Rapid Publication
Glucose-Induced Accumulation of Fructose-2,6-Bisphosphate in Pancreatic Islets

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SUMMARY

Rat islets contain the acid-labile activator of phosphofructokinase, fructose-2,6-bisphosphate. The islet content in activator is higher in islets exposed to glucose (16.7 mM) than in islets deprived of glucose. The islets display fructose-6-phosphate,2-kinase activity with a Km for fructose-6-phosphate close to 0.08 mM. Glucose fails to affect the activity of this enzyme. It is proposed that the effect of glucose to increase the islet content of fructose-2,6-bisphosphate is attributable, in part at least, to the glucose-induced increase in the concentration of fructose-6-phosphate in the islet cells. DIABETES *31*:90–93, January 1982.

The secretion of insulin by pancreatic islets exposed to glucose tightly depends on the rate of glycolysis in the islet cells.[1] It was recently reported that, in pancreatic islet homogenates, the activity of phosphofructokinase is higher when the homogenate is derived from islets that were incubated in the presence rather than absence of glucose, provided that the reaction velocity is not measured at saturating concentrations of fructose-6-P.[2] It was speculated that the activation of phosphofructokinase might be attributable to fructose-2,6-bisphosphate (fructose-2,6-P_2), a novel hexosephosphate which was identified as activator of phosphofructokinase in liver.[3,4] This interpretation is supported by the following observations. First, fructose-2,6-P_2 dramatically increases the velocity of fructose-6-P conversion to fructose-1,6-P_2 in islet homogenates when the activity of phosphofructokinase is measured at physiologic concentrations of fructose-6-P.[2] Second, the enzyme that catalyzes the synthesis of the activator from fructose-6-P and ATP-Mg, namely fructose-6-P, 2-kinase,[5–7] is present in pancreatic islets.[8] The present study indicates that glucose augments the islet content in an acid-labile activator of phosphofructokinase, suggesting that glucose indeed provokes the accumulation of fructose-2,6-P_2 in islet cells.

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MATERIALS AND METHODS

All experiments were performed with pancreatic islets removed from fed albino rats and isolated by the collagenase technique.[9] In some experiments, paired groups of 250–300 islets each were first incubated for 90 min at 37°C, in the absence or presence of glucose (16.7 mM), in 1.0–1.5 ml of a bicarbonate-buffered medium containing bovine albumin (5 mg/ml),[10] prior to homogenization. In all other experiments, the freshly isolated islets were immediately homogenized.

For measurement of the islet content in fructose-2,6-P_2, groups of 250 islets each were placed in 60 μl of a Tris-HCl buffer (200 mM; pH 7.4), which contained EGTA (0.5 mM) and $MgCl_2$ (5.0 mM) and was mixed with 15 μl of NaOH (0.5 M). For homogenization, the tubes containing the islets were placed in liquid N_2 and the islets homogenized by mechanical vibration.[11] After heating for 20 min at 80°C and centrifugation for 3 min at 5,000 × g, aliquots (20 μl each) of the supernatant solution were examined for their content in fructose-2,6-P_2, as described below. In each experiment, one aliquot (20 μl) of the supernatant solution was mixed with 5 μl HCl (0.5 M) and allowed to stand for 20 min at room temperature to destroy fructose-2,6-P_2.[12] These samples were brought back to neutral pH prior to measurement of their content in fructose-2,6-P_2.

For measurement of fructose-6-P,2-kinase activity, groups of 250–300 islets each were placed in 100 μl of the Tris-HCl buffer (200 mM; pH 7.4) containing EGTA (0.5 mM) and $MgCl_2$ (5.0 mM). The islets were homogenized and centrifuged as described above, aliquots (24 μl) of the supernatant solution being added to a reaction mixture (final volume 35 μl) containing (final concentrations) Tris-HCl (200 mM, pH 7.4), EGTA (0.5 mM), $MgCl_2$ (5.0 mM), ATP (5.0 mM), and fructose-6-P (0.08–4.1 mM). Under the present experimental conditions, fructose-6-P is rapidly converted to glu-

cose-6-P, due to the high activity of phosphoglucose isomerase in the islet homogenate.[13] When the initial concentration of fructose-6-P amounted to 4.1 mM, the concentration of glucose-6-P in the assay medium averaged 3.1 ± 0.1 mM, a value close to that expected from the equilibrium constant of phosphoglucose isomerase.[14] These considerations were taken into account in establishing the true concentrations of fructose-6-P in the assay medium, quoted in RESULTS and DISCUSSION. After a 20-min incubation at 37°C, the reaction mixture (35 μl) was mixed with 5 μl NaOH (0.7 M), heated for 20 min at 80°C, centrifuged (see above), and eventually examined for its content in fructose-2,6-P_2 (see below). We have previously reported that, under the present experimental conditions, the reaction velocity is constant over a 20-min incubation and proportional to the volume of islet homogenate.[8]

For measurement of their content in fructose-2,6-P_2 the alkali-treated (and further acidified) samples were added to an assay cuvette containing 0.98 ml of a Hepes-NaOH buffer (50 mM; pH 7.4). This buffer contained EDTA (0.2 mM), $MgCl_2$ (10 mM), NH_4Cl (1 mM), dithiothreitol (2.5 mM), fructose-6-P (0.25 mM), ATP (10 mM), NADH (0.16 mM), desalted aldolase (0.4 U), triose-P-isomerase (2.4 U) and α-glycero-P-dehydrogenase (0.4 U). Prior to addition of the samples, the reaction was initiated by addition of purified muscle phosphofructokinase (0.015 U) and the basal reaction velocity recorded spectrophotometrically over a 10–15-min incubation at 30°C. The increment in reaction velocity attributable to the addition of the sample was again monitored over 10–15 min, and expressed relative to the increment in reaction velocity (above basal value) enregistered when fructose-2,6-P_2 (kindly given by Drs. E. Van Schaftingen and H.-G. Hers, see ref. 15) was added to the same cuvette to yield a final concentration of 250 nM. The reaction velocity was judged from the decrease in NADH absorbance at 340 nm. The concentration of fructose-2,6-P_2 in the samples was then calculated by reference to the abacus established at increasing concentrations of fructose-2,6-P_2 (standards).[8]

All results are expressed as the mean (± SEM) together with the number of individual observations (N).

RESULTS

Islet content in fructose-2,6-P_2. When pancreatic islets were incubated for 90 min in the presence of glucose (16.7 mM) and then homogenized, the alkali-treated homogenates contained an acid-labile activator of purified muscle phosphofructokinase. In the presence of fructose-6-P (0.25 mM) and ATP (10 mM), the activity of the muscle enzyme averaged 918 ± 48 pmol of NADH consumed/min (N = 34). The islet homogenate increased the reaction velocity by 402 ± 141 pmol of NADH consumed/min (paired difference; N = 8; P < 0.025). Such an increase in reaction velocity represented 7.86 ± 1.81% (N = 8) of the paired increment in velocity measured in the same cuvette in the presence of 250 nM fructose-2,6-P_2. From these data, it was calculated that the glucose-stimulated islets apparently contained 147 ± 35 fmol/islet of fructose-2,6-P_2.

When the islets were incubated for 90 min in the absence rather than presence of glucose, the increase in the velocity of the reaction catalyzed by muscle phosphofructokinase was less marked. In a series of 8 experiments, the paired difference in fructose-2,6-P_2 content of glucose-deprived and glucose-stimulated islets averaged 59.6 ± 21.7 fmol/islet (P < 0.05). The apparent fructose-2,6-P_2 content of glucose-deprived islets represented 64.2 ± 5.6% (P < 0.001) of the paired value found in glucose-stimulated islets.

Whether in glucose-deprived or glucose-stimulated islets, the capacity of the islet homogenate to activate muscle phosphofructokinase was abolished by acid treatment of the homogenates. The acid-treated homogenates failed to increase significantly (paired difference : + 6 ± 24 pmol of NADH consumed/min, N = 14) the velocity of the reaction catalyzed by muscle phosphofructokinase.

Activity of islet fructose-6-P,2-kinase. When the islet homogenate was incubated for 20 min at 30°C in the presence of 1.0 mM fructose-6-P and 5.0 mM ATP-Mg, the rate of for-

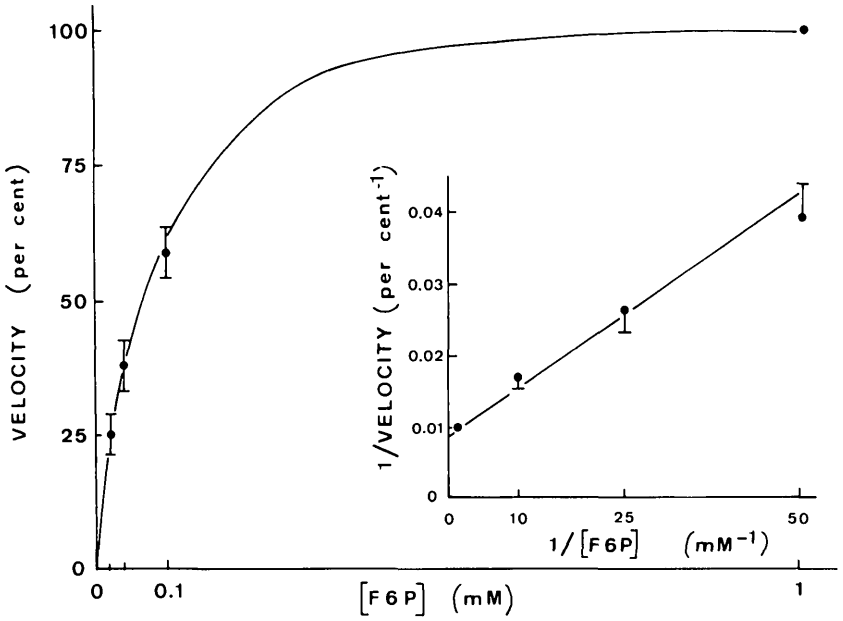

FIGURE 1. Saturation curve and double reciprocal plot for the activity of fructose-6-P,2-kinase at increasing concentrations of fructose-6-P. The velocity of the reaction is expressed as a percent of the paired value found within the same experiment in the presence of 1.0 mM fructose-6-P. Such a control value averaged 79.6 ± 9.1 fmol/min per islet (N = 14). Mean values (± SEM) refer to 6–8 individual observations.

mation of fructose-2,6-P_2 averaged 79.6 ± 9.1 fmol/min per islet (N = 14). The Km of fructose-6-P,2-kinase for fructose-6-P was close to 0.08 mM (Figure 1).

No direct effect of glucose upon the activity of fructose-6-P,2-kinase was observed. The islet homogenate was incubated for 20 min at 30°C in the presence of fructose-6-P (0.1–1.0 mM) and ATP-Mg (5.0 mM), with or without glucose (20 mM). After incubation in the absence and presence of glucose, respectively, the increment in the velocity of the reaction catalyzed by muscle phosphofructokinase, as observed after addition of the alkali-treated samples derived from the first reaction mixture, averaged 25.77 ± 2.94 and 23.14 ± 3.14% (N = 6 in each case, P > 0.5) of the paired increment evoked in the same cuvette by 250 nM fructose-2,6-P_2.

In the last series of experiments, intact islets were incubated for 90 min at 37°C in the absence or presence of glucose (16.7 mM), prior to homogenization and measurement of fructose-6-P,2-kinase activity. At three concentrations of fructose-6-P (0.02, 0.04 and 1.0 mM), the reaction velocity (fmol of fructose-2,6-P_2 formed per min and per islet) in the homogenates prepared from glucose-stimulated islets averaged 98.4 ± 9.8% (N = 12; P > 0.8) of the paired value found, within the same experiment and at the same concentration of fructose-6-P, in a homogenate prepared from glucose-deprived islets.

In all experiments designed to measure the activity of fructose-6-P,2-kinase in islet homogenates, the activator of muscle phosphofructokinase generated during the first reaction was examined for its lability at acid pH. When the first reaction was conducted in the presence of 1.0 mM fructose-6-P and when the alkali-treated samples derived from this first reaction were acidified, the increment in the velocity of the reaction catalyzed by muscle phosphofructokinase averaged 197 ± 63 pmol of NADH consumed/min (N = 15), representing less than 10% of the increment evoked by the nonacidified samples (2,391 ± 197 pmol of NADH consumed/min; N = 17). The residual effect of the acidified samples (P < 0.01) is likely to reflect the incomplete destruction of fructose-2,6-P_2 under the present experimental conditions.[12]

DISCUSSION

The present data indicate that pancreatic islets contain an acid-labile activator of phosphofructokinase, presumably fructose-2,6-P_2. The islet content in this activator is higher in islets exposed to glucose (16.7 mM) than in glucose-deprived islets. For the assay of the activator, the crude islet homogenate was added to a cuvette containing purified muscle phosphofructokinase. It is conceivable, therefore, that the activity of the muscle enzyme was affected to a limited extent by factors other than fructose-2,6-P_2.

The islet also display fructose-6-P,2-kinase activity. We have been unable to detect any obvious effect of glucose on the activity of this enzyme, whether glucose was directly added to the reaction mixture or the enzymatic activity measured in homogenates derived from glucose-deprived and glucose-stimulated islets, respectively. These negative findings do not formally rule out a regulatory effect of glucose upon the activity of fructose-6-P,2-kinase in intact islet cells.

The activity of fructose-6-P,2-kinase in pancreatic islets was of the same order of magnitude as that reported in liver (Table 1). The Km for fructose-6-P of the islet enzyme (0.08 mM) was lower than that measured with partially purified liver fructose-6-P,2-kinase (0.4–0.5 mM; see refs. 6 & 7). However, in view of the islet content in Pi and the activity of the islet Mg-ATPase,[16] the Pi content of our reaction mixture amounted to more than 2 mM. According to Van Schaftingen and Hers,[7] Pi (5 mM) lowers the Km of fructose-6-P,2-kinase for fructose-6-P from 0.4 to 0.05 mM. Hence, the Km derived from our experiments is in fair agreement with that found in liver.

TABLE 1
Fructose-6-P,2-kinase activity in liver and pancreatic islets

Tissue	Activity (1 mU = 1 nmol/min)	Reference
Liver	0.044 mU/mg protein	(5)
Liver	0.071 mU/mg protein	(6)
Liver	0.100 mU/mg protein*	(7)
Islets	0.080 mU/mg protein†	(this report)

* Assuming a protein content of 0.2 g/g wet weight liver.
† Assuming a protein content of 1.0 μg/islet.[17]

Our kinetic data suggest that the velocity of the reaction catalyzed by fructose-6-P,2-kinase may be markedly affected by changes in the concentration of fructose-6-P in the range of values to be found in intact islet cells (0.03–0.06 mM; see ref. 2). In other words, the glucose-induced increase in the islet content of fructose-6-P might represent a major determinant of the increase in fructose-2,6-P_2 formation and, hence, activation of phosphofructokinase in intact islets stimulated by glucose.

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