IS THE GLUCOSE-INDUCED STIMULATION OF GLYCOLYSIS IN PANCREATIC ISLETS ATTRIBUTABLE TO ACTIVATION OF PHOSPHOFRUCTOKINASE BY FRUCTOSE 2,6-BISPHOSPHATE?

Willy J. MALAISSE, Francine MALAISSE-LAGAE, Abdullah SENER, Emile VAN SCHAFTINGEN and Henri-Géry HERS

Laboratory of Experimental Medicine, Brussels Free University, Laboratory of Physiological Chemistry, Louvain University and *International Institute of Cellular and Molecular Pathology, Brussels 1000, Belgium*

Received 2 February 1981

1. Introduction

It is currently thought that the process through which glucose is identified in the pancreatic B-cell as a stimulus for both proinsulin biosynthesis and insulin release strictly depends on the integrity of glucose metabolism in the islet cells [1,2]. However, the mechanism by which the glycolytic and oxidative flux is increased in response to a rise in the extracellular concentration of glucose is not fully elucidated. In hepatocytes, the rate of glycolysis is regulated at the level of fructose 6-phosphate phosphorylation by a newly discovered hexose phosphate, fructose 2,6-bisphosphate, which activates phosphofructokinase [3–5]. Here, we propose that a similar situation prevails in pancreatic islets.

2. Materials and methods

For the assay of phosphofructokinase [3], groups of 1200–1500 pancreatic islets each isolated from fed albino rats were homogenized in Potter-Elvehjem tubes with 0.65 ml Hepes–NaOH buffer (25 mM, pH 7.4) containing NaF (50 mM) and EGTA (7.5 mM). The homogenate was centrifuged (30 min, 30 000 × *g*) and the supernatant used in 0.1 ml samples/assay cuvette. The reaction mixture (1.0 ml) contained (final concentration): fructose 6-phosphate (0.1–5.0 mM); glucose 6-phosphate (glucose 6-phosphate/fructose 6-phosphate concentration ratio of 3.0); ATP (1.5 mM); NADH (0.075 mM); AMP (0.1 mM); Hepes (50 mM, pH 7.0); NaF (5.0 mM); EGTA (0.75 mM); KCl (100 mM); $MgCl_2$ (5.0 mM); KH_2PO_4 (5.0 mM); NH_4Cl (1.0 mM); the auxiliary enzymes (phosphoglucose-isomerate 1 U/ml, aldolase 0.5 U/ml, triose phosphate isomerase 5 U/ml, and glycerophosphate dehydrogenase 0.5 U/ml); and, as required, fructose 2,6-bisphosphate prepared from fructose 1,6-bisphosphate by an adaption of the method in [6]. The reaction was initiated by the addition of ATP and the fall in NADH concentration was recorded at room temperature by spectrophotometry at 340 nm.

In a second series of experiments, paired groups of 600 islets each were incubated for 60 min at 37°C in the absence or presence of D-glucose (20 mM) in 2.0 ml bicarbonate-buffered medium equilibrated against a mixture of O_2 (95%)–CO_2 (5%) [7]. After incubation, the islets were briefly washed with a glucose free medium and homogenized. The homogenate was prepared and examined for its phosphofructokinase activity as described above.

3. Results

Fig.1 illustrates the effect of fructose 2,6-bisphosphate upon phosphofructokinase activity in islet homogenate. In the presence of 0.25 mM fructose 6-phosphate, the reaction velocity was increased in a dose-related fashion by fructose 2,6-bisphosphate (fig.1, left). The increment in velocity attributable to fructose 2,6-bisphosphate reached 50% of its maximal value at ~0.2 μM activator. The capacity of fructose 2,6-bisphosphate to increase the reaction velocity was much more marked at low than at high concentration of fructose 6-phosphate (fig.1, right). In the absence of fructose 2,6-bisphosphate, the curve relating the

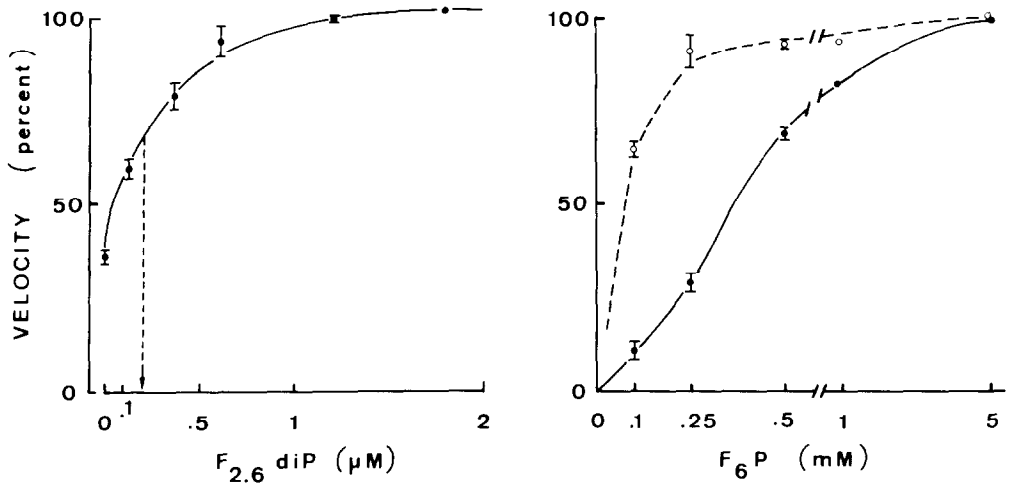

Fig.1. (Left) Dose–action relationship for the effect of fructose 2,6-bisphosphate (F2,6diP) on phosphofructokinase activity in islet homogenates. All measurements were performed in the presence of fructose 6-phosphate 0.25 mM, and are expressed in % of the paired control value found in the presence of fructose 2,6-bisphosphate 1.2 μM. Such a control value averaged 337 ± 55 pmol . islet^{-1} . h^{-1}. The vertical dotted line corresponds to the concentration of fructose 2,6-bisphosphate required to cause an increase in phosphofructokinase activity representing 50% of the increment seen at the higher concentration of the activator (i.e., 1.8 μM). (Right) Dose–action relationship for phosphofructokinase activity in islet homogenates at increasing concentrations of fructose 6-phosphate (F6P) in the absence (•,——) or presence (○,– –) of fructose 2,6-bisphosphate (1.2 μM). All data are expressed in % of the paired control value found at the highest concentration of fructose 6-phosphate (5.0 mM). Such a control value was the same in the absence or presence of fructose 2,6-bisphosphate, and averaged 412 ± 54 pmol . islet^{-1} . h^{-1}. Mean values (± SEM) refer to ⩾2 determinations.

reaction velocity to the concentration of fructose 6-phosphate appeared sigmoidal, failing to yield a straight line in a double reciprocal plot (not shown). In the presence of fructose 2,6-bisphosphate, however, the curve appeared hyperbolic with an app. K_m for fructose 6-phosphate below 0.1 mM. At a saturating concentration of fructose 6-phosphate (5.0 mM), no significant effect of fructose 2,6-bisphosphate upon reaction velocity was observed. The absolute values for phosphofructokinase activity at high concentrations of fructose 6-phosphate were in fair agreement with those in [8].

The above results refer to data obtained with islets which were homogenized immediately after having been isolated. In a second series of experiments, the isolated islets were first incubated for 60 min in the absence or presence of glucose (20 mM), prior to homogenization. The activity of phosphofructokinase was measured at 0.25 and 5.0 mM fructose 6-phosphate, so that the ratio in reaction velocity at these two concentrations could be established for each homogenate. This ratio can be used as an index of phosphofructokinase activation by fructose 2,6-bisphosphate [3]. It averaged (mean ± SEM) 40.5 ± 4.2% and 61.5 ± 6.8% (n = 8 in both cases, $P < 0.02$) in islets previously deprived of glucose and in islet first exposed to D-glucose 20 mM, respectively. By comparison with the data illustrated in fig.1 (left), it would appear that the effect of D-glucose in the intact islets was close to that seen when fructose 2,6-bisphosphate 0.12 μM was added to a homogenate prepared from islets not submitted to any prior incubation.

4. Discussion

The influence of fructose 2,6-bisphosphate upon reaction velocity in the islet homogenate (fig.1) was similar to that seen with liver phosphofructokinase [5]. The findings illustrated in fig.1 are essential for our understanding of glucose metabolism in pancreatic islets. In these islets, the intracellular concentration of free glucose rapidly equilibrates with its extracellular concentration [9,10] and the phosphorylation of glucose is catalyzed by a glucokinase-like enzyme with a high K_m for glucose [8,11]. The rate of glucose

6-phosphate formation may thus increase, as a function of the extracellular concentration of glucose, through a mass action phenomenon. The concentration of glucose 6-phosphate is indeed increased in islets exposed to high concentrations of glucose [12]. Taking into account the glucose 6-phosphate content of rat islets [12], the ratio of glucose 6-phosphate to fructose 6-phosphate [13] and the intracellular H_2O space of the islets [10], it can be calculated that a rise in extracellular glucose from 2.8–18.9 mM would cause an increase in fructose 6-phosphate from ~0.03–0.06 mM. In this concentration range the reaction catalyzed by phosphofructokinase in the absence of fructose 2,6-bisphosphate, as measured in islet homogenates (fig.1), occurs at a rate well below that recorded an intact islets. Indeed, even if corrected for the difference in temperature (25 *vs* 37°C) and despite the fact that the experimental conditions (e.g., concentration of ATP and AMP) were selected to achieve optimal enzymatic activity, the velocity of the reaction at the low concentrations of fructose 6-phosphate did not exceed 34 ± 8 pmol . $islet^{-1}$. h^{-1} in the islet homogenate, as distinct from a glycolytic rate of 128 ± 8 pmol . $islet^{-1}$. h^{-1} in intact islets exposed to glucose 16.7 mM [13]. Thus, if the islet phosphofructokinase were not to be somehow activated, the estimated concentration of fructose 6-phosphate would be too low to account for the rate of glycolysis indeed recorded in intact islets. In the presence of fructose 2,6-bisphosphate, however, the rate of fructose 6-phosphate phosphorylation, at the same low concentration of substrate and in the presence of the islet homogenate equals or exceeds that found in intact islets.

In view of these considerations, we investigated whether the activity of phosphofructokinase is indeed higher in glucose-stimulated islets than in glucose-deprived islets. For this purpose, paired groups of islets were incubated in the absence or presence of glucose and, in each case, the phosphofructokinase activity ratio at two concentrations of fructose 6-phosphate was then established. This activity ratio was indeed higher in the islets first exposed to glucose than in the islets previously deprived of glucose. The values found in the islets were almost identical to those found in hepatocytes removed from starved rats, in which case D-glucose (20 mM) also provokes a significant increase in the activity ratio [4].

In conclusion, these data suggest that the glucose-induced stimulation of glycolysis in intact rat islets is attributable, at least in part, to activation of phosphofructokinase and that such an activation is mediated by fructose 2,6-bisphosphate.

Acknowledgements

This work was supported by the Belgian Foundation for Scientific Medical Research and by the US Public Health Service (grant AM9235). E. V. S. is Aspirant of the Belgian Foundation for Scientific Research.

References

[1] Malaisse, W. J., Sener, A., Herchuelz, A. and Hutton, J. C. (1979) Metabolism 28, 373–386.
[2] Ashcroft, S. J. H. (1980) Diabetologia 18, 5–15.
[3] Van Schaftingen, E., Hue, L. and Hers, H.-G. (1980) Biochem. J. 192, 263–271.
[4] Van Schaftingen, E., Hue, L. and Hers, H.-G. (1980) Biochem. J. 192, 887–895.
[5] Van Schaftingen, E., Hue, L. and Hers, H.-G. (1980) Biochem. J. 192, 897–901.
[6] Pontis, H. G. and Fischer, C. L. (1963) Biochem. J. 89, 452–459.
[7] Malaisse, W. J., Brisson, G. R. and Malaisse-Lagae, F. (1970) J. Lab. Clin. Med. 76, 895–902.
[8] Malaisse, W. J., Sener, A. and Levy, J. (1976) J. Biol. Chem. 251, 1731–1737.
[9] Hellman, B., Sehlin, J. and Täljedal, I.-B. (1971) Biochim. Biophys. Acta 241, 147–154.
[10] Malaisse, W. J., Sener, A., Levy, J. and Herchuelz, A. (1976) Acta Diabet. Lat. 13, 202–215.
[11] Ashcroft, S. J. H. and Randle, P. J. (1970) Biochem. J. 119, 5–15.
[12] Ashcroft, S. J. H., Capito, K. and Hedeskov, C. J. (1973) Diabetologia 9, 299–302.
[13] Sener, A. and Malaisse, W. J. (1978) Diab. Métab. 4, 127–133.