Effects of glucose refeeding and glibenclamide treatment on glucokinase and GLUT2 gene expression in pancreatic B-cells and liver from rats

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The mutual role of glucose and insulin in the regulation of glucokinase and GLUT2 glucose transporter gene expression in pancreatic B-cells and liver has been studied *in vivo* in the rat. Glucokinase mRNA was quantified by competitive reverse-transcriptase PCR analysis, and GLUT2 mRNA by Northern-blot analysis in total RNA fractions. As in the liver, glucokinase mRNA decreased by 64% in pancreatic B-cells after starvation for 2 days and was induced 3-fold by short-term treatment (1 h) of the rats with oral glucose (4 g/kg body wt.). In contrast the sulphonylurea compound glibenclamide (0.1 mg/kg body wt.) did not significantly stimulate glucokinase gene expression in pancreatic B-cells. But glibenclamide caused a 4-fold increase of

glucokinase mRNA in liver which was abolished by concomitant administration of diazoxide, a drug which antagonizes glibenclamide-stimulated insulin secretion. GLUT2 gene expression was decreased by 50% in pancreatic B-cells and liver after starvation of the rats for 2 days. Neither short-term treatment (1 h) with glucose nor glibenclamide resulted in a significant increase of GLUT2 gene expression in pancreatic B-cells and liver. The results suggest that it is glucose which stimulates glucokinase gene expression in pancreatic B-cells whereas the transcriptional regulation of the glucokinase gene in liver is directed by insulin.

INTRODUCTION

The signal for glucose-induced insulin secretion in pancreatic B-cells is generated in its metabolism [1–3]. The signal recognition apparatus is composed of the low-affinity glucose transporter GLUT2 [4,5] and the low-affinity glucose phosphorylating enzyme glucokinase [1,2,6–8]. Glucokinase enzyme activity is nutrient dependent (for reviews see [1,2]). Glucose has been reported to be a physiological regulator of the glucokinase enzyme activity in pancreatic B-cells [9–11] while liver glucokinase enzyme activity is controlled by insulin [12]. In studies on cultured pancreatic islets [13–15] and insulin-producing tumour cells [16], glucose has also been considered a potent regulator of GLUT2. GLUT2 and glucokinase gene expression are likewise dependent on the nutritional status [17]. Starvation reduces and refeeding normalizes gene expression in pancreatic B-cells [17] and liver [17,18]. Molecular biological studies of the glucokinase gene revealed a tissue-specific regulation due to different promoter regions [18–20]. Glucokinase gene transcription in liver is regulated by insulin and cyclic AMP [21–23] while in pancreatic B-cells glucose has been hypothesized to regulate glucokinase gene transcription [20]. Thus differential regulation of glucokinase may be the basis for a distinct function of this enzyme in pancreatic B-cells and liver. This may be important not only for the understanding of the physiological regulation of glucose-induced insulin secretion but also for the pathogenesis of certain types of human diabetes such as the MODY diabetes which is related to glucokinase gene defects (for review see [24]). In accordance with Matschinsky's hypothesis [11] studies on MODY diabetes [25] and transgenic animals [26] provide evidence that deficiencies in glucokinase gene expression may disturb glucose homeostasis in non-insulin-dependent diabetes mellitus (NIDDM). In order to elucidate the mutual role of glucose and

insulin in nutrient-dependent regulation of glucokinase and GLUT2 gene expression in pancreatic B-cells and liver we have treated starved rats with glucose or glibenclamide, a sulphonylurea compound, and diazoxide, a drug which antagonizes glibenclamide-stimulated insulin secretion. Using a sensitive competitive reverse-transcriptase (RT)-PCR technique [27–30] it was possible to show that in pancreatic B-cells glucose rather than insulin is apparently the crucial regulator of glucokinase gene expression.

EXPERIMENTAL

Materials

Guanidine thiocyanate was from Fluka (Neu-Ulm, Germany); restriction enzymes, Klenow DNA polymerase, the DIG RNA transcription kit, DIG-dUTP, random hexamer primer and the DIG nucleic acid detection kit were from Boehringer (Mannheim, Germany); Hybond N nylon membranes and autoradiography films from Amersham (Braunschweig, Germany); DNA ligase, MMLV reverse transcriptase and agarose from GIBCO BRL (Eggenstein, Germany); dNTPs from Pharmacia (Freiburg, Germany); and RNasin RNase inhibitor from Promega (Heidelberg, Germany). Thermostable DNA polymerases were obtained from Boehringer (Mannheim, Germany) or Pharmacia (Freiburg, Germany). Glibenclamide was kindly provided by Boehringer (Mannheim, Germany), diazoxide by Essex Pharma (München, Germany) and D-penicillamine by Heyl (Berlin, Germany). The cDNAs coding for the rat liver glucokinase and the rat GLUT2 were generously provided by P. Iynedjian (Geneva, Switzerland) and B. Thorens (Lausanne, Switzerland) respectively. All other chemicals were of highest purity from Merck (Darmstadt, Germany), Sigma (Deisenhofen, Germany) or Boehringer (Mannheim, Germany).

Animals

Male Wistar rats weighing 220–280 g were fed a choline- and copper-deficient diet supplemented with D-penicillamine (Altromin, Lippe, Germany) for 12 weeks as described previously [17]. During this time, the acinar portion of the exocrine pancreas was replaced by fat and connective tissue without affecting morphological or functional characteristics of the islets [31–33]. The animals were subdivided into six experimental groups: (1) rats fed *ad libitum*; (2) rats fasted for 2 days; (3) rats fasted for 2 days and treated with an oral glucose load (4 g/kg body wt.) 1 h before pancreas isolation; (4) rats fasted for 2 days and treated with a single dose of glibenclamide (0.1 mg/kg body wt. subcutaneously) 1 h before pancreas isolation; (5) rats fasted for 2 days and treated with a single dose of glibenclamide (0.1 mg/kg body wt. subcutaneously) plus diazoxide (4.5 mg/kg body wt. subcutaneously) [34] administered twice (0.5 h before and concomitantly with glibenclamide) before pancreas isolation; (6) rats fasted for 2 days and treated twice with diazoxide (4.5 mg/kg body wt. subcutaneously) [34] 1.5 and 1 h before pancreas isolation. Immediately after killing, pancreas and liver were removed, rinsed twice in ice-cold phosphate-buffered saline and homogenized in buffered guanidine-thiocyanate solution for RNA extraction. Serum glucose concentrations were determined by the glucose oxidase method.

Northern-blot analysis

Total RNA was isolated according to Chomczynski and Sacchi [35]. Total RNA (20 µg) from liver and pancreas were separated in a 1% denaturing formamide/formaldehyde agarose gel and subsequently transferred to Nylon membranes. Hybridization was performed as described before [17] but with non-radioactive digoxigenin-labelled cRNA probes (Boehringer, Mannheim, Germany) coding for the rat liver glucokinase [12] and the rat GLUT2 [4]. The chemiluminescent detection process was performed according to manufacturer's instructions with PPD Lumigen (Boehringer, Mannheim, Germany) as the luminescent substrate. The autoradiograms were quantified by densitometry using the Image 1.52 analysis program (NIH, Bethesda, MD, U.S.A.). The arbitrary units of the glucokinase and GLUT2 bands were corrected to the 28 S ribosomal bands of the RNA gel documented on a Polaroid 667 film.

RT-PCR analysis of glucokinase gene expression

RT-PCR was performed as described previously [27–30,36] with some modifications. Briefly a glucokinase competitor of the liver glucokinase cDNA [37] was constructed by restriction endonuclease digestion with *Mlu*I and *Bfr*I. The cohesive ends were made blunt-ended by Klenow DNA polymerase and the plasmid was recirculized by ligation. This 50 bp deletion was within the primer binding sites of the sense primer P1 (GTCAGCAGCTGTACGAGAAG, 969–989 bp) and the antisense primer P2 (CTCCTTGAAGCTCGGGTGAC, 1379–1359 bp) (the cDNA sequences refer to [37]). Thus the shorter competitive PCR product of 359 bp could be distinguished from the template PCR product (410 bp). Both primers spanned intron–exon junctions of the glucokinase gene [19,22] to exclude the amplification of genomic DNA. cDNA was synthesized using 10 µg of total RNA from pancreas and 2 µg of total RNA from liver in a reaction mixture containing 50 mM KCl, 4 mM MgCl₂, 10 mM Tris/HCl (pH 9), 1 mM of the dNTPs each, 40 units of RNase inhibitor, 6 µg of random hexamer primer and 100 units/µg of RNA MMLV RT in a total volume of 40 µl at 37 °C for 1 h. DIG-dUTP was included to

confirm an equal transcription efficiency and the labelled cDNAs were detected by dot-blot analysis. Samples (4 µl; 1 µg of RNA equivalent from pancreas and 200 ng of RNA equivalent from liver) of the synthesized cDNAs were subjected to PCR titration in a reaction mixture containing 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris/HCl (pH 9), 0.2 mM of the dNTPs each, 0.2 µM P1 and P2 primer each, and 1 unit of *Taq* polymerase in a total volume of 50 µl. Different amounts of the deleted competitor cDNA standards were included in the PCR mixture. Samples were amplified for 33 cycles at 93 °C for 1 min, at 56 °C for 1 min and at 72 °C for 1 min. Aliquots (5 µl) of the amplification mixture from all pancreas and liver reverse-transcribed cDNA samples of the fasted animals were subjected to a second round of amplification using the nested primer pairs N1 (CGAGAAGATCATCGGTGGGA) and N2 (GGCTTTCGC GCATGCGATTT) for 25 cycles under the same conditions. The length of the amplified glucokinase template PCR product was 322 bp, the length of the glucokinase competitor PCR product 271 bp. Finally, 10 µl of the final amplification mixture were separated in a 2% agarose gel containing ethidium bromide and documented on a Polaroid 667 film. The intensities of the template and competitor bands were quantified by densitometry using the Image 1.52 analysis program. The amount of template cDNA in the amplification mixture was extrapolated by a linear regression analysis of the log(template/competitor ratio) to the log(competitor concentration).

Data analysis

Data are presented as mean values ± S.E.M. and were tested for statistical significance with Student's *t* test. Linear regression analysis was performed by the method of least-squares. *r*² was > 0.9 with a statistical significance of *P* < 0.05 for the quantification of RT-PCR experiments.

RESULTS

Glucokinase gene expression

RT-PCR analysis of glucokinase gene expression is an extremely sensitive method for the quantification of minimal amounts of mRNA in the range of a few femtograms. For this purpose

Figure 1 Competitive RT-PCR analysis of glucokinase gene expression in pancreatic B-cells from rats after fasting, glucose refeeding and glibenclamide treatment

(a) Animal fasted for 2 days; (b) animal fasted for 2 days and glucose refeed (4 g/kg body wt. for 1 h); (c) rat fasted for 2 days and treated with glibenclamide (0.1 mg/kg body wt. subcutaneously for 1 h). Abbreviation: MW, molecular-mass marker (base pairs). cDNA was synthesized from 10 µg of total RNA from pancreas and a 10% aliquot of the reaction mixture was subjected to a competitive nested PCR amplification. The amplification products were separated in a 2% agarose gel. The amount of glucokinase competitor is indicated in each panel.

Figure 2 Quantification of glucokinase gene expression in pancreatic B-cells from rats by RT-PCR analysis after fasting, glucose refeeding and glibenclamide treatment

Key to symbols: ●, animal fasted for 2 days; ■, animal fasted for 2 days and glucose refeed (4 g/kg body wt. for 1 h); ▲, rat fasted for 2 days and treated with glibenclamide (0.1 mg/kg body wt. subcutaneously for 1 h). The intensities of the bands of the glucokinase template and competitor amplification products were quantified by computer imaging of the polaroid photos of the ethidium bromide-stained agarose gel. The logarithm of the ratio of endogenous glucokinase template to glucokinase competitor is depicted as a function of the logarithm of the amount of glucokinase competitor added to the PCR reaction. The equal molar ratio of glucokinase template and competitor corresponds to the log ratio of 0.

internal standards of a glucokinase competitor cDNA fragment were co-amplified with the reverse-transcribed pancreas RNA to yield typical titration curves of glucokinase cDNA (Figures 1 and 2). The 322 bp glucokinase template amplification product could be distinguished from the 271 bp glucokinase competitor amplification product using gel electrophoresis. The glucokinase template/competitor ratio was determined by densitometric analysis of the documented DNA gels and plotted against the amount of internal competitor standard. The exact amount of reverse-transcribed glucokinase cDNA was calculated by linear regression analysis of the glucokinase template/competitor ratio (Figure 2). Using RNA isolated from liver, which provides a sufficient amount for such a comparison, glucokinase gene expression by RT-PCR analysis and by Northern-blot analysis revealed a significant correlation of both methods ($r^2 = 0.99$). Thus quantification of glucokinase mRNA in samples of 10 μ g of total RNA by competitive RT-PCR analysis yields valid results.

A fasting period of 2 days significantly reduced glucokinase mRNA levels in pancreatic B-cells by 64% (Figure 2 and Table 1) confirming results which we have previously also obtained by Northern-blot analysis [17]. Refeeding of 2-day-fasted rats increased glucokinase mRNA significantly by 300% within 1 h of oral glucose administration (4 g/kg body wt.) (Figures 1 and 2; Table 1). In contrast glibenclamide treatment (0.1 mg/kg body wt.) induced no significant increase of glucokinase gene expression in pancreatic B-cells of 2-day-fasted rats 1 h after subcutaneous administration (Figures 1 and 2; Table 1). Diazoxide treatment (4.5 mg/kg body wt.), either alone or in combination with glibenclamide, did not affect glucokinase gene

Table 1 Effects of fasting, glucose refeeding, glibenclamide and diazoxide treatment on glucokinase and GLUT2 gene expression in pancreatic B-cells and on serum glucose from rats

After a fasting period of 2 days the animals were treated either with glucose (4 g/kg body wt. for 1 h), glibenclamide (0.1 mg/kg body wt. for 1 h), glibenclamide plus diazoxide (diazoxide 4.5 mg/kg body wt. 30 min before and concomitantly with glibenclamide 0.1 mg/kg body wt. for 1 h), or diazoxide (4.5 mg/kg body wt. for 1 h). Glucokinase gene expression was quantified in 10 μ g of total RNA by competitive RT-PCR analysis, GLUT2 gene expression in 20 μ g of total RNA by Northern-blot analysis. Values are presented as means $\pm$ S.E.M., expressed as percentages of the fed controls with the numbers of experiments in parentheses. Statistical significance was assessed by Student's *t* test. * $P < 0.05$; ** $P < 0.01$ as compared with the fasted state.

Conditions	Glucokinase (% of fed control)	GLUT 2 (% of fed control)	Serum glucose (mmol/l)
Fed	100 $\pm$ 4 (3)*	100 $\pm$ 8 (3)*	5.9 $\pm$ 0.1 (3)*
Fasted	36 $\pm$ 5 (9)	51 $\pm$ 2 (9)	3.5 $\pm$ 0.2 (9)
Glucose refeed	361 $\pm$ 71 (5)**	58 $\pm$ 7 (7)	11.1 $\pm$ 0.8 (7)**
Glibenclamide	50 $\pm$ 8 (7)	54 $\pm$ 6 (6)	3.2 $\pm$ 0.1 (7)
Glibenclamide plus diazoxide	55 $\pm$ 6 (5)	49 $\pm$ 8 (5)	3.4 $\pm$ 0.3 (5)
Diazoxide	38 $\pm$ 10 (4)	44 $\pm$ 5 (4)	3.9 $\pm$ 0.2 (3)

Figure 3 Competitive RT-PCR analysis of glucokinase gene expression in liver from rats after fasting, glucose refeeding and glibenclamide treatment

(a) Animal fasted for 2 days; (b) animal fasted for 2 days and glucose refeed (4 g/kg body wt. for 1 h); (c) rat fasted for 2 days and treated with glibenclamide (0.1 mg/kg body wt. subcutaneously for 1 h). Abbreviation: MW, molecular-mass marker (base pairs). Total RNA (2 μ g) from pancreas was reverse transcribed and a 10% aliquot of the reaction mixture was subjected to competitive PCR amplification. The amplification products were separated in a 2% agarose gel. The amount of glucokinase competitor is indicated in each panel.

expression in pancreatic B-cells of 2-day-fasted rats 1 h after subcutaneous administration (Table 1).

Glucokinase gene expression in liver was reduced by more than 90% after a 2 day fasting period (Figures 3 and 4; Table 2). This observation is in agreement with previous results [17,21,38]. Both glucose refeeding (4 g/kg body wt.) and glibenclamide treatment (0.1 mg/kg body wt.) significantly increased glucokinase gene expression in liver of 2-day-fasted rats (Figures 3 and 4). In the RT-PCR analysis and Northern-blot analysis 9–13-fold increases were observed for glucose and 4–7-fold

Figure 4 Northern-blot analysis of glucokinase gene expression in liver from rats after fasting, glucose refeeding, glibenclamide and diazoxide treatment

Lane 1, fed control; lane 2, fasted for 2 days; lane 3, fasted for 2 days and glucose re-fed (4 g/kg body wt. for 1 h); lane 4, fasted for 2 days and treated with glibenclamide (0.1 mg/kg body wt. for 1 h); lane 5, fasted for 2 days and treated with diazoxide and glibenclamide [diazoxide (4.5 mg/kg body wt.) 30 min before the treatment with glibenclamide (0.1 mg/kg body wt.) concomitantly with a second dose of diazoxide (4.5 mg/kg body wt. for 1 h)] [34]; lane 6, treated with diazoxide (4.5 mg/kg body wt.). The upper panel shows the ribosomal bands stained by ethidium bromide. A sample (10 μ g) of total RNA was applied to each lane and hybridized with a digoxigenin-labelled cRNA probe coding for rat liver glucokinase [12]. The intensities of the glucokinase bands in the autoradiograms were corrected to the intensities of the 28 S ribosomal bands for equal loading.

Table 2 Effects of fasting, glucose refeeding, glibenclamide and diazoxide treatment on glucokinase and GLUT2 gene expression in liver from rats

Animals were treated as described in the legend to Table 1. Glucokinase gene expression was quantified in 2 μ g of total RNA by competitive RT-PCR analysis, GLUT2 gene expression in 20 μ g of total RNA by Northern-blot analysis. Values are presented as means $\pm$ S.E.M., expressed as percentages of the fed controls with the numbers of experiments in parentheses. Statistical significance was assessed by Student's *t* test. * $P < 0.05$; ** $P < 0.01$ compared with the fasted state.

Conditions	Glucokinase (% of fed control)	GLUT2 (% of fed control)
Fed	100 $\pm$ 24 (3)*	100 $\pm$ 2 (3)*
Fasted	4 $\pm$ 1 (6)	43 $\pm$ 2 (7)
Glucose re-fed	1290 $\pm$ 32 (7)**	46 $\pm$ 3 (7)
Glibenclamide	695 $\pm$ 12 (7)**	43 $\pm$ 3 (6)
Glibenclamide plus diazoxide	72 $\pm$ 22 (5)	43 $\pm$ 4 (5)
Diazoxide	28 $\pm$ 6 (4)	37 $\pm$ 7 (4)

increases for glibenclamide treatment (Table 3). In order to prove that the stimulatory effect of glibenclamide was mediated by the endogenous insulin released by this blood-glucose-lowering sulphonylurea compound, diazoxide was used. Diazoxide is a compound which specifically antagonizes glibenclamide-induced insulin secretion through binding to the ATP-sensitive K channel in the pancreatic B-cell membrane. Concomitant administration of diazoxide (4.5 mg/kg body wt.) and glibenclamide (0.1 mg/kg body wt.) suppressed the stimulatory effect of glibenclamide on glucokinase gene expression in liver from 2-day-fasted rats (Table 3). Diazoxide (4.5 mg/kg body wt.) alone had no significant effect (Table 3).

GLUT2 gene expression

GLUT2 gene expression was determined by a sensitive Northern-blot hybridization technique in total RNA fractions from pan-

Table 3 Comparison of RT-PCR analysis of glucokinase gene expression in liver with Northern-blot analysis

Animals were treated as described in the legend to Table 1. Glucokinase gene expression was quantified in identical RNA probes from liver tissue. A 2 μ g sample of total RNA was subjected to RT-PCR analysis and 20 μ g of total RNA to Northern-blot analysis. Values are presented as means $\pm$ S.E.M., expressed as percentages of the fed controls with the numbers of experiments in parentheses. Statistical significance was assessed by Student's *t* test. * $P < 0.05$, ** $P < 0.01$ compared with the fasted state. RT-PCR analysis and Northern-blot analysis have demonstrated a positive correlation in the linear regression analysis ($r^2 = 0.99$).

Conditions	Glucokinase (% of fed controls)	
	RT-PCR	Northern blot
Fed	100 $\pm$ 24 (3)*	100 $\pm$ 11 (3)*
Fasted	4 $\pm$ 1 (6)	2 $\pm$ 0.8 (8)
Glucose re-fed	1290 $\pm$ 32 (7)**	903 $\pm$ 213 (7)**
Glibenclamide	695 $\pm$ 12 (7)**	387 $\pm$ 65 (7)**
Glibenclamide plus diazoxide	49 $\pm$ 8 (4)	31 $\pm$ 6 (5)
Diazoxide	28 $\pm$ 6 (4)	10 $\pm$ 5 (4)

creas and liver. The treatment schedule was the same as in the glucokinase gene expression studies. Densitometric analysis of the autoradiograms revealed that a fasting period of 2 days reduced GLUT2 mRNA levels both in pancreatic B-cells and liver by 50 % (Table 2). Glucose refeeding (4 g/kg body wt.) after a 2 day fasting period did not significantly affect GLUT2 mRNA levels in pancreatic B-cells and liver within 1 h of administration. Treatment with glibenclamide (0.1 mg/kg body wt.) also failed to increase GLUT2 mRNA levels in pancreatic B-cells and liver after a 2 day fasting period and diazoxide (4.5 mg/kg body wt.) was without effect as well (Table 2). Thus neither glucose nor endogenous insulin appear to have an inductive effect on GLUT2 gene expression in pancreatic B-cells and liver within a 1 h treatment period (Table 2).

DISCUSSION

The regulation of the glucokinase and the GLUT2 glucose transporter gene is of central importance for glucose responsiveness of the pancreatic B-cell and thus for the pathogenesis of diabetes. In the present study we have studied glucokinase and GLUT2 gene expression in pancreatic B-cells and liver from rats. The animals were fed a D-penicillamine-supplemented choline and copper-deficient diet to induce a lipomatosis of the exocrine portion [31,39]. The islets are morphologically and functionally intact [31–33]. The pancreas lipomatosis increased the endocrine portion of the pancreas in relation to the total amount of tissue and thus prevented RNA degradation and avoided misleading results originating from fluctuations in the exocrine RNA content. This made it possible to avoid a time-consuming islet isolation procedure and thus changes of glucokinase mRNA levels during the long isolation process. Thus this model is particularly suited for the study of genes such as glucokinase and GLUT2, which are expressed only in pancreatic B-cells [18,40]. With the competitive RT-PCR methodology we could quantify glucokinase mRNA between 10 and 100 fg which is far below the detection limit of standard Northern-blot analysis. In a Northern-blot experiment RNA from several pancreases would have been required for a single determination. In pancreatic B-cells glucokinase mRNA levels are dependent on the nutritional status. A 2 day fasting period reduced glucokinase mRNA levels by more than 50 % and short-term glucose refeeding normalized glucokinase mRNA levels in pancreatic B-cells as well as in liver,

thus confirming results previously obtained by Northern-blot analysis [17].

In order to distinguish between the effects of glucose and insulin on tissue-specific glucokinase gene expression, glibenclamide was administered to fasted rats in order to stimulate endogenous insulin secretion. Glibenclamide, like glucose, caused a drastic stimulation of glucokinase gene expression in liver. In pancreatic B-cells only glucose, but not glibenclamide, was effective. Thus it is glucose rather than insulin which during refeeding stimulates glucokinase gene expression in the pancreatic B-cell. Our results are in agreement with the concept of glucose as the crucial regulatory element in pancreatic B-cell function. In liver, glucokinase gene expression was responsive to insulin released by glibenclamide stimulation from the endocrine pancreas. Our *in vivo* data confirm earlier experiments [21,22], which proved that glucokinase gene expression in liver is under dual control of insulin and glucagon. Regulation of the glucokinase gene in pancreatic B-cells by glucose appears to mirror a universal principle of gene regulation in these endocrine cells. Recent studies on the transcriptional regulation of the acetyl-CoA carboxylase gene [41] and the pyruvate kinase gene [42] in insulin-secreting tumour cells also revealed a transcriptional activation by glucose. In addition glucose exerts stimulatory effects on regulatory B-cell proteins and insulin on the translational and post-translational level [10,15]. The observation in the present *in vivo* study that glucose rather than insulin induces glucokinase gene expression in pancreatic B-cells is at variance from earlier studies on glucokinase gene expression. Studies on cultured pancreatic islets kept under various glucose concentrations in the culture medium [10] and on freshly isolated islets from fasted and refed rats [18] displayed no significant changes of glucokinase mRNA levels. These discordant observations on glucokinase gene regulation may be due to the extremely low abundance of glucokinase mRNA in pancreatic B-cells. This makes a quantification of glucokinase mRNA in pancreatic islets by standard Northern-blot hybridization very difficult in comparison with mRNA species which are expressed at higher levels. The discrimination of small changes in mRNA levels by Northern-blot analysis is hampered by the narrow linearity range of autoradiographic films, whereas competitive RT-PCR analysis exhibits linearity over a wide concentration range. Thus the limitations of the quantification of glucokinase mRNA by Northern-blot analysis in isolated islets may provide an explanation for the negative findings in these studies.

The glucokinase gene in pancreatic B-cells is less sensitive towards nutritional regulation than the liver gene. But the importance of glucokinase gene transcription for glucose responsiveness of pancreatic B-cells is reinforced by studies on the linkage of the glucokinase gene locus to human MODY diabetes [24]. Due to the autosomal dominant mode of inheritance of MODY diabetes even a heterogeneous defect of glucokinase gene expression can alter the stimulus-secretion coupling, and can contribute significantly to defective glucose metabolism in diabetes [25]. The rate-limiting character of glucose phosphorylation in pancreatic B-cells by glucokinase implies that a moderate reduction of glucokinase enzyme activity can significantly affect the glycolytic flux and is linked to an impaired glucose-induced insulin secretion. Recent studies on transgenic mice, in which glucokinase mRNA levels had been reduced through introduction of an antisense glucokinase mRNA construct, revealed a 45% reduction of glucokinase mRNA levels, which leads to a 50–70% reduction in glucokinase protein and enzyme activity [26]. In these transgenic mice the insulin secretory response to glucose was impaired in a manner similar to human MODY diabetes. Thus the 'thrifty gene' glucokinase seems to

lack a compensatory mechanism if a mutated allele of the glucokinase gene is present in a patient with human MODY diabetes [43]. So there is strong evidence that the regulation of glucokinase mRNA levels in pancreatic B-cells can significantly contribute to the regulation of glucokinase enzyme activity and glucose phosphorylation. Studies on glucokinase protein levels and glucokinase enzyme activity in pancreatic B-cells point to additional regulatory mechanisms at the post-translational level [18,44]. Western-blot studies of glucokinase protein did not detect any nutrient-dependent increase in freshly isolated islets from starved and refed rats [18] and after infusion of glucose [44]. Only in tissue culture of pancreatic islets for 3–7 days at high glucose concentrations were glucokinase protein levels increased significantly [10]. In contrast to these observations on the regulation of the glucokinase protein there is consensus that glucokinase enzyme activity in pancreatic B-cells and liver is regulated in dependence on the nutrient status [1,2,10,18,44,45]. However, at variance from the effects on the mRNA level the changes of the glucokinase enzyme activity are more sluggish during starvation, and glibenclamide and glucose administration.

GLUT2 gene expression in pancreatic B-cells and liver is also dependent upon the nutritional status. A 2 day fasting period reduced GLUT2 mRNA by 50%. However, short-term (1 h) glucose refeeding, as well as glibenclamide treatment, did not normalize GLUT2 mRNA in pancreatic B-cells and liver. In a previous study we have demonstrated a normalization of GLUT2 mRNA levels in pancreatic B-cells and liver after a 4 h glucose refeeding period of fasted rats [17]. This observation is in accordance with studies on cultured pancreatic islets showing a significant induction of GLUT2 gene expression only after a high-glucose culture period of at least 8 h [15]. Thus glucose is also the major regulator of GLUT2 gene expression in pancreatic B-cells. However, a 50% reduction of the GLUT2 gene expression cannot explain a starvation-induced reduction of the metabolic flux rate, as the glucose transport capacity by the GLUT2 exceeds the glucose phosphorylation capacity by a factor of around 100 [46,47]. Thus the results support the concept that the phosphorylation by glucokinase, rather than glucose uptake via GLUT2, is the rate-limiting step for initiation of glucose-induced insulin [2,3].

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