Changes induced by sucrose administration on glucose metabolism in pancreatic islets in normal hamsters

M L Massa, M I Borelli, H Del Zotto and J J Gagliardino

CENEXA – Centre of Experimental and Applied Endocrinology (UNLP-CONICET, PAHO/WHO Collaborating Center), University of La Plata
School of Medicine, La Plata, Argentina

(Requests for offprints should be addressed to J J Gagliardino, CENEXA (UNLP-CONICET), Facultad de Ciencias Médicas, Calles 60 y 120, 1900 La Plata, Argentina; Email: gagliardino@infovia.com.ar)

Abstract

We correlated the changes in glucose-induced insulin secretion with those observed in glucose metabolism and hexokinase/glucokinase activity in islets from normal sucrose-fed hamsters. Blood glucose and insulin levels were measured in normal male hamsters fed with (S5) or without (C5) 10% sucrose in the drinking water for 5 weeks. Isolated islets (collagenase digestion) from both groups of animals were used to study insulin secretion, $^{14}\text{CO}_2$ and $^3\text{H}_2\text{O}$ production from D-[U- ^{14}C]-glucose and D-[5- ^3H]-glucose respectively, with 3.3 or 16.7 mM glucose in the medium, and hexokinase/glucokinase activity (fluorometric assay) in islet homogenates.

Whereas S5 and C5 animals had comparable normal blood glucose levels, S5 showed higher insulin levels than C5 hamsters (2.3 ± 0.1 vs 0.6 ± 0.03 ng/ml, $P < 0.001$). Islets from S5 hamsters released significantly more insulin than C5 islets in the presence of low and high glucose (3.3 mM glucose: 0.77 ± 0.04 vs 0.20 ± 0.06 pg/ng DNA/min, $P < 0.001$; 16.7 mM glucose: 2.77 ± 0.12 vs 0.85 ± 0.06 pg/ng DNA/min, $P < 0.001$) and produced significantly higher amounts of $^{14}\text{CO}_2$ and $^3\text{H}_2\text{O}$ at both glucose concentrations ($^{14}\text{CO}_2$: 3.3 mM glucose: 0.27 ± 0.01 vs 0.18 ± 0.01 , $P < 0.001$; 16.7 mM glucose: 1.44 ± 0.15 vs 0.96 ± 0.08 , $P < 0.02$; $^3\text{H}_2\text{O}$: 3.3 mM glucose: 0.31 ± 0.02 vs 0.15 ± 0.01 , $P < 0.001$; 16.7 mM glucose: 1.46 ± 0.20 vs 0.76 ± 0.05 pmol glucose/ng

DNA/min, $P < 0.005$). The hexokinase K_m and $V_{\max}$ values from S5 animals were significantly higher than those from C5 ones (K_m : 100.14 ± 7.01 vs 59.90 ± 3.95 μM , $P < 0.001$; $V_{\max}$: 0.010 ± 0.0005 vs 0.008 ± 0.0006 pmol glucose/ng DNA/min, $P < 0.02$). Conversely, the glucokinase K_m value from S5 animals was significantly lower than in C5 animals (K_m : 15.31 ± 2.64 vs 35.01 ± 1.65 mM, $P < 0.001$), whereas $V_{\max}$ figures were within a comparable range in both groups ($V_{\max}$: 0.048 ± 0.009 vs 0.094 ± 0.035 pmol glucose/ng DNA/min, not significant). The glucose phosphorylation ratio measured at 1 and 100 mM (hexokinase/glucokinase ratio) was significantly higher in S5 (0.26 ± 0.02) than in C5 animals (0.11 ± 0.01 , $P < 0.005$), and it was attributable to an increase in the hexokinase activity in S5 animals.

In conclusion, sucrose administration increased the hexokinase/glucokinase activity ratio in the islets, which would condition the increase in glucose metabolism by β -cells, and in β -cell sensitivity and responsiveness to glucose. These results support the concept that increased hexokinase rather than glucokinase activity causes the β -cell hypersensitivity to glucose, hexokinase being metabolically more active than glucokinase to up-regulate β -cell function.

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Introduction

Glucose homeostasis depends on the balance between insulin secretion and the response of the target tissues to the hormone. This balance is further modulated by different hormones and metabolites, physical activity, physiological states and dietary components (Like & Chick 1970, Stauffacher *et al.* 1970, Lombardo *et al.* 1983, Parsons *et al.* 1992, Luo *et al.* 1998).

Among those modulators, insulin resistance is a common denominator of several pathological conditions (Reaven 1988) that can strongly affect glucose homeostasis

and islet function (DeFronzo 1997). Under its influence, normal blood glucose levels can only be achieved by attaining high levels of circulating insulin. To cope with this increased demand for insulin, islets undergo marked functional and morphological changes, increasing the release of insulin in response to glucose and the β -cell mass (Lombardo *et al.* 1996, Massa *et al.* 1997, Pick *et al.* 1998).

In this regard, we have demonstrated that administration of sucrose to both normal rats (Lombardo *et al.* 1996, Pick *et al.* 1998) and hamsters (Massa *et al.* 1997, Del Zotto *et al.* 1999) induces insulin resistance, which in time triggers an increase in insulin secretion and in the β -cell

mass through an increase in the replication rate of β -cells and islet neogenesis. These changes were accompanied by a significant increase in the content of islet neogenesis-associated protein (INGAP) (Del Zotto *et al.* 2000), a cellular compound originally described by Vinik *et al.* (1996, 1997). However, we do not know the possible mechanism by which all these changes occurred.

Glucose-induced insulin release results from an increase in the rate of glucose metabolism in pancreatic β -cells (Ashcroft *et al.* 1970). Since the kinetics of glucose transport in the islets rapidly equilibrates its concentration across the cell membrane (Thorens *et al.* 1990), glucose phosphorylation becomes the rate-limiting step in the control of glucose metabolism. Further phosphorylation of glucose in the islets depends on the activity of two different kinases: hexokinase and glucokinase (Lenzen 1992, Lenzen & Panten 1988, Matschinsky 1990) of low and high Michaelis constant (K_m) for glucose respectively.

Because of this knowledge and in an attempt to further characterize the mechanism by which sucrose administration modifies the secretory function of the islets, we have currently studied insulin secretion elicited by glucose, glucose metabolism ($^{14}\text{CO}_2$ production from $\text{U-}^{14}\text{C}$ -glucose and $^3\text{H}_2\text{O}$ production from ^3H -glucose), and the hexokinase/glucokinase activity in islets isolated from normal hamsters fed with or without sucrose.

Materials and Methods

Chemicals and drugs

Collagenase was obtained from Serva Feinbiochemica, Heidelberg, Germany; D-[$\text{U-}^{14}\text{C}$]-glucose and D-[$5\text{-}^3\text{H}$]-glucose (10 $\mu\text{Ci}/\text{ml}$ (300 $\mu\text{Ci}/\text{mM}$)) were from New England Nuclear, Boston, MA, USA; bovine serum albumin (fraction V; BSA) and other reagents of the purest grade available were from Sigma Chemical Co., St Louis, MO, USA.

Experimental groups

Male Syrian hamsters of 23 days of age (30 ± 2 g), maintained in a temperature-controlled room (23°C) with a fixed 12 h light:12 h darkness cycle (lights on 0600–1800 h), were randomly divided into two groups of 20 animals each. The treated group had free access to a standard commercial diet plus 10% (w/v) sucrose in the drinking water for 5 weeks (S5), whereas the control group received the same diet and tap water for the same period (C5). Water intake was measured daily in both groups, while individual body weight was recorded once a week throughout the experimental period. The experiments were carried out in tissues from different animals from both control and treated groups.

Blood measurements and pancreas removal

At the time of death, blood samples were obtained from each animal (retro-orbital plexus) for determination of glucose (glucose-oxidase GOD-PAP method; Roche Diagnostics, Mannheim, Germany) and radioimmunoactive insulin levels by radioimmunoassay (RIA) (Herbert *et al.* 1965). The whole pancreas was also removed from each animal to isolate islets by collagenase digestion (Lacy & Kostianovsky 1967). The islets obtained from each isolation were used to study insulin secretion, glucose metabolism and enzymatic activity in different assays.

Insulin secretion in vitro

Groups of five isolated islets were incubated for 60 min at 37°C in 0.6 ml Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, previously gassed with a mixture of CO_2/O_2 (5%/95%) and containing 1% (w/v) BSA and different concentrations of glucose (3.3 and 16.7 mM) (Gagliardino *et al.* 1974). At the end of the incubation period, aliquots were taken from the medium to measure insulin by RIA (Herbert *et al.* 1965), using an antibody against rat insulin standard (Linco Research Inc., St Charles, MO, USA), and highly purified porcine insulin labelled with ^{125}I (Linde *et al.* 1980). Using this procedure, parallel tracer-displacement curves were obtained using either the rat insulin standard, or partially purified insulin extracted from a pool of hamster pancreata (Massa *et al.* 1997).

Glucose oxidation and utilization

Groups of 20 islets were incubated in a glass vial containing 40 μl KRB buffer supplemented with 10 mM Hepes (pH 7.4) containing D-[$\text{U-}^{14}\text{C}$]-glucose and D-[$5\text{-}^3\text{H}$]-glucose (10 $\mu\text{Ci}/\text{ml}$ (300 $\mu\text{Ci}/\text{mM}$)) in the presence of 3.3 or 16.7 mM glucose. This vial was placed inside an airtight-sealed 20 ml glass scintillation vial (500 μl distilled water in the bottom) which contained another empty glass vial; after 2 h at 37°C the reaction was stopped by adding 20 μl metabolic poison (400 mM citric acid, 10 mM rotenone, 10 mM antimycin and 3 mg KCN, pH 4.9 injected through the rubber seal) to the incubation vial; at the same time 250 μl hyamine was added to the empty tube. After incubation for 60 min at 37°C , the $^{14}\text{CO}_2$ fixed to hyamine was measured in vials containing 5 ml scintillation liquid. The islets were then incubated overnight at room temperature and glucose utilization was measured as $^3\text{H}_2\text{O}$ production captured by water in 5 ml scintillation liquid. The reaction tube was frozen at -20°C (Malaisse & Sener 1988).

Hexokinase/glucokinase assay

Groups of 20 isolated islets were homogenized (1 islet/ μl) in Hepes-NaOH buffer (50 mM, pH 7.5) containing

6 mM MgCl_2 , 60 mM KCl, 10 mM KH_2PO_4 , 1 mM EDTA, 1 mM L-cysteine and 0.02% BSA. Twenty microlitres of islet homogenate were dropped into another 20 μl of the reaction mixture (Hepes-NaOH, 10 mM ATP, 20 $\mu\text{Ci/ml}$ (300 $\mu\text{Ci/mM}$) D-[U- ^{14}C]-glucose and 1–100 mM unlabelled D-glucose) and incubated for 60 min at 37 °C. The reaction was stopped by the addition of 1 ml iced water and the diluted reaction medium was then passed through a column of AG 1-X8 (0.5 ml; Bio-Rad Laboratories, Hercules, CA, USA) to separate D-[U- ^{14}C]-glucose-6-phosphate from D-[U- ^{14}C]-glucose by ion exchange chromatography (Giroix *et al.* 1984). The column was later rinsed with 5×1 ml water, and the hexose phosphate was eluted with 3.0 ml 1 M ammonium formate/0.1 M formic acid. The eluate was mixed with 10 ml scintillation fluid (Ultima Gold XR; Packard, Meriden, CT, USA) and its radioactive content was determined. Blank values were obtained under identical conditions but in the absence of islet homogenate. Only 0.5% of the initial radioactivity was found in such control samples. Using this procedure, the enzymic activity measured in the presence of low (up to 1 μM) and high glucose concentrations corresponded to hexokinase and glucokinase respectively.

DNA content

Several 10 μl aliquots of islet homogenates from C5 and S5 hamsters were collected in tubes and stored at -70°C for subsequent measurements of DNA content by the fluorometric assay described by Labarca & Paigen (1980).

Statistical analysis

The experimental data were analyzed using Student's *t*-test. Data are expressed as the means $\pm$ S.E.M. Differences were considered significant when $P < 0.05$.

Results

Body weight and water intake

Similar body weights were recorded in S5 ($n=20$) and C5 ($n=20$) animals (66.1 ± 8.6 vs 68.3 ± 7.2 g) at the end of the experiment. Animals from the S5 group drank a significantly larger volume of water than those from the C5 group (30.4 ± 3.7 vs 22.1 ± 1.3 ml/day, $P < 0.001$).

Blood glucose and serum insulin levels

There were no significant differences in the glucose levels measured at the time of death in both groups of animals (S5 ($n=20$): 5.7 ± 0.3 vs C5 ($n=20$): 5.9 ± 0.22 mM). Conversely, S5 hamsters showed higher insulin levels than C5 hamsters (2.3 ± 0.1 vs 0.6 ± 0.03 ng/ml ($n=20$ animals in each group), $P < 0.001$).

Figure 1 Insulin secretion (pg insulin/ng DNA/min) is represented in each group as the mean value $\pm$ S.E.M.; the number of cases is shown above each bar, and was obtained in three different experiments performed with islets isolated from six hamsters from each experimental group. $P < 0.001$: a vs b and c vs d.

Since blood glucose levels were comparable in S5 and C5 animals, the uneven serum insulin values increased the insulin–glucose molar ratio in the S5 group, as compared with the corresponding control values (2.24×10^{-6} vs 5.6×10^{-7} respectively).

Insulin secretion

Islets incubated with high glucose released significantly more insulin than those incubated with low glucose in both groups. On the other hand, islets isolated from S5 hamsters released significantly larger amounts of insulin than those from C5 animals in response to either a low or high glucose concentration (3.3 mM glucose: 0.77 ± 0.04 vs 0.20 ± 0.06 pg insulin/ng DNA/min, $P < 0.001$; 16.7 mM glucose: 2.77 ± 0.12 vs 0.85 ± 0.06 pg insulin/ng DNA/min, $P < 0.001$) (in all cases $n=9$) (Fig. 1).

Glucose metabolism

The production of $^{14}\text{CO}_2$ from D-[U- ^{14}C]-glucose and $^3\text{H}_2\text{O}$ from D-[5- ^3H]-glucose by the isolated islets increased significantly when the glucose concentration in the incubation medium was raised from 3.3 to 16.7 mM in both experimental groups. Both $^{14}\text{CO}_2$ and $^3\text{H}_2\text{O}$ production was significantly higher in islets isolated from S5 than from C5 animals in the presence of either low or high glucose in the medium ($^{14}\text{CO}_2$: 3.3 mM glucose: 0.27 ± 0.01 ($n=11$) vs 0.18 ± 0.01 ($n=12$), $P < 0.001$; 16.7 mM glucose: 1.44 ± 0.15 ($n=18$) vs 0.96 ± 0.08 ($n=18$) pmol glucose/ng DNA/min, $P < 0.02$; $^3\text{H}_2\text{O}$: 3.3 mM glucose: 0.31 ± 0.02 ($n=18$) vs 0.15 ± 0.01 ($n=18$), $P < 0.001$; 16.7 mM glucose: 1.46 ± 0.20 ($n=18$)

Figure 2 (A) Glucose oxidation and (B) glucose utilization measured as $^{14}\text{CO}_2$ from D-[U- ^{14}C]-glucose and $^3\text{H}_2\text{O}$ from D-[5- ^3H]-glucose respectively. Each group represents the mean value $\pm$ S.E.M. expressed as pmol glucose/ng DNA/min. The number of cases is shown above each bar, and was obtained in three different experiments performed with islets isolated from six hamsters from each experimental group. $P < 0.001$ in A and B: a vs b and c vs d.

vs 0.76 ± 0.05 ($n=17$) pmol glucose/ng DNA/min, $P < 0.005$) (Fig. 2A and B respectively).

Hexokinase and glucokinase activity

At increasing concentrations of glucose (up to 1 mM), the rate of glucose phosphorylation by islet homogenates was compatible with the participation of a hexokinase-like enzyme, with a K_m for glucose close to 59 μM . On the other hand, when increasing the concentration of glucose up to 100 mM, the rate was compatible with the participation of a glucokinase-like enzyme, with a K_m close to 35 mM. The K_m of hexokinase measured in S5 vs C5 islets (100.14 ± 7.01 vs 59.90 ± 3.95 μM , $P < 0.001$) as well as its maximal velocity ($V_{\max}$) (0.010 ± 0.0005 vs 0.008 ± 0.0006 pmol glucose/ng DNA/min, $P < 0.02$, 25% increase) were significantly higher in S5 islets

Figure 3 Glucose phosphorylation kinetics of islet homogenates. Each value represents the mean of 12 replicates performed in four different experiments with islets isolated from eight hamsters from each experimental group. The plot ($v_i = K_m \times v_i / \text{Eadie-Hofstee } S + V_{\max}$) (where v_i is initial velocity and S is substrate concentration) was used to estimate the K_m and $V_{\max}$ values for hexokinase and glucokinase. In both experimental groups (A) hexokinase activity was measured at 0.05, 0.01, 0.25, 0.5 and 1 mM glucose, while (B) glucokinase activity was tested at 5, 10, 50 and 100 mM glucose. The comparison of v_i/S values (means $\pm$ S.E.M.) for hexokinase and glucokinase measured at each glucose concentration within each experimental group showed significant differences ($P < 0.001$), except in the case of 50 vs 100 mM glucose in the control group (C5).

(Fig. 3A). Conversely, glucokinase K_m from S5 was significantly lower than that from C5 islet homogenates (15.31 ± 2.64 vs 35.01 ± 1.65 mM, $P < 0.001$), whereas no significant differences in $V_{\max}$ were found between both groups (0.048 ± 0.009 vs 0.094 ± 0.035 pmol glucose/ng DNA/min, not significant) (Fig. 3B).

On the other hand, the glucose phosphorylation ratio measured at 1 and 100 mM glucose was significantly higher in S5 than in C5 hamsters (0.26 ± 0.02 vs 0.11 ± 0.01 , $P < 0.005$), suggesting a higher hexokinase/glucokinase activity ratio in S5 animals. Such a difference could be attributed to an increased hexokinase activity in S5 animals.

DNA content

The DNA content of islets isolated from C5 and S5 hamsters was 57 ± 2.85 and 41.5 ± 2.02 ng DNA/islet respectively ($P < 0.005$). Thus, islets from C5 animals would have a larger number of cells than those from S5 hamsters.

Discussion

Our results show that islet β -cells from sucrose-treated animals released more insulin than those from control animals, in the presence of low or high glucose. This effect was observed either *in vitro* or *in vivo* (normoglycaemia with hyperinsulinaemia), and is in agreement with that previously reported by our group using the same experimental model (Massa *et al.* 1997, Del Zotto *et al.* 1999). These functional changes were accompanied by a significant increase (100%) in the β -cell mass due to an increased β -cell replication rate and islet neogenesis without changes in the percentage of α - and β -cells (Massa *et al.* 1997, Del Zotto *et al.* 1999, 2000). The latter process resulted in an increment of small-sized islets, which is reflected in the current experiments in the lower DNA content measured in S5 islets. Sucrose-induced insulin resistance might be the underlying mechanism that triggered these functional and morphological β -cell changes (Massa *et al.* 1997, Del Zotto *et al.* 1999, 2000).

Glucose stimulates insulin secretion in a dose-response sigmoidal fashion (Ashcroft *et al.* 1970), and the shape of this curve follows the same pattern as that of glucose phosphorylation in the islets (Ashcroft *et al.* 1970, Matschinsky 1990).

Glucose phosphorylation is the rate-limiting step for glucose metabolism and for triggering the release of insulin (Lenzen & Panten 1988, Matschinsky 1990, Lenzen 1992). It is accomplished by two different enzymes, with high and low affinity for glucose (hexokinase and glucokinase respectively) (Lenzen & Panten 1988). The presence of these two kinases in β -cells enables the islets to effectively phosphorylate glucose in the presence of a wide range of glucose concentrations, adjusting appropriately the release of insulin to the actual glucose level.

Changes in the release of insulin are accompanied by simultaneous changes in glucose metabolism (and islet phosphorylating activity), as it occurs in different physiological conditions, such as pregnancy (Brelje & Sorenson 1988, Cockburn *et al.* 1997), and in several experimental conditions characterized by β -cell overload and hyperglycaemia (Loubatieres 1964, Lee *et al.* 1989, Epstein *et al.* 1992, Becker *et al.* 1996, Leahy 1996). In such circumstances, β -cells undergo a simultaneous increase in glucose sensitivity and responsiveness, in glucose metabolism and in the hexokinase/glucokinase ratio. The islets from our sucrose-treated hamsters showed all these features, namely, a higher release of insulin in response to glucose, together with an increased glucose metabolism and hexokinase/glucokinase ratio, but in the presence of normal blood glucose levels. Therefore, the overload of β -cells *per se* rather than hyperglycaemia would trigger the changes in β -cell function.

The hexokinase/glucokinase ratio in S5 animals increased 279 times compared with controls; the magnitude of this increment was similar to that reported by

Hosokawa *et al.* (1995) in islets from 90% pancreatectomized rats. The 25% increase in islet hexokinase $V_{\max}$ measured in our S5 animals – comparable with that stated by Hosokawa *et al.* (1995) – without concomitant changes in glucokinase activity, can account for the increased hexokinase/glucokinase ratio, and in time for the increased glucose metabolism and β -cell hypersensitivity and hyper-responsiveness to glucose. The fact that hexokinase overexpression in β -cells (Epstein *et al.* 1992, Becker *et al.* 1996) induces similar changes in their secretory function lends support to the latter assumption.

Together, these results reinforce the concept that (a) under conditions of β -cell overload, hexokinase partially takes control over the glucose set-point for insulin secretion, causing the β -cell hypersensitivity to glucose (Lenzen & Panten 1988, Epstein *et al.* 1992, Lenzen 1992, Newgard 1992, Hosokawa *et al.* 1995, Leahy 1996), and (b) hexokinase is metabolically more active than glucokinase to up-regulate the β -cell function. Consequently, it could be assumed that sucrose induces an increase in the hexokinase/glucokinase activity ratio in the islets, which is responsible for the increase in β -cell glucose metabolism, and in the β -cell sensitivity and responsiveness to glucose. These changes make the islets capable of overcoming the increased demand of insulin – elicited by sucrose feeding – keeping blood glucose levels within a normal range at the expense of an increased release of insulin (hyperinsulinaemia), characteristic of the insulin-resistant state (Reaven 1988).

In our model, the morphological and functional changes depicted in the islets were accompanied by a significant increment in the mass of INGAP-positive cells (Del Zotto *et al.* 2000). It remains to be demonstrated whether this compound might also play a role in the control of the metabolic changes currently described.

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