Fructokinase activity in rat liver, ileum, parotid gland, pancreas, pancreatic islet, B and non-B islet cell homogenates

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Abstract. The presence of fructokinase (ketohexokinase) in rat pancreatic islet homogenates was previously documented. However, no information was so far available on the activity of this enzyme in islets relative to that in other tissues and on the respective contribution of insulin-producing B cells and non-B islet cells. The present study provides such an information. The activity of fructokinase, as assessed by the phosphorylation of 1.0 mM D-fructose, was compared to that of hexokinase isoenzyme(s), as measured in the presence of 1.0 mM D-glucose, and further characterized by its heat-resistance, K+ dependency and resistance to the inhibitory action of D-manno-heptulose. As judged from the results obtained in heated homogenates, the activity of fructokinase, expressed relative to protein content (nmol/min per mg protein) was highest in liver (21.5±2.5; n=11) and lowest in parotid gland (0.16±0.07; n=6). The paired ratio between fructokinase and hexokinase isoenzyme activity was also highest in liver (548±45%; n=8) and lowest in parotid gland (0.93±0.52%; n=11). Such a ratio was not significantly different in pancreas, islets and purified B or non-B islet cells, with an overall mean value of 2.57±0.46% (n=12). The present findings thus unambiguously document the presence of fructokinase activity in all cell types under consideration, except possibly parotid cells, with the following hierarchy: liver > ileum > pancreas. Relative to paired hexokinase activity, no obvious difference was found for fructokinase activity in B versus non-B islet cells.

Introduction

Over the last two decades, several studies aimed at investigating the metabolism and insulinotropic action of D-fructose in pancreatic islets. In terms of metabolism, attention was drawn to the participation of different enzymes to the phosphorylation of the ketohexose, including fructokinase (1), the low-Km hexokinase (2), and the high-Km glucokinase with emphasis on the glucose-induced cooperativity of this enzyme towards D-fructose (3,4) and the anomic specificity of such a process (5,6). The possible participation of D-fructose 1-phosphate in the regulation of glucokinase activity at the intervention of its regulatory protein (7) was also examined (Iwashiga K, et al, Diabetologia 38 (Suppl 1): abs. 394, A102, 1995) (8). In terms of stimulus-secretion coupling, puzzling findings relate to the dissociation both between the effects of D-fructose on proinsulin biosynthesis and insulin release (9) and between the changes evoked by the ketohexose in the ATP and ADP content, as well as ATP/ADP ratio, and its insulinotropic action (Giroix M-H, et al, Diabetologia 47 (Suppl 1): abs. 457, A168, 2004) in isolated islets. From the physiological standpoint, a recent study revealed that both the immediate and delayed effects of D-fructose, as compared to D-glucose, upon secretory activity may differ in insulin-, somatostatin- and glucagon-producing cells (10). The possible participation of D-fructose insulinotropic action in the improvement of D-glucose tolerance evoked in vivo by the ketohexose was also investigated (11). Last, in terms of the perturbation of islet function in type 2 diabetes, several studies aimed at comparing the fate of D-fructose in islets obtained from either control rats or animals currently used as models for this disease (12-14).

The present study deals with one of these issues, i.e. the activity of fructokinase in rat pancreatic islets. In a prior study, it was already documented that rat islets indeed display fructokinase activity (1). The ATP-dependent phosphorylation of D-fructose in homogenates of rat pancreatic islets that had been heated for 5 min at 70°C in order to inactivate hexokinases was found to be dependent on the presence of K+ and inhibited by D-tagatose. The phosphorylation product was identified as D-fructose 1-phosphate through its conversion to a bisphosphate ester by Clostridium difficile fructose 1-phosphate kinase (1). In this prior study, however, the fructokinase activity in islet homogenates was not compared to that found in other organs, and the respective contribution of B and non-B islet cells to such an activity was not investigated. The results of the present study provide this missing information.

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Materials and methods

D-[U-14C]glucose and D-[6-14C]glucose were purchased from NEN Life Science Products (Boston, MA) and Amersham International (Aylesbury, UK), respectively. The radioactive D-fructose tracers (D-[U-14C]fructose or D-[6-14C]fructose) were prepared from the corresponding 14C-labelled D-glucose using an alkaline isomerization procedure and were further purified by HPLC, as described elsewhere (15).

Adult albino Wistar rats of both sexes were given free access to tap water and a standard pellet chow (Km-04-K12; Pavan Service, Oud Turnhout, Belgium) up to the time of sacrifice. All animal experimentation was conducted in accordance with accepted standards of animal care, as established by the local animal experimentation ethics committee.

Islets were isolated from the pancreas of 3-6 rats using a collagenase (EC 3.4.24.3 from Clostridium histolyticum, type P; Roche Molecular Biochemicals, Mannheim, Germany) digestion technique (16) and subsequently separated from the remaining exocrine tissue by hand-picking under a stereomicroscope. Hanks’ balanced salt solution saturated with a heat-resistant enzyme, presumably fructokinase. The phosphorylation of D-fructose by the non-heated homogenates is attributable, at the low concentration of the reaction velocity for D-fructose phosphorylation averaged, after heating, 113.0±11.4% (n=4; p>0.3) of the paired value recorded in non-heated homogenates. Thus, pooling all available data, the phosphorylation of D-fructose by heated homogenates averaged 100.0±6.2% (n=15; p>0.99) of the paired control value. In the case of D-fructose, however, such a percentage averaged 95.2±7.1% (n=11; p>0.5). Likewise, in the presence of D-mannoheptulose or absence of K+, the reaction velocity for D-fructose phosphorylation averaged, after heating, 113.0±11.4% (n=4; p>0.3) of the paired value found in fresh homogenates. These findings suggest that the phosphorylation of D-fructose by liver homogenates is attributable, at the low concentration of the islet cells per ml.

For the measurement of D-glucose or D-fructose phosphorylation, aliquots (50 μl) of the homogenates were mixed with a tracer amount (2.0 μCi/ml) of the 14C-labelled corresponding hexose (D-[U-14C]glucose or D-fructose, mixed with a tracer amount (2.0 μCi/ml) of D-[U-14C]glucose containing 10 mM ATP (sodium salt), 2 mM of either D-fructose or D-[6-14C]fructose, and 0.4 mg/ml bovine serum albumin and, as required, 40 mM D-mannoheptulose (CMS Chemical Ltd., Oxford, UK). After a 20 min incubation at 30°C, the reaction was halted by adding 1.0 ml of ice water, the diluted reaction mixture being then passed through a column (0.5 ml) of AG1-X8 resin (chloride-form; Bio-Rad Laboratories, Inc., Hercules, CA, USA) for separation of the hexose phosphate(s), generated during incubation, by anion exchange chromatography (19). The column was successively rinsed with 3x0.25 ml and 5x1.5 ml of water. The hexose phosphate(s) were then eluted with 2x1.5 ml of a solution of 1.0 M ammonium formate/0.1 M formic acid. The eluate was mixed with 8.0 ml of scintillation fluid and its radioactive content measured. The results were calculated, after correction for the blank value obtained under identical conditions in the absence of cell or tissue homogenates, by reference to the specific radioactivity of the incubation medium. All measurements were performed in triplicate.

The protein content of the homogenates was measured as previously described (20), using bovine serum albumin as standard.

All results are presented as mean values (± SEM), together with the number of individual observations (n) or degree of freedom (df). The statistical significance of differences between mean values was assessed by use of Student’s t-test.

Results

Liver. In non-heated liver homogenates, the phosphorylation of D-glucose (1.0 mM) averaged 17.5±1.9% (n=8; p<0.001) of that found, within the same experiment(s) with D-fructose (also 1.0 mM). D-mannoheptulose (20 mM) failed to affect the phosphorylation of D-fructose, which averaged 98.6±1.8% (n=3; p>0.5) of the paired value found in the absence of the ketohexose. The omission of K+ in the assay medium, however, decreased D-fructose phosphorylation to 20.0±1.8% (n=3; p<0.001) of the paired control value. Prior heating of the liver homogenates for 5 min at 70°C decreased D-glucose phosphorylation to 1.15±0.27% (n=8; p<0.001) of its paired control value. In the case of D-fructose, however, such a percentage averaged 95.2±7.1% (n=11; p>0.5). Likewise, in the presence of D-mannoheptulose or absence of K+, the reaction velocity for D-fructose phosphorylation averaged, after heating, 113.0±11.4% (n=4; p>0.3) of the paired value recorded in non-heated homogenates. Thus, pooling all available data, the phosphorylation of D-fructose by heated homogenates averaged 100.0±6.2% (n=15; p>0.99) of the paired value found in fresh homogenates. These findings suggest that the phosphorylation of D-fructose by liver homogenates is attributable, at the low concentration of the phosphorylation of D-fructose by fructokinase is resistant to inhibition by D-mannoheptulose.

Ileum. In non-heated homogenates of ileum segments, the phosphorylation of D-fructose (1.0 mM) represented 64.2±6.8% (n=3; p=0.05) of the paired value found with D-glucose (also 1.0 mM). After heating, the phosphorylation of D-fructose was decreased to 0.05±0.02% (n=3) of the paired value found in non-heated homogenates. Such a percentage only represented 0.48±0.28% (n=3; p<0.001) of that found, within the same experiment(s), in the case of D-fructose. Thus, a sizeable fraction of the phosphorylation of D-fructose by the non-heated ileum homogenates (12.4±4.7%; n=3) appeared attributable to a heat-resistant enzyme, presumably fructokinase. The
remaining fraction of D-fructose phosphorylation by the non-heated homogenates was presumably catalyzed by a low-Km hexokinase.

Parotid cells. In non-heated homogenates of parotid glands, the phosphorylation of D-fructose (1.0 mM) only represented 50.4±1.7% (n=3) of that found within the same experiments with D-glucose (also 1.0 mM). The absolute values averaged 1364±283 and 641±145 pmol/min per mg wet weight (n=3 in both cases) with D-glucose and D-fructose, respectively. After 5 min heating at 70˚C, the phosphorylation of D-glucose was virtually abolished, averaging not more than 0.04±0.02% (n=3) of the paired value recorded before heating. In the case of D-fructose phosphorylation, such a percentage was increased to not more than 1.66±0.63% (n=3; p<0.07). These findings argue against the presence of any biologically significant amount of fructokinase in parotid glands. If so, the comparison of the results obtained in homogenates of parotid glands and other organs (e.g. liver) indicates that the heating procedure was indeed adequate to assess the possible presence of fructokinase in such homogenates.

Pancreas. In non-heated pancreatic homogenates, the phosphorylation of D-glucose and D-fructose (1.0 mM each), when expressed per mg wet weight, averaged 2.57±0.56 (n=8) and 1.82±0.28 (n=11) nmol/min. The phosphorylation of D-fructose represented 72.8±7.8% (n=8; p<0.02) of the paired value found with D-glucose. D-mannoheptulose (20 mM) decreased D-fructose phosphorylation to 7.7±1.7% (n=3; p<0.001) of its paired control value (no D-mannoheptulose). In the absence of K+, however, the phosphorylation of D-fructose averaged 98.8±9.9% (n=3) of the paired value found in the presence of K+. After heating for 5 min at 70˚C, the phosphorylation of D-glucose was decreased to 0.09±0.04% (n=8) of the paired value found in fresh homogenates. In the case of D-fructose, however, a sizeable phosphorylating activity remained present in the heated homogenates. It averaged 89.7±10.4 pmol/min per mg wet weight (n=11), representing 6.96±1.56% (n=11) of the paired value found in fresh homogenates. The latter percentage is obviously higher (p<0.005) than that recorded in the case of D-glucose. D-mannoheptulose (20 mM) failed to affect significantly (p>0.8) the phosphorylation of D-fructose by the heated pancreas homogenates, in which case the reaction velocity averaged 74.1±16.4% (n=3; p>0.2) of the paired value found in fresh homogenates. In the case of D-fructose, however, a sizeable phosphorylating activity remained present in the heated homogenates. It averaged 98.8±9.9% (n=3) of the paired value found in the presence of K+.

In the absence of K+, the reaction velocity found in the heated homogenates averaged 1.70±0.39% (n=3) of the paired value found in fresh homogenates. The latter percentage only represented 29.9±5.9% (n=3; p<0.01) of the paired value found in the presence of K+. These findings indicate that the major fraction of D-fructose phosphorylation by fresh pancreatic homogenates was attributable to D-mannoheptulose-sensitive and K+-independent enzyme(s), such as hexokinase isoenzyme(s). Nevertheless, the results recorded in heated pancreas homogenates reveal the presence of a sizeable heat-resistant, D-mannoheptulose-resistant and K+-dependent D-fructose phosphorylating enzymatic activity, presumably fructokinase. The hierarchy of the heated/fresh ratio in D-fructose phosphorylation, i.e. 74.1±16.4% in the presence of D-mannoheptulose, 7.0±1.7% in the absence of the ketoheptose but presence of K+ and 1.7±0.4% in the absence of K+, further supports such a conclusion.

Pancreatic islets. In the next step, it was investigated whether the results recorded in whole pancreas homogenates may correspond, in part at least, to the presence of fructokinase in the endocrine moiety of the pancreatic gland. In non-heated islet homogenates, the phosphorylation of D-fructose (1.0 mM) averaged 89.2±6.4% (n=3; p>0.2) of the paired value found with D-glucose (also 1.0 mM). The major fraction of D-fructose phosphorylation by the fresh islet homogenates appeared attributable to hexokinase isoenzyme(s). In the non-heated islet homogenates, the phosphorylation of D-fructose was decreased to 8.2±1.5% (n=3; p<0.001) of its paired control value by D-mannoheptulose (20 mM), but not significantly affected (p>0.95) in the absence of K+ in which case the reaction velocity averaged 99.8±3.9% (n=3) of the paired value found in the presence of K+. A vastly different situation prevailed in heated islet homogenates. Thus, in this case, the mean reaction velocity was virtually identical in the absence (456±8 fmol/min per islet) and presence (457±139 fmol/min per islet; n=3 in both cases) of D-mannoheptulose (20 mM), but decreased (p<0.05) to about one-third of its paired control value (K+ present) in the absence of K+ (146±107 fmol/min per islet; n=3). These features are consistent with the presence of fructokinase activity in the
heated islet homogenates. Such a view was borne out by the following findings. Prior heating of the islet homogenates for 5 min at 70°C abolished D-glucose phosphorylation. In the presence of K+, it decreased D-fructose phosphorylation to 4.24±0.89% (n=6; p<0.001) of the paired value found in fresh homogenates. Such a decrease was even more pronounced in the absence of K+, in which case the heated/fresh ratio in reaction velocity averaged 1.70±1.10% (n=3), as distinct (p<0.05) from 5.90±0.89% (n=3) found within the same experiment(s). Most importantly, in the presence of D-mannoheptulose, the heated/fresh ratio in D-fructose phosphorylation averaged 66.15±6.89% (n=3; p<0.05), a value about ten times higher (p<0.001) than that found within the same experiment(s) in the absence of the ketoheptose. Fig. 1 illustrates the close analogy between pancreatic islets and the whole pancreas in terms of the effects of heating upon D-fructose phosphorylation in the presence of K+, absence of K` or presence of D-mannoheptulose.

**Purified B and non-B islet cells.** In the last set of experiments in this series, the phosphorylation of D-glucose and D-fructose was compared in semi-purified B and non-B islet cells. Fig. 2 illustrates the results of a representative experiment. In non-heated homogenates of purified B and non-B islet cells, respectively, the phosphorylation of D-glucose (1.0 mM) was 4.46±0.79 and 3.02±0.08 times higher (n=2 in both cases) than that of D-fructose (also 1.0 mM). Expressed per cell, the phosphorylation of D-glucose was decreased to 2.25±0.96% (n=4; p<0.001) of the paired value found prior to heating. In the case of D-fructose, however, such a percentage was 6.09±2.07 times higher (d.f.=4; p<0.05) than that found with D-glucose. In these two respects, no significant difference was found between B and non-B islet cells. These findings are compatible, therefore, with the presence of a limited contribution of fructokinase to the phosphorylation of D-fructose in islet cell homogenates. The major fraction of D-fructose phosphorylation by non-heated islet cell homogenates appears attributable, however, to heat-sensitive enzymes, e.g. hexokinase isoenzyme(s).

**Comparison between different cell types.** Table I shows the protein content and rates of D-glucose (1.0 mM) and D-fructose (1.0 mM) phosphorylation in fresh homogenates of liver, ileum, parotid gland, pancreas and pancreatic islets. The protein content of islet cells averaged 249±29 ng per 10^3 cells (n=4). Incidentally, the protein content in heated homogenates of liver, ileum, parotid gland, pancreas, pancreatic islets and
purified B-cells averaged 69.4±5.0% (n=39; p<0.001) of the paired value found in fresh homogenates.

Relative to protein content, the phosphorylation of D-glucose (1.0 mM) by fresh homogenates was lowest in liver and highest in ileum, with in-between values in parotid glands, pancreas and pancreatic islets. In the case of D-fructose (1.0 mM), the highest mean value was found in liver and the lowest in parotid gland.

In order to compare, in each cell type, the activity of fructokinase to that of hexokinase isoenzyme(s), the reaction velocity recorded in the presence of D-fructose (1.0 mM) and K⁺ using heated homogenates was expressed relative to the paired value found in the presence of D-glucose (also 1.0 mM) with non-heated homogenates. As illustrated in Fig. 3, such a paired ratio ranged between the extreme values of 548±45% (n=8) in liver homogenates and 0.93±0.52% (n=3) in parotid gland homogenates. The mean ratios found in pancreas, islets, purified islet B and non-B cells were not significantly different from one another with an overall mean value of 2.57±0.46% (n=12).

Last, in order to estimate the specific activity of fructokinase in the different cell types, the reaction velocity recorded in the presence of D-fructose (1.0 mM) and K⁺ in heated homogenates was expressed relative to protein content (as measured in the corresponding fresh homogenate). Such a specific activity, expressed as nmol/min per mg protein ranged between the extreme mean values of 21.54±2.47 (n=11) in liver and 0.158±0.089 (n=3) in parotid gland. In ileum, pancreas and islets, it averaged, respectively, 2.45±0.53 (n=3), 0.82±0.11 (n=11) and 0.46±0.07 (n=6) nmol/min per mg protein. In this respect, the differences between liver versus ileum (p<0.005), ileum versus pancreas (p<0.001), pancreas versus islets (p>0.05) and islets versus parotid gland (p<0.05) were all statistically significant. Such a specific activity was not significantly different, however, in B and non-B cells (e.g. 0.14±0.01 nmol/min per mg protein in the experiment illustrated in Fig. 2).

Discussion

The phosphorylation of D-glucose, at the low concentration of D-glucose (1.0 mM) used in the present experiments, informs mainly on the activity of low-Km hexokinase isoenzyme(s). In this respect, the lower specific activity (p<0.001) found in the liver than in other organs (Table I) and the lower activity found in purified B islet cells than in non-B islet cells is consistent with a relatively low activity of the low-Km hexokinase(s), as distinct from high-Km glucokinase, in hepatocytes and insulin-producing islet B cells.

Relative to the activity of the hexokinase isoenzyme(s), as assessed by the phosphorylation of D-glucose (1.0 mM) by fresh homogenates, that of fructokinase, as estimated from the phosphorylation of D-fructose (also 1.0 mM) by heated homogenates, displayed the following hierarchy: liver > ileum > pancreas ~ islets ~ B and non-B islet cells ≥ parotid gland. A comparable hierarchy prevailed when the specific activity of fructokinase was estimated from the phosphorylation of D-fructose (1.0 mM) in the presence of K⁺ by heated homogenates and the protein content of the corresponding non-heated homogenates.

In the heated homogenates of either liver, pancreas or islets, the absence of K⁺ decreased, but did not abolish, the phosphorylation of D-fructose. Thus, pooling the results recorded in these three cases, the reaction velocity measured in the absence of K⁺ averaged 25.3±8.0% (n=9) of the paired value found in the presence of K⁺. Such a percentage is indeed significantly different from both unity (p<0.001) and zero (p<0.02). This finding is consistent with the knowledge that other monovalent cations than K⁺, such as Na⁺ contributed by both the ATP salt and NaOH used to bring the pH of the Hepes buffer to 7.5, may to a certain extent substitute for K⁺ to allow the expression of fructokinase catalytic activity (21).

At this point, it should be underlined that the present study does not inform on the isoform(s) of fructokinase found in each cell type. According to Hayward and Bonthron (22), in both rat and human, tissues expressing high levels of fructokinase (liver, kidney, duodenum) utilize exclusively the 3c exon isoform, while all other tissues (except pancreas) which express fructokinase at a low level used the 3a exon isoform. In the pancreas, a mixture of the two isoforms was observed, possibly reflecting low expression of the 3a form in exocrine pancreatic cells and higher expression of the 3c form in islet B-cells (22).

In both pancreatic gland and islet homogenates, but not in liver homogenates, prior heating of the homogenates decreased to a limited extent the phosphorylation of D-fructose as measured in the presence of D-mannohexulose (Fig. 1). Under
this experimental condition, the heated/fresh paired ratio in reaction velocity averaged 70.1±8.1% (pooled results recorded in pancreatic gland and islet homogenates; n=6; p<0.02). This is consistent with the knowledge that D-mannoheptulose, at the concentration tested in the present study, severely inhibits but does not totally suppress the phosphorylation of hexoses by hexokinase(s) (23).

As already alluded to, in the non-heated homogenates of islet cells, the phosphorylation of D-glucose, as well as that of D-fructose, was higher in non-B cells than in B cells (Fig. 2), with a paired ratio of 6.84±0.57 (n=2). This suggests a higher specific activity of the low-Km hexokinase in non-B cells than in islet B cells. The fact that this ratio was comparable in the case of D-glucose and D-fructose phosphorylation is consistent with the view that the latter enzyme was mainly responsible for the phosphorylation of D-fructose in the non-heated homogenates of islet cells (2).

In conclusion, the present study confirms the presence of fructokinase in rat pancreatic islets. It documents that the specific activity of fructokinase is lower in the islets than in liver, ileum or the whole pancreatic gland, but higher than in the parotid gland. It also suggests that there is no obvious difference between B and non-B islet cells in terms of either the specific activity of fructokinase or its activity relative to that of hexokinase isoenzyme(s).

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