Time Course Studies of Glucose-Induced Changes in Glucose-6-Phosphate and Fructose-1,6-Diphosphate Content of Mouse and Rat Pancreatic Islets

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Summary. The concentrations of glucose 6-phosphate (G6P) and fructose 1,6-diphosphate plus triose-phosphates (FDP + TPs) were measured in isolated islets of Langerhans from mice and rats after a sudden increase in extracellular glucose concentration from 0.5 to 3.4 mg/ml. In mouse islets, the contents of G6P and (FDP + TPs) were both raised after a two minute incubation at the high glucose concentration and remained elevated for at least 30 min. In rat islets, the G6P but not the (FDP + TPs) content was increased after a 5 min exposure to high glucose. After a 30 min incubation, both G6P and (FDP + TPs) contents were higher than at low glucose concentration. The (G6P)/(FDP + TPs) ratio was some tenfold

higher in mouse islets than in rat islets. Increasing extracellular glucose concentration was associated with an increase in the (G6P)/(FDP + TPs) ratio. The results are consistent with the increased glycolytic rate in response to a raised extracellular glucose concentration arising primarily from an increase in the rate of phosphorylation of glucose, and with the hypothesis that the insulin secretory response to glucose may be mediated by a metabolite of the sugar.

Key words: Islet metabolism, insulin release, hexose phosphate, luciferase assay of pyridine nucleotides.

Introduction

To substantiate the hypothesis that metabolites of glucose or conditions associated with glucose metabolism, rather than glucose itself, trigger insulin release from the islets of Langerhans [1, 2], it would be necessary to show that changes in concentrations of metabolites or cofactors occur at least as fast as insulin secretion switches on after a glucose pulse.

In *in vivo* experiments with islets from obese hyperglycemic mice a 400% rise in G6P¹ concentration within 90 sec has been demonstrated [3]. However, later Matschinsky *et al.* [4] again using an *in vivo* approach — found no changes in the concentrations of G6P, 6PG¹, ATP or phosphocreatine in rat islets within 1–5 min after a glucose pulse.

We felt that this species difference in metabolic response possibly could be explained by differences in anaesthesia, in the degree of hypoxia and stress and/or other experimental conditions difficult to control *in vivo* and have therefore in *in vitro* experiments measured the concentrations of G6P and (FDP + TPs)¹ in isolated islets of Langerhans from both mice and rats after a sudden rise in extracellular glucose concentration from 0.5 to 3.4 mg/ml.

Significant and fast changes in the concentration of metabolites were demonstrated in both rat- and mouse islets although most pronounced in mouse islets.

Materials and Methods

Purified luciferase from *Achromobacter fischerii* and FMN were from Sigma Chemical Co. Myristic aldehyde was from Aldrich Chemical Co. Other enzymes and coenzymes used were from Boehringer. Human albumin was from Behringwerke AG. All other reagents were of analytical grade.

All glassware used was cleaned in 1.5 N nitric acid and washed in double distilled water.

Preparation of Islets

Islets were isolated by a collagenase method [5] from the pancreas of male Wistar rats weighing 150–175 g or of male T.O. mice weighing 22–26 g. All animals were starved overnight.

Incubation of Islets

Batches of 7–10 islets (for glucose-6-phosphate assay) or 20–40 islets (for fructose-1,6-diphosphate assay) were preincubated for 30 min at 37° in stoppered glasstubes with 21 μ l gassed (O₂:CO₂ 95:5) Krebs bicarbonate buffer [6] containing 0.5 mg glucose/ml and 1 mg human albumin/ml.

After preincubation, the glucose concentration of the medium was raised by injecting 7 μ l Krebs bicarbonate buffer containing 12 mg glucose/ml and 1 mg human albumin/ml. After the time indicated in the tables the metabolism was stopped by injecting 28 μ l 0.1 N HCl (for G6P assay) or 1 N HCl (for FDP assay). The low glucose controls were prepared by adding the HCl immediately after the preincubation and then later the 7 μ l Krebs buffer with high glucose content. Blank values were obtained by incubating medium without islets.

Before assaying for G6P or FDP all tubes were sonicated for 20 sec at position 1 on a Branson Sonifier B-12.

FDP and G6P standards were made up in a Krebs bicarbonate medium-HCl mixture similar in composition to the acidified incubation medium in order to account for

¹ Abbreviations used: G6P: glucose-6-phosphate, FDP + TPs: fructose-1,6-diphosphate plus triose phosphates, 6PG: 6-phosphogluconate.

any interference by the medium reagents in the metabolite assays.

Glucose-6-Phosphate Assay

40 μ l of the sonicate or standard G6P solutions were analysed for G6P by adding 27 μ l of 0.2 M trisbuffer pH 8.0, 1.5 μ l 1 mM NADP⁺ and 1.5 μ l glucose-6-phosphate dehydrogenase (0.35 U/ml). After incubation for 60 min at room temperature the content of NADPH was measured in triplicate by the luciferase assay described below.

Fructose-1,6-Diphosphate Assay

40 μ l of the sonicate or standard FDP solutions were analysed for FDP and triose phosphates by adding 27 μ l 0.4 M trisbuffer containing 0.5 N KOH, 1 mM mercapto-

saturated solution of myristic aldehyde in ethanol and 20 μ l of the sample to be determined were added to the tubes. The tubes were allowed to stand at room temperature in the dark for 10–15 min and the assay was started by adding 40 μ l of a luciferase-solution containing 1 mg luciferase/ml phosphate buffer pH 7.0 to the tube. The tube was quickly removed from the dark, mixed on a Vortex-Genie and transferred to a conventional glass scintillation vial placed in the liquid scintillation spectrometer and counted for 0.1 min.

The time passing (15 sec) from the adding of the luciferase until the start of the counting was accurately standardized by always adding the luciferase when the printing out of the results of the preceding tube started. A Packard TriCarb scintillation spectrometer, model 2002

Table 1. *Glucose-6-phosphate and fructose-1, 6-diphosphate in islets of Langerhans*
Mouse Islets

	Control (glucose 0.5 mg/ml)	Duration of incubation with glucose 3.4 mg/ml		
		2 min	5 min	30 min
G6P (pmol/10 islets)	5.0 $\pm$ 0.9 (11)	12.4 $\pm$ 0.5 ^b (3)	12.1 $\pm$ 1.0 ^a (12)	15.5 $\pm$ 1.5 ^a (17)
% above control		148	142	210
FDP + TPs (pmol/10 islets)	0.37 $\pm$ 0.05 (26)	0.71 $\pm$ 0.09 ^c (5)	0.72 $\pm$ 0.12 ^c (32)	0.63 $\pm$ 0.05 ^a (30)
% above control		92	95	70
G6P FDP + TPs ratio	13.5	17.5	16.8	24.6
<i>Rat Islets</i>				
G6P (pmol/10 islets)	2.5 $\pm$ 0.2 (28)		4.2 $\pm$ 0.3 ^a (26)	5.5 $\pm$ 0.3 ^a (30)
% above control			68	120
FDP + TPs (pmol/10 islets)	1.32 $\pm$ 0.12 (33)		1.59 $\pm$ 0.15 ^d (31)	2.06 $\pm$ 0.20 ^b (32)
% above control			20	56
G6P FDP + TPs ratio	1.9		2.6	2.7

^a Different from control with $p < 0.001$

^b Different from control with $p < 0.005$

^c Different from control with $p < 0.02$

^d NS, $p > 0.2$

Batches of mouse or rat islets were preincubated for 30 min in 21 μ l bicarbonate medium containing albumin (1 mg/ml) and glucose (0.5 mg/ml). The medium glucose concentration was then raised to 3.4 mg/ml by injection of 7 μ l medium containing 12 mg/ml of glucose. After the times indicated islet metabolism was stopped by the injection of HCl and the islet contents of G6P and (FDP + TPs) were measured as described in the text. The data are expressed as mean $\pm$ S.E.M. with the number of batches of islets under each condition given in parentheses.

ethanol and 1 mM NaH₂AsO₄, 1.5 μ l 1 mM NAD⁺ and 1.5 μ l of an enzyme mixture containing triosephosphate-isomerase (120 U/ml), glyceraldehyde-3-phosphate dehydrogenase (40 U/ml) and aldolase (4.5 U/ml).

After incubation for 90 min at room temperature the content of NADH was measured in duplicate by the luciferase assay described below. The assay measures the sum of fructose-1,6-diphosphate and triose phosphates (FDP + TPs).

Luciferase Assay

Determination of NADH and NADPH was based on the method described by P.E. Stanley [7].

Counting procedure: Polypropylene tubes (1.3 $\times$ 5.0 cm) were soaked overnight in water and washed with double distilled water. 1 ml of a 0.1 M trisbuffer pH 7.3 containing 1 mM mercaptoethanol was mixed with 2 μ l FMN (0.1 mg FMN/ml 0.1 M potassium phosphate buffer pH 7.0) and placed in a polypropylene tube. 10 μ l of a

operated at room temperature was used. The photomultipliers were switched out-of-coincidence. Amplification was 100% and the discriminators were set between 10 and infinity.

Results

Since islet G6P and FDP were determined from standard curves prepared using authentic G6P and FDP taken through the same procedures, no corrections for recovery were needed. Typical standard curves for G6P and FDP in the luciferase assay are shown in the Figure.

The concentrations of G6P and (FDP + TPs) in mouse and rat islets at various times after raising the

extracellular glucose concentration from 0.5 to 3.4 mg/ml are given in the Table. In mouse islets, the contents of G6P and (FDP + TPs) were both significantly raised after a two minute incubation at the high glucose concentration and remained elevated for at least 30 min.

In rat islets, the G6P but not the (FDP + TPs) content was significantly increased after a 5 min exposure to high glucose (the shortest time interval tested). After a 30 min incubation both G6P and (FDP + TPs) were significantly higher than at low glucose concentrations.

Fig. 1. Standard curves for G6P and FDP using the luciferase assay. NADPH was generated from G6P using NADP⁺ and glucose-6-phosphate dehydrogenase. NADH was generated from FDP using NAD⁺, aldolase, triose phosphate isomerase and triose phosphate dehydrogenase. The reduced pyridine nucleotides formed were then determined by the luminescence produced by bacterial luciferase using a scintillation counter as detector. See the text for experimental details. Each point plotted is the mean of six determinations. ●—● FDP, ▲—▲ G6P

Although mouse and rat islets are of similar size, there were differences between the absolute and relative contents of G6P and (FDP + TPs) in the two species. The G6P content was higher in mouse islets than in rat islets whereas the reverse was true for the (FDP + TPs) content. As a result the [G6P]/[(FDP + TPs)] ratio was some tenfold higher in mouse islets than in rat islets. In mouse islets, increasing extracellular glucose concentration was associated with an increase (approx. 100%) in the [G6P]/[(FDP + TPs)] ratio; in rat islets this increase was much less marked.

Discussion

The view that glucose must be metabolized in order to elicit insulin release envisages that effects on insulin release of changes in the extracellular glucose concentration are mediated, at least in part, by changes in the intracellular concentration of one or more metabolites

formed from glucose in the β -cell. It should be emphasized that neither the magnitude nor the direction of the changes in such a hypothetical trigger metabolite need parallel the changes in insulin secretion rate; however, the rapidity of the metabolite concentration change should be comparable to that of the secretory response.

In islets from several species, increasing extracellular glucose concentrations have been shown to give rise to increased islet content of hexose phosphates [3, 8, 9]. In an earlier study [8], we showed that a correlation existed between insulin release rate and hexose phosphate content of mouse islets under various conditions. Glucose-6-phosphate was shown to attain a steady state level in mouse islets after 15 min of incubation but the rapidity of changes in the G6P level in response to changes in extracellular glucose concentration was not further investigated. Earlier studies of the kinetics of changes in islet content of glycolytic intermediates have yielded conflicting results; whereas very rapid (< 90 sec) changes were observed in islets from obese-hyperglycemic mice on rapid elevation of blood glucose *in vivo* [3], no changes were found in rat islets after 5 min [4]; 60 min after elevation of blood glucose rat islets did show an increase in hexose phosphate content. When similar measurements were carried out on islets from a perfused rat pancreas preparation, no significant changes in islet metabolic intermediates were seen at any time up to 40 min after acute elevation of the extracellular glucose concentration [4]. It was argued that the rapid initial insulin secretory response to sugars could not be mediated by these intermediates. A recent report, however, has described fast (1 min) *decreases* in the islet content of glycolytic intermediates in islets from duct-ligated rat pancreas in response to high glucose [10].

Our data show that mouse and rat islets both evince significant and rapid changes in G6P content after elevation of extracellular glucose concentration. (FDP + TPs) content is also increased after 30 min; however, whereas the change in (FDP + TPs) content occurs in less than 2 min in mouse islets the rate of increase of (FDP + TPs) concentration in rat islets is sluggish, no significant increase being seen after 5 min. These results confirm that there are indeed differences in the metabolic responses to an increased extracellular glucose concentration between mouse and rat islets but suggest that these differences are quantitative rather than qualitative. In particular they indicate that changes in hexose phosphate content of rat islets although less marked than for mouse islets occur within 5 min after raising the glucose concentration. These findings are thus consistent with the hypothesis that the insulin secretory response to glucose may be mediated by a metabolite of the sugar.

The relative amounts of G6P and (FDP + TPs) in isolated rat islets in the present study are similar to those reported for islets in a perfused rat pancreas preparation [4]. It is striking that in mouse islets the

[G6P]/[FDP + TP] ratio is some ten-fold higher than in the rat islets. The enzymic basis for this difference is not known. In mouse islets the high K_m for glucose utilization [11] is to some extent explicable in terms of the high K_m glucose-phosphorylating activity and the glucose-inhibited glucose-6-phosphatase activity [3, 12]; in view of the failure to demonstrate these activities in rat islets [4, 13] and the low K_m for glucose of hexokinase, the increased glucose oxidation rate in rat islets as the glucose concentration is raised from 3 to 20 mM [14] is puzzling. It has been suggested that the phosphofructokinase reaction may be the step that is stimulated by the addition of glucose; the evidence for this was a decrease in the [G6P]/[FDP + TP] ratio in rat islets when the extracellular glucose concentration was raised from zero to 20 mM [4]. Under the conditions of our experiments, however, no such decrease was observed; indeed in mouse islets a marked increase was found. These data do not support the view that phosphofructokinase was activated other than by a rise in substrate concentration. They are consistent with the increased glycolytic rate in response to a raised extracellular glucose concentration arising primarily from an increase in the velocity of the rate-determining step for glucose uptake, which in view of the rapid permeation of glucose into islets [3, 15], is likely to be phosphorylation of glucose.

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