in potency and the vastly different acid-lability of the two hexose-bisphosphates are appropriate attributes to distinguish between the effects of fructose-2,6- P_2 and glucose-1,6- P_2 , respectively, when both esters are present in the same biological sample.

Role of Fructose-2,6- P_2 in the Regulation of Glycolysis in Pancreatic Islets

Pancreatic islets display fructose-6-P,2-kinase activity [12]. This enzyme catalyzes the synthesis of fructose-

Fig. 1. The glycolytic cascade: the black arrow depicts the synthesis of fructose-2,6- P_2 ; the forked arrow depicts the activation of phosphofructokinase by fructose-2,6- P_2

Fig. 2. Effect of fructose-2,6-P₂ (●—●) and glucose-1,6-P₂ (○—○) upon the velocity of the reaction catalyzed by muscle phosphofructokinase in the presence of 0.25 mmol/l fructose-6-P and 10 mmol/l ATP. The increments in reaction velocity are expressed as a percentage of their paired maximal values which, in absolute terms, were not significantly different in the presence of fructose-2,6-P₂ and glucose-1,6-P₂, respectively

Fig. 3. Saturation curve and double reciprocal plot for the activity of islet fructose-6-P,2-kinase at increasing concentrations of fructose-6-P. The reaction velocity is expressed as a percentage of the paired value found within the same experiment in the presence of 1.0 mmol/l fructose-6-P. Such a control value averaged 80 ± 9 fmol/min per islet

2,6-P₂ from fructose-6-P and ATP. At optimal concentration of these substrates, the reaction velocity averages 80 ± 9 fmol/min per islet [13]. The latter value, when expressed relative to the protein content of the islets (approximately 1.0 μ g/islet), is close to that found in liver [9–11]. In the presence of ATP (5 mmol/l) the velocity of the reaction markedly increases as the concentration of fructose-6-P is raised from 0.02 to 0.04 and 0.1 mmol/l (Fig. 3). In intact islets, the intracellular concentration of fructose-6-P increases in the 0.03 to 0.06 mmol/l range as the extracellular concentration of glucose is raised from 2.8 to 16.7 mmol/l [8]. Thus, the glucose-induced increase in the islet content of fructose-6-P occurs over a range of concentrations such that a parallel increase in fructose-2,6-P₂ generation would be expected.

It is also conceivable that glucose affects the islet content of fructose-2,6-P₂ by altering the activity of fructose-6-P,2-kinase. However, we have so far been unable to detect any significant effect of glucose on the activity

of the insular fructose-6-P,2-kinase. Thus, no significant difference in reaction velocity was observed either when glucose was added directly to the assay cuvette or when the activity of fructose-6-P,2-kinase was measured in homogenates derived from islets first incubated in the absence or presence of glucose, respectively [13].

Exposure of intact islets to glucose results in an increase in their fructose-2,6-P₂ content [13]. Pancreatic islets indeed contain this acid-labile activator of phosphofructokinase. The amount of activator is higher in islets incubated in the presence of glucose (16.7 mmol/l) than in islets incubated in the absence of glucose. Under steady-state conditions, the glucose-induced increment in fructose-2,6-P₂ content of the islets averages 60 ± 22 fmol/islet. If one takes into account the activity of fructose-6-P,2-kinase at physiological concentrations of fructose-6-P (about 0.05 mmol/l), it appears that no more than 1 or 2 min of exposure to glucose would be re-

quired to achieve such an increase in the fructose-2,6-P₂ content of the islets.

In islet homogenates, fructose-2,6-P₂ is a potent activator of phosphofructokinase, as little as 0.1–0.2 $\mu\text{mol/l}$ being sufficient to cause a half-maximal activation of the enzyme [8]. In the islet homogenate as in the case of purified muscle phosphofructokinase, the fructose-2,6-P₂ augments the reaction velocity at low but not at saturating concentrations of fructose-6-P [8]. This behaviour provides an opportunity to assess whether phosphofructokinase is indeed activated in intact cells [17]. For instance, when islets are first incubated in the absence or presence of glucose and then homogenized, the ratio between reaction velocities at low and at saturating concentrations of fructose-6-P will be higher in the glucose-stimulated than in the glucose-deprived islets if exposure of the islets to glucose indeed leads to activation of phosphofructokinase. Figure 4 illustrates that such is indeed the case.

The convergent observations summarized so far strongly suggest that activation of phosphofructokinase by fructose-2,6-P₂ represents a critical event in the regulation of glycolytic rate at increasing concentrations of extracellular glucose.

Glucagon and the Glycolytic Cascade

Exposure of hepatocytes to glucagon results in a rapid and dramatic fall in their fructose-2,6-P₂ content, even when the concentration of extracellular glucose is maintained at a high level [18]. This fall appears to be due to inactivation of fructose-6-P,2-kinase by a cyclic AMP-dependent protein kinase [19]. It is unlikely that glucagon acts in a comparable manner in islet cells, since exogenous glucagon is reported to exert little if any effect upon the oxidation of glucose by pancreatic islets [20]. In our hands, also, the rate of D-[U-¹⁴C]glucose oxidation by islets exposed to glucose (16.7 mmol/l) was virtually identical in the absence (32.0 ± 1.8 pmol/120 min per islet; $n = 24$) and presence (32.7 ± 1.6 pmol/120 min per islet; $n = 24$) of glucagon (10 $\mu\text{g/ml}$), respectively. The well-known enhancing action of glucagon upon glucose-stimulated insulin release [21, 22], a phenomenon currently ascribed to activation of adenylate cyclase [22, 23], also argues against an inhibitory effect of glucagon upon the rate of glycolysis in the islet cells.

Nevertheless, the knowledge that glucagon lowers the fructose-2,6-P₂ content of hepatocytes and that glucagon release is stimulated at low concentrations of glucose, raises the idea that locally released glucagon could prevent the stimulation of glycolysis in adjacent B cells exposed to low concentrations of extracellular glucose. By doing so, glucagon could be responsible, in part at least, for the sigmoidal appearance of the curve relating the rates of glycolysis and glucose oxidation by isolated islets to the concentration of extracellular glucose [20, 24, 25], thus accounting for the existence of a threshold phenomenon in the relation between insulin release and glucose concentration [26]. These considerations led us to explore whether glucagon affected the activity of fruc-

Fig. 4. The activity of phosphofructokinase in homogenates derived from islets first incubated in either the absence (left column) or presence of glucose (right column) was measured in the presence of 0.25 and 5.0 mmol/l fructose-6-P, so that the ratio in reaction velocity at these two concentrations could be established for each homogenate

Fig. 5. Insulin output by rat islets removed from the part of the pancreas derived from the ventral anlage. Mean values $\pm$ SEM are shown with the number of individual determinations in parentheses

tose-6-P,2-kinase in pancreatic islets and whether the threshold value for the stimulant action of glucose upon insulin release was unusually low in islets containing few glucagon-producing cells.

In the first approach, we measured the activity of fructose-6-P,2-kinase in homogenates prepared from islets which had been incubated for 60 min either in the presence of glucose alone (5.6 or 16.7 mmol/l) or in the simultaneous presence of glucose and either glucagon (20 $\mu\text{g/ml}$) or theophylline (1.4 mmol/l). The activity of

fructose-6-P₂-kinase was measured at a low pH (7.0) and a low concentration of fructose-6-P (0.2 mmol/l), i. e. under suitable conditions for detection of the inactivation of liver fructose-6-P₂-kinase treated by the catalytic subunit of protein kinase [19]. The rate of fructose-2,6-P₂ synthesis was not higher in the islets exposed solely to glucose (8.2 ± 2.3 fmol/min per islet; $n = 5$) than in the islets incubated in the simultaneous presence of glucose and either glucagon (10.0 ± 1.9 fmol/min per islet; $n = 7$) or theophylline (9.2 ± 1.1 fmol/min per islet; $n = 7$). Thus, there was no indication that agents raising the cyclic AMP content of the islets [23, 27] would decrease the activity of fructose-6-P₂-kinase.

In the second approach, we have investigated whether islets removed from the part of the pancreas derived from the ventral anlage, and therefore containing relatively few glucagon-producing cells [28], would display an unusually low threshold value for the stimulant action of glucose upon insulin release. As illustrated in Figure 5, the concentration of glucose had to exceed 5.6 mmol/l in order to stimulate insulin release above basal value in this population of islets. Such behaviour is similar to that of islets taken at random from the entire pancreatic gland and thus, in their majority, relatively rich in glucagon-producing cells. Likewise, in a purified B cell population [29], the concentration of glucose must still exceed a threshold value (between 4.2 and 8.3 mmol/l) to stimulate insulin release (D.G. Pipeleers, personal communication). Incidentally, Trimble and Renold [30] reported that islets derived from the dorsal area of rat pancreas and exposed to high concentrations of glucose (8.3 and 16.7 mmol/l) secrete more insulin than islets derived from the ventral area and containing about eight times less glucagon. These authors concluded that locally secreted glucagon facilitates glucose-stimulated insulin release. None of these observations support the idea that glucagon affects the glycolytic cascade in islet cells in a manner comparable to that described in hepatocytes.

Alternative Activators of Phosphofructokinase

The knowledge that glucose-1,6-P₂, like fructose-2,6-P₂, is able to activate phosphofructokinase (Fig. 2) led us to examine whether these two hexose-bisphosphates may not act in concert to regulate the activity of phosphofructokinase in intact islets.

The glucose-1,6-P₂ content of glucose-deprived islets is extremely low (not exceeding 10 fmol/islet). When the extracellular concentration of glucose is raised to 5.6 or 16.7 mmol/l, the glucose-1,6-P₂ content increases above basal value by 19.7 ± 2.6 and 41.0 ± 3.6 fmol/islet, respectively. Since the intracellular water space of an islet does not exceed 3 nl [31], the concentration of glucose-1,6-P₂ would amount to at least 6 and 14 μ mol/l, respectively, at glucose 5.6 and 16.7 mmol/l.

Glucose-1,6-P₂ activates phosphofructokinase in islet homogenates. As is the case with purified muscle phosphofructokinase, the maximal response to glucose-1,6-P₂ is not significantly different from that evoked by

fructose-2,6-P₂ [14]. Glucose-1,6-P₂, like fructose-2,6-P₂, augments the reaction velocity at low concentrations of fructose-6-P, but does not enhance the maximal velocity recorded at a saturating concentration of fructose-6-P [14]. The magnitude of the increment in reaction velocity is dose-related in the range of glucose-1,6-P₂ concentrations between 2.5 and 50 μ mol/l, with a half-maximal response at a concentration close to 8.5 μ mol/l [14]. It is evident, therefore, that the concentration of glucose-1,6-P₂ in intact islets is sufficiently high to cause activation of phosphofructokinase, despite the lesser sensitivity of this enzyme to glucose-1,6-P₂ as distinct from fructose-2,6-P₂ (Fig. 2). The latter two hexose-bisphosphates may act in concert to regulate the activity of phosphofructokinase. Indeed, when they are used at submaximal concentrations, glucose-1,6-P₂ and fructose-2,6-P₂ act synergistically in causing activation of purified muscle phosphofructokinase [14].

Since the synthesis of glucose-1,6-P₂ is catalyzed by phosphoglucomutase [32], we have examined whether glucose itself might activate the latter enzyme. The activity of purified muscle phosphoglucomutase (1 mU/ml) was measured using glucose-1-P (3 mmol/l) as the substrate and yeast glucose-6-P dehydrogenase (125 mU/ml) as the auxiliary enzyme. In the absence of glucose, the reaction velocity increased from 46 ± 1 to 143 ± 1 and 384 ± 9 pmol of NADH formed/min as the concentration of glucose-1,6-P₂ in the reaction mixture was raised from zero to 1.5 and 7.3 nmol/l, respectively. In the presence of glucose (20 mmol/l), the reaction velocity averaged $98.2 \pm 3.9\%$ ($n = 6$) of the paired control value recorded in the absence of glucose. These findings indicate that glucose does not exert any major effect on the activity of purified phosphoglucomutase.

Conclusion

The present report emphasizes the view that the acceleration of glycolysis occurring in islets stimulated by a rise in the extracellular concentration of glucose involves increases in both the availability of glycolytic intermediates, such as glucose-6-P and fructose-6-P, and the activity of the key glycolytic enzyme phosphofructokinase. We initially thought that such a dual mechanism could help to reconcile the substrate-site and regulatory-site hypotheses for the process of glucose-induced insulin release [33]. However, our more recent study on the properties of fructose-6-P₂-kinase now suggests that the increase in the rate of fructose-2,6-P₂ synthesis may be mainly due to an elevation in the fructose-6-P content of the islet cells, an elevation itself attributable to a mass action phenomenon. We were also unable to detect any direct effect of glucose on the activity of phosphoglucomutase, the enzyme catalyzing the synthesis of glucose-1,6-P₂, another activator of phosphofructokinase. Therefore, there is as yet no convincing evidence that the molecule of glucose, whether located in the extracellular or intracellular fluid, interacts with a specific receptor to induce activation of a hypothetical glucosensor system. Obvi-

ously, the latter statement is not meant to deny that glucose binds to the stereospecific carrier mediating glucose transport across the B cell plasma membrane and to those enzymes (hexokinase, glucokinase, aldose reductase) catalyzing the first steps of its intracellular metabolism. Such binding phenomena, however, cannot be equated with the situation in which the insulin secretory response depends solely on the allosteric activation of an enzyme by a given secretagogue, as appears to be the case in islets stimulated by the nonmetabolized analogue of L-leucine, 2-aminobicyclo [2, 2, 12] heptane-2-carboxylic acid [34, 35].

Acknowledgements. Studies from this laboratory mentioned in this article were supported by grants from the Belgian Foundation for Scientific Medical Research. We are grateful to Mrs. C. Demesmaeker for secretarial help.

Note added in proof. The participation of glucose-1,6-P₂ and/or mannose-1,6-P₂ as activators of islet phosphofructokinase was recently found to account, in part at least, for the higher insulin release evoked by the α -anomer (as distinct from β -anomer) of either D-glucose or D-mannose [36, 37].

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