Increased glucocorticoid sensitivity in islet beta-cells: effects on glucose 6-phosphatase, glucose cycling and insulin release

Z-C. Ling[1], A. Khan[1], F. Delauny[2], B. Davani[2], C-G. Östenson[1], J-Å. Gustafsson[2], S. Okret[2], BR. Landau[3], S. Efendic[1]

[1] Department of Molecular Medicine, Endocrine and Diabetes Unit, Karolinska Hospital, Stockholm, Sweden
[2] Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden
[3] Departments of Medicine and Biochemistry, Case Western Reserve University, Cleveland, Ohio, USA

Summary Glucose-6-phosphatase (G6Pase) activity and the rate of glucose cycling are increased in islets from animal models of Type II (non-insulin-dependent) diabetes mellitus. Glucocorticoid treatment further stimulates these processes and inhibits glucose-induced insulin release. To determine whether these effects result from a direct action of glucocorticoids on the beta-cells, we used isolated islets. The islets were from transgenic mice overexpressing the glucocorticoid receptor in their beta-cells to increase the cells' sensitivity to glucocorticoid. Islets from transgenic and non-transgenic control mice utilized and oxidized the same amount of glucose. In contrast, islet G6Pase activity was 70 % higher, glucose cycling was increased threefold and insulin release was 30 % lower in islets from transgenic mice. Hepatic G6Pase activity was the same in transgenic and control mice. Dexamethasone administration increased G6Pase activity and glucose cycling and decreased insulin release in both transgenic and control mouse islets. We conclude that glucocorticoids stimulate islet G6Pase activity and glucose cycling by acting directly on the beta-cell. That activity may be linked to the inhibition of insulin release. [Diabetologia (1998) 41: 634–639]

Keywords Glucose-6-phosphatase, insulin release, glucose metabolism, glucocorticoid sensitivity, transgenic mice.

Glucose-6-phosphatase (G6Pase) and glucokinase activities can be demonstrated in pancreatic islets from mammalian species [1–4]. Glucokinase appears to be the rate limiting enzyme of glycolysis in the beta-cell, regulating the dose-response relationship between glucose utilization and insulin release [4]. Since G6Pase opposes the action of glucokinase, a possible role for G6Pase in the metabolic regulation of insulin release was postulated [2, 3]. There are several reports of relatively low G6Pase activity in homogenates of normal mammalian islets [1–4], one

of activity exceeding that in liver [5] and one of no activity [6]. We found low activity in permeabilized and sonicated islets from normal rats and mice [7]. Sweet et al. [8] also found low G6Pase activity in intact islets from normal rats.

Glucose cycling is the simultaneous phosphorylation of glucose to glucose-6-P and dephosphorylation of glucose-6-P to glucose with the resulting consumption of ATP [9]. In accordance with reports of low G6Pase activity, we demonstrated a low rate of glucose cycling in intact islets from normal mice, dephosphorylation occurring at only 3 % of the rate of phosphorylation [10]. However, in islets from ob/ob mice, 30–40 % of phosphorylated glucose was dephosphorylated [10]. Glucose cycling was also increased in islets from other animal models of Type II diabetes [11, 12]. In accordance with these results, G6Pase activity was several fold higher in islets from diabetic than normal animals [7, 13] and islet G6Pase gene expression was increased during the development of diabetes in Zucker rats [14].

Glucocorticoids increase hepatic glucose production and decrease glucose uptake in man [15]. Increased glucose production is accompanied by increased gluconeogenesis and associated with enhanced G6Pase gene expression and enzyme activity [16, 17]. Recently, a glucocorticoid response element was identified in the human G6Pase gene promoter, suggesting that the effects of glucocorticoids on G6Pase are directly mediated by the glucocorticoid receptor (GR) [18]. We previously reported an increase of G6Pase activity in islets from ob/ob mice treated with dexamethasone [7], indicating the enzyme's activity is also regulated in islets by glucocorticoids. This effect was associated with increased glucose cycling and decreased insulin release suggesting a role for G6Pase in insulin release. However, those findings could not be ascribed to a direct action of glucocorticoids on the beta-cell, since glucocorticoids have multiple effects on whole body glucose homeostasis as well as on non beta-cells, including glucagon-producing cells [15, 19].

Recently, Trinh et al. overexpressed the hydrolase catalytic subunit of the hepatic G6Pase complex in an insulinoma cell line [20]. They observed increased glucose cycling and decreased insulin secretion, but not the marked alteration in beta-cell metabolism found in the rodent models of obesity and Type II diabetes. Therefore, they postulated the possible involvement of genes, in addition to the hydrolase, in the etiology of beta-cell dysfunction in these models.

By overexpressing GR in the beta-cell, we recently generated transgenic mice with increased beta-cell glucocorticoid sensitivity [21]. The mice exhibited mild glucose intolerance and a dramatic decrease in acute insulin response. In the present study, we used isolated islets from the mice to examine the consequence of an increased beta-cell glucocorticoid sensitivity on G6Pase activity, glucose cycling and insulin release.

Materials and methods

Materials. [1-^{14}C]Glucose-6-P (specific activity 60 mCi/mmol), [5-^{3}H]glucose (15.7 Ci/mmol) and tritiated water (100 mCi/ml) were purchased from DuPont NEN (Boston, Mass. USA). Glucose-6-P, β-glycerol-P, (N-[2-hydroxyethyl]piperazine-N'-2-ethane sulphonic acid) (HEPES) and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Amberlite MB-3 monobed resin was purchased from BDH Ltd. (Poole, UK). Collagenase was purchased from Boehringer Mannheim GmbH (Mannheim, Germany).

Animals. Control and transgenic mice, 3–4 months old, weighing 20–29 g, were used. Increased beta-cell sensitivity to glucocorticoids in the transgenic mice was achieved by overexpressing the GR under the control of the insulin promoter [22]. The characteristics of these animals have been described previously [21].

Isolation of islets. One group of control and transgenic mice was killed to isolate pancreas and liver. A second group was injected i. p. with dexamethasone, 25 μg in 0.25 ml saline or saline only, at 0900 hours on the first day of the study and again at 0900 hours on the second and third days. The mice were killed by decapitation 2 h after injection on the third day. Blood was collected for determination of plasma glucose concentration. Pancreata were digested for 10–12 min under continuous shaking (150 strokes/min) at 37 °C in 2.5–3.0 ml of Hank's balanced salt solution containing 6–8 mg collagenase. An additional 15–20 ml of the cold balanced salt solution was added and the suspension was allowed to settle. The sediment was washed several times with the cold solution and the islets from the sediment were collected using a glass pipette under stereomicroscopy.

Preparation of hepatic microsomes. All procedures were performed at 0–4 °C. Livers from control and TG mice were homogenized in 250 mmol/l sucrose, 10 mmol/l HEPES buffer (pH 7.5) and the microsomes were collected by differential centrifugation. They were re-suspended in the sucrose-HEPES buffer at a final concentration of 3–5 μg protein/10 μl. Disrupted microsomes were prepared by preincubating the microsomes with 0.1 % Triton for 20 min in ice-water and then diluting with sucrose-HEPES to the same protein concentration as for the intact microsomes. Microsomes were regarded as "intact" if the latency of mannose-6-phosphohydrolase activity was more than 90 %, i.e less than 10 % of the activity exhibited in the absence of Triton was observed in its presence [23].

Glucose cycling, glucose utilization and oxidation. Glucose cycling was determined from the incorporation of ^{3}H at position 2 of glucose after incubation of islets with ^{3}H$_2$O and unlabelled glucose. Estimates depend on the assumption that before each molecule of glucose 6-P is hydrolysed to glucose, because of rapid equilibration between glucose 6-P and fructose 6-P, a hydrogen from the medium is bound to carbon 2 of the glucose 6-P. Rapid equilibration of glucose 6-P with fructose 6-P is expected because of a relatively high activity of phosphohexose isomerase in islets [24]. Isotopic equilibration in human liver was estimated to reach 80–92 % [25]. To the extent isotopic equilibration is incomplete, cycling is underestimated.

For the determination of glucose cycling, 25 islets were placed in an incubation vial with 16.7 mmol/l glucose, 0.5 % bovine albumin and 2.5 mCi of ^{3}H$_2$O in 50 μl of Krebs-bicarbonate buffer (pH 7.4). Each vial with its content was placed in a scintillation bottle, sealed and gassed with O$_2$/CO$_2$ (19:1 by volume) for 3 min. After 2 h incubation at 37 °C, 100 μl of perchloric acid was injected into the vial. The vial was removed from the scintillation bottle and 2 mg of glucose was added. The contents were centrifuged for 2 min (10 000 g), the supernatant neutralized with KOH and the precipitated potassium perchlorate removed by centrifugation. The resulting supernatant was passed through the MB-3 resin. The effluent was freeze dried several times and the residue dissolved in water and subjected to HPLC (HPX-87P system, Bio-Rad, Hercules, Calif. USA) to purify the glucose. A portion of the purified glucose was assayed for radioactivity and glucose concentration determined using a glucose oxidase method (Glucose Analyser, Model 23A, Yellow Springs Co., Yellow Springs, Ohio, USA). Another portion of the purified glucose was incubated with glucose oxidase and the resulting reaction mixture was deionized, concentrated and the concentrate chromatographed. No radioactivity was found with the mobility of glucose after incubation with glucose oxidase.

For the measurements of glucose oxidation and utilization, 25 islets were incubated in an identical manner to that with ^3H_2O, except that instead the islets were incubated with [U-^{14}C]glucose and [5-^{3}H]glucose. The incubation time was 1.5 h. After incubation, 100 µl of 10 % perchloric acid was injected into the vial and 250 µl of Hyamine (Packard Instrument Co., Meriden, Conn., USA) and 250 µl of water were injected into the bottle to absorb $^{14}CO_2$ and ^3H_2O. Parallel incubation was performed, but without islets.

$^{14}CO_2$ and ^3H_2O were collected overnight. The vial with its acidified contents was removed and scintillation fluid (Ultima Gold, Packard Instrument Co.) added to the bottle, which was then assayed for ^{14}C and ^{3}H in dual channels using a liquid scintillation spectrophotometer (Packard Instrument Co., Tri-Carb 1900 TR Liquid Scintillation Analyser). The recovery of $^{14}CO_2$ and ^3H_2O was assayed by incubating medium containing sodium ^{14}C bicarbonate and ^3H_2O without islets and collecting $^{14}CO_2$ and ^3H_2O in an identical manner.

Islet G6Pase activity. Batches of 25 islets were placed in tubes containing 50 mmol/l HEPES (pH 7.4) with 20 mmol/l β-glycerol-P and sonicated 10 s to disrupt the islets [7]. β-glycerol-P was added to the incubation medium to inhibit non-specific phosphatase activity. 0.5 µCi [1-^{14}C]glucose-6-P (4 mmol/l) was added to each tube, mixed and incubated for 20 min at 37 °C. The volume of incubation medium was 200 µl. Parallel incubations were performed without islets. Incubation was stopped by placing the incubate in ice-water and adding 200 µl of 0.3 mol/l $ZnSO_4$, and after mixing, 200 µl of a saturated solution of $Ba(OH)_2$. Then, 2 mg of carrier glucose was added to each incubation vial and glucose isolated from the reaction mixture as described above.

Hepatic G6Pase activity. Hepatic microsomes were incubated for 10 min at 37 °C in a medium containing 4 mmol/l glucose 6-P, 0.1 µCi [1-^{14}C]glucose 6-P, 2–5 µg of microsomal protein, 50 mmol/l sodium acetate in a total volume of 50 µl. Incubation was stopped by placing the tubes in ice-water and adding 50 µl 0.3 mol $ZnSO_4$ and after mixing 50 µl of a saturated solution of $Ba(OH)_2$. Then again 2 mg of carrier glucose was added to each incubation vial and glucose isolated as described above. The activity of G6Pase in intact endoplasmic reticulum membrane estimates the translocase function, whereas disruption of the membrane by Triton provides a measure of the hydrolase component [23].

Insulin release. Batches of three islets were incubated in triplicate for 1 h at 37 °C in 300 µl Krebs bicarbonate buffer (pH 7.4), containing 2 mg/ml bovine albumin and 5.5 or 16.7 mmol/l glucose. After incubation, an aliquot of the medium was stored at − 70° for insulin assay. Insulin was measured by radioimmunoassay with the addition of charcoal to separate free and bound antibody [26]. The sensitivity of the assay was 6 µU/ml, and the inter- and intra-assay coefficients of variation were 10 %. The islets from the incubation medium were washed with Krebs bicarbonate buffer and insulin was extracted with acid-ethanol. Islet DNA content was assayed in batches of 25 islets using a fluorometric method modified by Hinegardner [27].

Calculations. Glucose cycling was calculated from the dpm per min from ^3H_2O in the glucose and the amount of [5-^{3}H]glucose (pmol) utilized by the islets. The dpm in the HPLC glucose peak multiplied by 2.15, i.e. 2.0 mg of glucose added at the end of the incubation plus 0.15 mg of glucose in the medium at the beginning of incubation, and divided by the milligrams of glucose in the peak fraction gave the dpm of ^{3}H from the

Table 1. Characteristics of control and transgenic mice

	Control (n = 17)	Transgenic (n = 17)
Weight (g)	29.6 ± 1.1	28.1 ± 1.9
Plasma glucose (mmol/l)	4.9 ± 0.3	5.6 ± 0.4
Islet DNA (ng/islet)	29.3 ± 5.8	26.8 ± 1.6
Insulin content (µU/islet)	214.8 ± 19.7	226.7 ± 26.6

Results are expressed as means ± SEM

Table 2. Glucose utilization, oxidation and cycling in control and transgenic mice islets incubated at glucose concentrations of 5.5 and 16.7 mmol/l

Parameters	n	Control	Transgenic	Glucose (mmol/l)
Glucose utilization (pmol × islet^{-1} × h^{-1})	6	24.8 ± 1.9[a]	30.7 ± 2.9[a]	5.5
		54.4 ± 3.3	61.4 ± 6.1	16.7
Glucose oxidation (pmol × islet^{-1} × h^{-1})	6	10.8 ± 0.7[b]	11.9 ± 1.2[b]	5.5
		29.5 ± 2.1	28.5 ± 2.5	16.7
Glucose cycling (%)	5	5.9 ± 1.0	16.5 ± 2.9[c]	16.7

Results are expressed as ± SEM. [a] $p < 0.001$ vs 16.7 mmol/l, glucose utilization; [b] $p < 0.001$ vs 16.7 mmol/l, glucose oxidation; [c] $p < 0.05$ vs control, glucose cycling

^3H_2O incorporated into glucose. Because there are 0.55×10^{10} dpm in 2.5 mCi of ^{3}H and 0.55×10^{10} picoatoms of hydrogen in 50 µl of water, the dpm in glucose equals the picoatoms of ^{3}H incorporated into glucose. Fraction of ^{3}H incorporated to carbon 2 of glucose was taken as 0.45 for both control and TG mice. Glucose cycling in percent was then 100 (^{3}H bound to carbon 2 of glucose)/(glucose utilization + ^{3}H bound to carbon 2 of glucose).

G6Pase activity in the incubations with islets and microsomes was taken as the amount of glucose 6-P hydrolysed to glucose, traced with ^{14}C. The specific activity of [1-^{14}C]glucose 6-P in the incubate was calculated from the quantity of glucose 6-P in the incubate (pmoles) and the amount of ^{14}C in that glucose 6-P. The dpm in the 2 mg glucose in the incubation medium after stopping the incubation and adding the 2 mg of carrier glucose was calculated from the ^{14}C activity (dpm) in the effluent from the HPLC column and the amount of glucose in the effluent. The pmoles of glucose 6-P hydrolysed to glucose were then calculated by dividing the dpm in 2 mg of glucose by the specific activity of [1-^{14}C]glucose 6-P in dpm/pmol after subtracting the dpm in 2 mg of glucose when the identical incubation was done, except in the absence of islets. Activities of hepatic G6Pase are expressed per mg of microsomal protein. Protein concentration in microsomes was determined using a Bio-Rad method [28]. Results are expressed as means ± SEM. Differences between groups were tested for significance using Student's *t* test for unpaired data.

A p value less than 0.05 was considered statistically significant.

Results

The transgenic mice overexpressing the glucocorticoid receptor in their beta-cells and non-transgenic control mice had similar body weights and plasma glucose concentrations (Table 1). Content of DNA and insulin in isolated islets did not differ between

Table 3. Glucose-6-phosphatase activity in islets from control and transgenic mice treated with saline or dexamethasone (Dex).

Treatment	n	Glucose-6-phosphatase activity ($pmol \times islet^{-1} \times min^{-1}$)	
		Control	Transgenic
Saline	8	2.3 ± 0.4	3.6 ± 0.4[a]
Dex	8	4.0 ± 0.5	6.3 ± 0.4[b]

Results are expressed as means ± SEM. [a]$p < 0.05$ vs control, saline treated; [b]$p < 0.01$ vs control, dexamethasone treated

Table 4. Hepatic glucose-6-phosphatase activity in control and transgenic mice treated with saline or dexamethasone (Dex)

Treatment	n	Glucose-6-phosphatase activity ($nmol \times mg\ protein^{-1} \times min^{-1}$)			
		Translocase		Hydrolase	
		Control	Transgenic	Control	Transgenic
Saline	5	110 ± 19	106 ± 15	182 ± 32	182 ± 23
Dex	5	150 ± 21	162 ± 26	230 ± 49	216 ± 25

Results are expressed as mean ± SEM
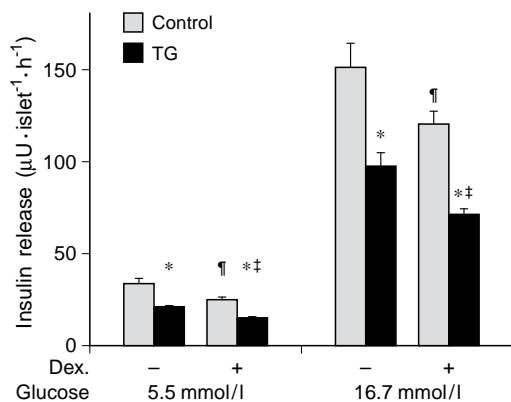
Fig. 1. Glucose induced insulin release in islets from control and TG mice treated with saline ($n = 8$) and dexamethasone (Dex;) ($n = 8$) at 5.5 and 16.7 mmol/l glucose. *$p < 0.05$ control vs TG mice; ¶ $p < 0.05$ saline vs dexamethasone treatment of control mice, ‡ $p < 0.05$ saline vs dexamethasone treatment of TG mice

the transgenic and control group (Table 1). Islets from transgenic mice utilized and oxidized as much glucose as islets from control mice, utilizing and oxidizing 2–3 times as much at 16.7 as at 5.5 mmol/l glucose (Table 2). In contrast, at 16.7 mmol/l glucose, 16 % of glucose phosphorylated by islets from transgenic mice was dephosphorylated, 3 times more than by control islets (Table 2). G6Pase activity in transgenic mice islets was 57 % higher than in control islets (Table 3). When groups of transgenic and control mice were treated with dexamethasone for 2 days prior to isolation of islets, G6Pase activity was increased about 75 % in both groups (Table 3). In con-

trast, G6Pase activity was similar in hepatic microsomes from transgenic and control mice and dexamethasone treatment increased translocase activity about 45 % and hydrolase activity about 25 % (Table 4). Similar G6Pase activity in livers of transgenic and control mice and increased activity in transgenic mouse islets demonstrate that the overexpression of GR is specific to the islet beta-cell. Islets from transgenic mice incubated with 5.5 and 16.7 mmol/l glucose released 30 % less insulin than islets from control mice, with 4–5 times as much insulin released at 16.7 as 5.5 mmol/l glucose (Fig. 1). Dexamethasone treatment decreased the insulin release from both groups by about 35 %. Thus, increased glucocorticoid sensitivity in islet beta-cells results in increased G6Pase activity and glucose cycling and decreased insulin release.

Discussion

Biological effects of glucocorticoids are mediated through the GR and the intracellular GR concentration is a major factor determining the sensitivity of target cells to the glucocorticoids [29]. Since in our transgenic mouse, the overexpression of the GR transgene is controlled through the insulin promoter, the increased effect of glucocorticoids in these animals should be limited to beta-cells [21]. In accordance with this, no GR transgene expression was found in brain and liver and high expression of the transgene was demonstrated in beta-cells of transgenic mice, but not in control mice [21].

Therefore, the present results indicate that glucocorticoids enhance islet G6Pase activity by acting directly on beta-cells. Previously reported increased enzyme activity in islets of ob/ob mice after dexamethasone treatment [2, 7] was most probably also due to a direct hormonal action on beta-cells and not secondary to alteration of metabolism at other sites. The same mechanism probably explains the increase in the enzyme activity in normal mice treated with dexamethasone which we observed. Previously reported increased activity of G6Pase in ob/ob mice islets [2, 7] may have been due to increased corticosterone availability found in the animals [30].

An increased activity of islet G6Pase by 70 % in transgenic mice increased islet glucose cycling threefold. Interestingly, islet glucose utilization and oxidation in transgenic mice were comparable with those in control animals, demonstrating that the enhanced glucose flux through G6Pase did not alter glucose utilization. Similarly, glucose utilization and oxidation were comparable in islets from ob/ob mice treated with dexamethasone or saline, although glucose cycling was increased from 36 % to 56 % following dexamethasone treatment [31]. These findings agree with in vitro studies, where dexamethasone had no ef-

fect on glucose oxidation and NAD(P)H changes [32].

Previous studies described increased [33], unchanged [34], and decreased [35] insulin secretion upon administration of glucocorticoids to rats and mice. These results are difficult to interpret because of differing strains of animals and of doses and durations of administration of glucocorticoids. Glucocorticoids in vitro have also been reported to inhibit [34] or had no effect [36] on insulin release. In transgenic compared with control islets, glucose-induced insulin release was decreased and dexamethasone further decreased insulin release. In experiments with transgenic mice in vivo, the early insulin response to a glucose challenge was suppressed and glucose tolerance was decreased [21]. This provides direct evidence that glucocorticoids exert a diabetogenic effect via the suppression of insulin response, in addition to their other diabetogenic actions such as decreased glucose uptake and increased gluconeogenesis [15, 17]. This may be relevant to the increased incidence of diabetes mellitus in patients with Cushing's disease and patients treated with glucocorticoids [15, 37]. Inhibition of insulin release was not due to decreased insulin content in the islets, since content was similar in transgenic and control islets.

Our results confirm recent reports that dexamethasone inhibited insulin secretion by the islets in a concentration dependent manner and the inhibition was prevented by a blocker of nuclear glucocorticoid receptor [32, 38]. In the insulinoma cell line, overexpressing 10-fold the hydrolase subunit of hepatic G6Pase, glucose cycling was increased 10-fold to 7.1 % and insulin secretion was decreased by 30 %. However, unlike findings in our transgenic and ob/ob mice, glucose utilization was decreased by 30 % [20]. The reason for this apparent discrepancy is not clear.

Glucose stimulates insulin release by generating an increased ATP/ADP ratio which closes ATP sensitive K^+-channels and depolarizes beta-cell membrane. This in turn results in increased Ca^{2+} influx and exocytosis of insulin [39]. Since an increased rate of glucose cycling in islets results in increased ATP consumption, a linkage is possible between the increase in cycling and G6Pase activity and the decrease in insulin release. The finding that in diabetic animal models increased cycling and phosphatase activity is associated with decreased glucose-induced insulin secretion [7, 10–13] supports this possibility.

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