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Regulation of glucokinase and GLUT-2 glucose-transporter gene expression in pancreatic B-cells

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Glucokinase (EC 2.7.1.2) is the signal-recognition enzyme in pancreatic B-cells for initiation of glucose-induced insulin secretion. We show here that both the glucokinase and glucose-transporter GLUT-2 genes are regulated physiologically. Fasting decreased B-cell glucokinase and glucose-transporter GLUT-2 mRNA in pancreatic B-cells as well as in liver, whereas refeeding induced expression of both genes. In pancreatic B-cells a ~ 4.4 kb glucokinase-related mRNA was detectable, in addition to the 2.8 kb form. This ~ 4.4 kb glucokinase transcript was drastically decreased during refeeding. The 2.8 kb mRNA, which is typical for pancreatic B-cells, was accompanied after refeeding by a 2.4 kb mRNA species typical for liver glucokinase. Starvation primarily decreased the 2.8 kb pancreatic B-cell glucokinase mRNA species. The concordant regulation of both genes may represent the basis for the physiological regulation of glucose-induced insulin secretion at a transcriptional level.

INTRODUCTION

Glucokinase (ATP:D-glucose 6-phosphotransferase, EC 2.7.1.2) is a low-affinity glucose-phosphorylating enzyme expressed in pancreatic B-cells and in liver (Lenzen & Panten, 1988; Iynedjian *et al.*, 1989). In the B-cells it functions as the so-called signal-recognition enzyme (Lenzen, 1991), which couples changes in the millimolar blood glucose concentration to corresponding signal-generating metabolic flux rates for initiation of glucose-induced insulin secretion (Meglasson & Matschinsky, 1986; Lenzen, 1990, 1991). Like glucose-induced insulin secretion, glucokinase enzyme activity in both pancreatic B-cells and liver is decreased during fasting and normalized during refeeding (Malaisse *et al.*, 1976; Weinhouse, 1976; Burch *et al.*, 1981; Bedoya *et al.*, 1986; Lenzen *et al.*, 1987; Lenzen & Panten, 1988; Lenzen, 1991). In contrast, glucose uptake by the B-cells via facilitated diffusion through the low-affinity glucose transporter GLUT-2 (Thorens *et al.*, 1988; Permutt *et al.*, 1989; Johnson *et al.*, 1990) is usually not rate-limiting and is therefore unlikely to be the primary site for regulation of glucose-induced insulin secretion (Meglasson & Matschinsky, 1986; Lenzen & Panten, 1988; Lenzen, 1991).

Although glucokinase in liver is known to be regulated at the transcriptional level (Iynedjian *et al.*, 1989) in dependence on the nutritional status, pancreatic B-cell glucokinase mRNA expression has been reported to be unaffected by fasting and refeeding (Iynedjian *et al.*, 1989). The high-K_m glucose transporter GLUT-2 in liver is regulated at a transcriptional level during the fasting–refeeding cycle (Thorens *et al.*, 1990), and expression of this transporter gene is apparently also regulated in pancreatic islets (Johnson *et al.*, 1990; Chen *et al.*, 1990; Orci *et al.*, 1990; Unger, 1991). This has caused the paradoxical situation that an unimpaired glucose transport rather than proper glucose phosphorylation has been considered to be the major component of the glucose-responsive insulin-secretory apparatus of the pancreatic B-cell required for correction of hyperglycaemia (Unger, 1991).

In contrast with glucose-transporter GLUT-2 mRNA, glucokinase mRNA is expressed in only a few copies in the B-cell. In conjunction with the small volume of pancreatic islet tissue embedded into a RNAase-rich exocrine pancreas, this situation represents the major obstacle for the analysis of glucokinase gene expression in pancreatic B-cells. Northern-blot analysis using special cRNAs under hybridization conditions of high stringency, in combination with RNA isolated from total pancreas and liver tissue from rats kept on a special diet in order to decrease the RNAase-rich exocrine portion of the pancreas, provided an adequate methodology to study the influence of fasting and refeeding on gene expression of glucokinase and glucose transporter GLUT-2 in pancreatic B-cells.

EXPERIMENTAL

Male Wistar rats (220–260 g) were fed on a choline- and copper-deficient diet (French, 1966) supplemented with choline citrate and D-penicillamine (Heyl Chemisch-Pharmazeutische Fabrik, Berlin, Germany) as described by Fölsch *et al.* (1980). After 3 months on this diet, the acinar portion of the exocrine pancreas was replaced by fat and connective tissue, without affecting morphological or functional characteristics of the islets (Fölsch *et al.*, 1980, 1988; Weaver *et al.*, 1988). The RNA content of the exocrine pancreas was decreased by 90%. Rats were fed *ad libitum* on a standard high-carbohydrate diet (51% carbohydrate, 19% protein, 4% fat) (Altromin, Lippe/Lage, Germany) during the last 2 days before the experiment, or were starved during this period. Refeeding was performed by provision of the standard high-carbohydrate diet *ad libitum* during the last 4 h before killing. Total RNA from pancreas and liver tissue homogenized with an Ultra-Turrax tissue homogenizer was extracted as described by Chomczynski & Sacchi (1987). Poly(A)-containing RNA was isolated by oligo(dT)–cellulose chromatography. Gel electrophoresis of total RNA and poly(A)-containing RNA was performed in 1%-agarose gels after denaturation with formamide/formaldehyde (Fourney *et al.*, 1988). Fractionated RNA was transferred to a nylon membrane and fixed by u.v. radiation. After hybridization the membranes were stained with Methylene Blue to make the ribosomal bands visible. Transcription of labelled antisense cRNA probes encoding for rat liver glucokinase (Iynedjian *et al.*, 1987) was performed by using T7 and T3 polymerase enzymes with [^{32}P]UTP (NEN, Dreieich, Germany) (Melton *et al.*, 1984). Hybridization at 65 °C for 21 h took place in a solution con-

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taining 50% (v/v) formamide, 5 × SSPE (1 × SSPE = 180 mM-NaCl/1mM-Na_2EDTA/10 mM-sodium phosphate, pH 7.7), 10 × Denhardt's solution, 0.5% (w/v) SDS, denaturated salmon sperm DNA (200 μg/ml) and labelled cRNA probes at 30–50 ng/ml with a specific radioactivity of 2×10^8 c.p.m./μg. Membranes were washed twice in 2 × SSPE containing 0.1% SDS for 15 min at 65 °C and in 0.2 × SSPE containing 0.1% SDS for 15 min at 65 °C. Washing of the membranes with 0.1% SDS for 10 min at 90 °C removed neither the small nor the large mRNA bands, thus confirming the stringency of the hybridization method. Hybridization of rat kidney and spleen mRNA samples with the glucokinase antisense cRNA probe did not yield a band, in particular no bands at 2.4 kb, 2.8 kb or 4.4 kb. Blots were exposed for autoradiography at −80 °C. Hybridization with a labelled antisense cRNA probe encoding for the rat liver glucose transporter (GLUT-2) (Thorens *et al.*, 1988) was performed as described above for glucokinase at 60 °C. The results of all experiments have been confirmed in at least three separate experiments. Control hybridizations were performed with insulin- and β-actin-coding probes as 'housekeeping' genes. The RNA transcription kit was from Stratagene (Heidelberg, Germany), guanidine thiocyanate from Fluka (Neu-Ulm, Germany), oligo(dT)–cellulose (type 7) from Pharmacia (Freiburg, Germany), and Hybond N nylon membranes were from Amersham (Braunschweig, Germany). All other chemicals of analytical grade were purchased from Merck (Darmstadt, Germany), Sigma (Deisenhofen, Germany) or Boehringer (Mannheim, Germany).

RESULTS AND DISCUSSION

The glucokinase gene was transcribed into two different mRNA species, a 2.8 kb mRNA specific for pancreatic B-cells and a 2.4 kb mRNA specific for liver (Fig. 1), thus confirming a previous observation by Iynedjian *et al.* (1989). Fasting of the rats for 2 days decreased B-cell glucokinase mRNA by 53%, while liver glucokinase mRNA became undetectable (Fig. 1). Refeeding for 4 h induced glucokinase gene expression in both tissues, resulting in a normalization of mRNA levels in B-cells and a nearly 500% over-expression in liver (Fig. 1). Fasting also decreased serum glucose concentrations of 6.5 ± 0.3 mM in fed rats to 4.6 ± 0.3 mM in fasted animals, and refeeding raised the values to 13.9 ± 5.3 mM (means ± S.E.M., $n = 4$ each).

An unexpected observation was a second ~ 4.4 kb glucokinase-related mRNA species in pancreatic B-cells (Fig. 1), which would not have been detected in dot-blot-type experiments. This form was not affected by fasting for 2 days, but was decreased by 80% during refeeding of the rats for 4 h, thus paralleling the refeeding-induced increase in the small mRNA forms (Fig. 1). This indicates that the ~ 4.4 kb pancreatic B-cell mRNA species might be converted into the small mRNA species under refeeding conditions, i.e. in a hyperglycaemic and hyperinsulinaemic state. This small pancreatic B-cell mRNA species from refed rats was composed of two bands, a weak band of 2.8 kb mRNA, as characteristic for pancreatic B-cell glucokinase mRNA of fed and fasted rats, and additionally a stronger band of approx. 2.4 kb mRNA, as characteristic for liver B-cell glucokinase mRNA. Refeeding produced the same changes in the pancreatic B-cell and liver mRNA bands after 1 h as those shown after 4 h in Fig. 1. Shorter refeeding times were insufficient for full expression of refeeding-induced changes. Such a ~ 4.4 kb glucokinase mRNA species was undetectable in liver from fed and fasted rats; only in liver from refed animals were two faint mRNA transcripts with sizes in the range 3–4 kb registered in addition to the 2.4 kb mRNA (Fig. 1). The different pancreatic B-cell and liver glucokinase mRNA forms observed might possibly indicate differences between these two tissues in transcription or splicing of the glucokinase mRNA (Magnuson & Shelton, 1989). Such a ~ 4.4 kb glucokinase mRNA species specific to pancreatic B-cells has been observed by Iynedjian *et al.* (1989) also in RIN insulin-producing tumour cells. This is in accordance with the origin of this tissue-culture cell line from a radiation-induced rat islet-cell tumour.

The glucose-transporter GLUT-2 gene was transcribed in both pancreatic B-cells and liver into a 2.8 kb mRNA species (Fig. 2) (Permutt *et al.*, 1989; Johnson *et al.*, 1990). Fasting of the rats for 2 days decreased B-cell glucose-transporter GLUT-2 mRNA by 58% and the liver glucose-transporter GLUT-2 mRNA by 69% (Fig. 2). Refeeding for 4 h induced glucose-transporter GLUT-2 gene expression, resulting in a normalization of mRNA levels in both tissues (Fig. 2). The results for liver glucose-transporter GLUT-2 mRNA regulation confirm results by Thorens *et al.* (1990).

The present results do not support an earlier contention that B-cell glucokinase is not regulated at the transcriptional level

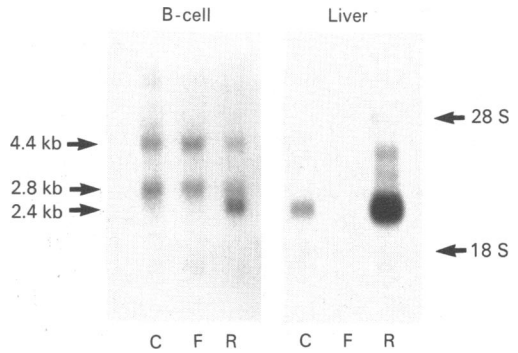

Fig. 1. Effects of a 2-day fasting (F) and a 4 h refeeding (R) period compared with fed controls (C) on the expression of glucokinase mRNA in pancreatic B-cells and liver

The mRNA levels determined by scanning densitometry were (in arbitrary units) for B-cells in controls 2050 and 1250, after fasting 970 and 1300, and after refeeding 1950 (composed of two sub-bands with 180 units at 2.8 kb and additionally 1770 units at 2.4 kb) and 250, respectively, for the 2.8 kb and the 4.4 kb mRNA forms, and for the 2.4 kb mRNA form from liver in controls 1420, after fasting 10, and after refeeding 7880. Northern-blot analysis was performed on 10 μg of poly(A)-containing RNA from pancreas tissue or 10 μg of total liver RNA per lane from pooled organs of four rats.

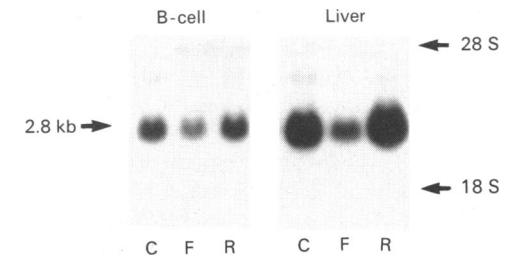

Fig. 2. Effects of a 2-day fasting (F) and a 4 h refeeding (R) period compared with fed controls (C) on the expression of glucose transporter GLUT-2 mRNA in pancreatic B-cells and liver

The mRNA levels determined by scanning densitometry were (in arbitrary units) for B-cell mRNA in controls 6220, after fasting 2640, and after refeeding 5540, and for liver mRNA in controls 18360, after fasting 5740, and after refeeding 22320. Northern-blot analysis was performed on 20 μg of total RNA from either pancreas tissue or liver per lane from pooled organs of four rats.

(Iynedjian *et al.*, 1989). Rather, this is the first demonstration of a nutrient-dependent gene regulation of B-cell glucokinase and glucose transporter GLUT-2. This gene regulation provides the transcriptional basis for a physiological regulation of glucose-induced insulin secretion. The existence of two different promotors within the glucokinase transcription unit, as reported for B-cell and liver tissue (Magnuson & Shelton, 1989; Hughes *et al.*, 1991), could be an explanation for the expression of the 4.4 kb mRNA species in pancreatic B-cells and for the alternative splicing during refeeding. Thus glucokinase gene regulation seems to be more complex at the level of transcription and post-transcriptional modifications in pancreatic B-cells than in liver. This will require the analysis of *cis*- and *trans*-acting factors (Boam *et al.*, 1990) of the glucokinase transcription unit.

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