Induction of PEPCK Gene Expression in Insulinopenia in Rat Small Intestine

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PEPCK is a key enzyme of gluconeogenesis in liver and kidney. Recently, we have shown that small intestine also contributes to the endogenous glucose production in insulinopenia in rats and that glutamine is the main precursor of glucose synthesized in this tissue. The expression of the PEPCK gene in rat and human small intestine and the effect of streptozotocin-induced diabetes and fasting have been studied using reverse transcriptase–polymerase chain reaction, Northern blot analysis, and determination of enzyme activity. The PEPCK gene is expressed along the whole small intestine in adult rat and human. The abundance of PEPCK mRNA was increased ~30 times in the duodenum, 15 times in the jejunum, and only 3 times in the ileum from diabetic rats. PEPCK mRNA was downregulated in all parts of the tissue upon insulin treatment for 10 h. In 48-h fasted rats, the PEPCK mRNA abundance was increased 17 times in the duodenum and the jejunum and 3 times in the ileum, and it was normalized upon refeeding for 7 h. PEPCK activity was also increased 2–5 times in diabetic and fasted rats in the duodenum and jejunum but not in the ileum. We conclude that PEPCK is a crucial enzyme contributing to the induction of gluconeogenesis in small intestine, just as it is well known to be in the liver and kidney. *Diabetes* 49:1165–1168, 2000

PEPCK (EC 4.1.1.32) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, the rate-limiting step of gluconeogenesis (GNG). The gene for PEPCK is mainly expressed in the liver and kidney cortex (1,2) and in white adipose tissue (3). In addition, the activity of this enzyme has been reported in brown fat (4), the mammary gland during lactation (5), and the small intestine (SI) (6,7). Lesser amounts of PEPCK have been reported in the colon, skeletal muscle, lung, certain smooth muscles, and sublingual gland (8). PEPCK is considered to be the first committed step in GNG in hepatic and renal cells. Its function in nongluconeogenic tissues remains less clear. In white adipose tissue, PEPCK could be involved in the supply of glycerol-3-phosphate for triglyceride synthesis. This pathway, termed glyceroneogenesis, is required for the re-esterification of free fatty acids to maintain an active level of triglyceride synthesis, even during periods of net lipolysis such as fasting (3).

The gene of PEPCK is acutely regulated by a variety of dietary and hormonal signals in a tissue-specific pattern. In the liver, the main factors that increase PEPCK gene expression include cAMP, glucocorticoids, and thyroid hormones, whereas insulin inhibits this process. Glucocorticoids are also inducers in the kidney, whereas cAMP and insulin are weak effectors in this tissue. In contrast, glucocorticoids inhibit the transcription of the PEPCK gene in adipocytes (9). Although the hormonal regulation of PEPCK in SI has not been studied, it has been shown that DNA sequences involved in the specific expression of PEPCK in the liver, kidney, and SI are located in the same proximal region of the promoter (–600 to 69 bp). In contrast, white fat– and brown fat–specific sequences are located distally (–2,088 to –888 bp) (10). This result suggests that the regulation of PEPCK expression in the SI might be similar to that in liver and kidney.

In keeping with the latter proposal, PEPCK mRNA is expressed at a high level in the liver, kidney, and SI during suckling in rat (11,12). Because all other enzymes involved in GNG, including glucose-6-phosphatase (G6Pase), are concomitantly highly expressed in the SI during this period, it is assumed that GNG takes place in this organ during development in rats (12,13). In contrast, it is generally considered that gluconeogenic genes are not expressed in SI after weaning, implying that GNG no longer occurs in this tissue in adult mammals.

At variance with this view, we have recently demonstrated that the G6Pase gene is still expressed at a low level in SI in adult rats and humans and is strongly induced in insulinopenia (14). This finding prompted us to ask if SI could be a glucose producer organ under insulinopenia. We found that SI may contribute 20–25% of total endogenous glucose release in fasted and diabetic rats and that glutamine is the main precursor of glucose synthesized in this tissue (15). Because PEPCK is required for the use of glutamine by GNG, in this study, we examined the hypothesis that an enhanced expression of the PEPCK gene might occur during fasting and diabetes in rat SI.

RESEARCH DESIGN AND METHODS

Tissue sampling. Male Sprague-Dawley rats weighing ~230–250 g were used. They were fed rat laboratory diet (50% starch, 23.5% proteins, 5% lipids, 4% cellulose, 5.5% mineral salts, 12% water, [wt/wt]) for 3 days before experiments. Diabetic rats were used 3 days after a single intraperitoneal injection of streptozotocin (70 mg/kg) (Sigma, La Verpillière, France). Insulin treatment consisted of 6 injections of insulin (Lilly-France S.A., St-Cloud, France): 60 nmol at 6:00 A.M. and 18 nmol at 8:00 A.M., 10:00 A.M., 12:00 P.M., 2:00 P.M., and 4:00 P.M. Tissues of insulin-treated rats were sampled at 5:00 P.M. Fasted rats were deprived of food for 48 h. Refed rats were then given free access to rat diet for 7 h before tissue sampling. Tissue sampling was performed in anesthetized animals (single injection of 7 mg pentobarbital/100 g). The tissues were

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G6Pase, glucose-6-phosphatase; GNG, gluconeogenesis; PCR, polymerase chain reaction; RT, reverse transcriptase; SI, small intestine.

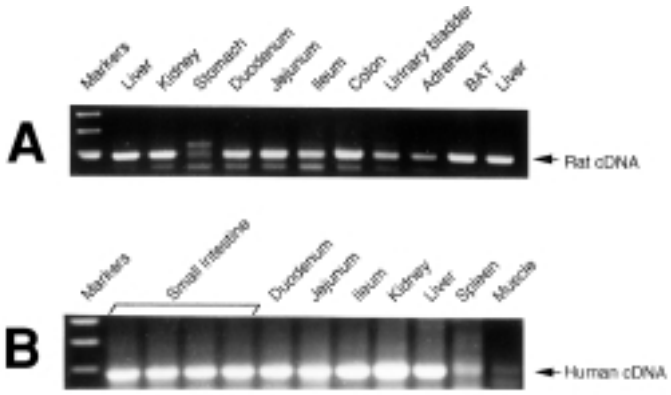

FIG. 1. Tissue specificity of PEPCK gene expression in normal fed rats. Amplification of the rat PEPCK cDNA (575 bp) by RT-PCR from 1 µg total RNA from all tissues in fed rats (*A*) and in humans (*B*). From a 50-µl amplification medium, 20 µl was loaded on a 2% agarose gel. On the left lane, molecular mass marker was loaded on the gel: DNA fragments appearing were 1,000, 800, and 600 bp long from the top to bottom. BAT, brown adipose tissue.

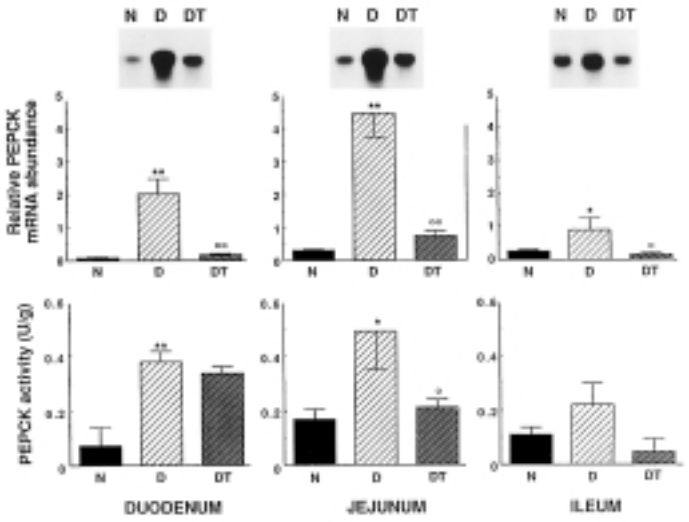

FIG. 2. Effect of diabetes and insulin treatment on PEPCK mRNA abundance and enzyme activity in rat small intestine. Upper panels: Northern blot analysis of PEPCK mRNA abundance in normal fed (N), diabetic (D), and diabetic insulin-treated (DT) rats. Middle panels: densitometric analysis of Northern blots obtained from 4 animals in each group. The PEPCK mRNA abundances were referred to a standard PEPCK mRNA chosen arbitrarily (from a diabetic rat duodenum) that was analyzed in parallel with other samples in all blots. Lower panels: specific PEPCK activity assayed in cytosolic tissue extracts. Results are expressed as mean micromoles per minute per gram (unit per gram) of tissue ± SE. Significantly different from N value: *$P < 0.05$ and **$P < 0.01$; significantly different from D value: °$P < 0.05$; °°$P < 0.01$.

rapidly rinsed using saline buffer, frozen in liquid nitrogen, and stored at –80°C until use. Human tissue samples were obtained from patients undergoing surgery. Patients were informed about the use of their tissue samples and gave their consent. Healthy parts of the tissues were taken far from the diseased part, frozen in liquid nitrogen, and stored at –80°C.

Reverse transcriptase–polymerase chain reaction and Northern blot analysis. For reverse transcriptase (RT)–polymerase chain reaction (PCR) analysis, 1 µg total RNA was extracted following the protocol described by Sambrook et al. (16) and was reverse-transcribed for 1 h at 37°C in 20 µl reaction medium made up of 50 mmol/l Tris/HCl, 75 mmol/l KCl, 3 mmol/l $MgCl_2$, 10 mmol/l dithiothreitol, pH 8.3, 1 mmol/l dNTP, 15 pmol antisense primer, and 200 U Moloney murine leukemia virus (M-MLV) RT (Promega, Charbonnieres, France). The PCR amplifications were performed in 50 µl medium containing 50 mmol/l Tris-HCl, 50 mmol/l KCl, 1 mmol/l $MgCl_2$, pH 9.0, 0.2 mmol/l dNTP, 15 pmol sense and antisense primers, and 2.5 U Taq DNA polymerase (Promega). The reaction mixtures were subjected to 40 cycles of PCR amplification consisting of denaturation for 60 s at 94°C, annealing for 60 s at 56°C, and elongation for 120 s at 72°C. The oligonucleotide primers (5′-AGCCTCGACAGCCTGCCCCAGG-3′ sense and 5′-CCAGTTGTTGACCAAAGGCTTTT-3′ antisense) allowed amplification of a 575-bp cDNA product. The procedure to analyze and quantify PEPCK mRNA by Northern blot analysis was as described in a previous report (17).

PEPCK activity determination. Frozen tissues were reduced to powder at liquid nitrogen temperature. The powder was homogenized in 10 mmol/l HEPES and 0.25 mol/l sucrose, pH 7.4 (9 volumes per gram tissue), by 10 × 1 s ultrasonication pulses and centrifuged at 100,000*g* for 1 h at 4°C. PEPCK activity was measured in 100,000*g* cytosolic supernatants by the decarboxylation assay described by Jomain-Baum and Schramm (18). The decarboxylation assay determines the formation of phosphoenolpyruvate by an equilibrium displacement generating oxaloacetate from malate by malate dehydrogenase in a mixture containing 50 mmol/l Tris, pH 8.0, 0.75 mmol/l $MnCl_2$, 1 mmol/l NAD^+, 6 U malate dehydrogenase, 1 mmol/l GTP, and 20 µl cytosolic tissue extract in a 1-ml final volume. Reaction was started by adding 10 mmol/l malate, and the oxaloacetate formation from malate by malate dehydrogenase was measured spectrophometrically at 37°C by measuring NADH formation. A reaction blank was obtained without malate by determination of NADH formation for each assay.

Statistical analyses were performed using Student's *t* test (unpaired values).

RESULTS

We have performed RT-PCR experiments with specific PEPCK oligonucleotides from total RNAs extracted from liver, kidney, stomach, duodenum, jejunum, ileum, colon, urinary bladder, adrenals, and brown adipose tissue were sampled in normal fed rats. PEPCK cDNA was amplified in all tissues as in the liver and kidney (control tissues), with the exception of stomach, in which a very low amount was detected (Fig. 1). Among RNAs extracted from human tissues, PEPCK cDNA was amplified in liver and kidney and all small intestine samples we have studied. PEPCK cDNA was also amplified in spleen, whereas a faint amount was detectable in skeletal muscle (Fig. 1). The abundance of PEPCK mRNA in the duodenum in normal rats, quantitatively analyzed by Northern blot, was about one-fourth to one-third that present in the jejunum and ileum (Fig. 2). Specific PEPCK activity was present along the whole SI (0.1–0.2 U/g wet tissue) (Fig. 2). It represented ~10–15% of the activity found in the liver of the same animals (not shown).

The quantification of PEPCK mRNA has been carried out by means of Northern blot analysis in the duodenum, jejunum, and ileum of diabetic rats and compared with normal and insulin-treated rats. The PEPCK mRNA abundance was increased ~30 times in the duodenum, 15 times in the jejunum, and only 3 times in the ileum from diabetic rats compared with normal fed rats (Fig. 2). The PEPCK mRNA abundance was decreased after insulin treatment for 10 h in all parts of the tissue, but it remained somewhat higher than that in the jejunum of control animals (Fig. 2). In the tissue from diabetic rats compared with normal rats, the total PEPCK activity (V_{max}) was significantly increased by 430% in the duodenum (0.37 ± 0.08 vs. 0.07 ± 0.09 µmol · min^{-1} · g^{-1} wet tissue [mean ± SE]) and 190% in the jejunum (0.49 ± 0.24 vs. 0.17 ± 0.07 µmol · min^{-1} · g^{-1} wet tissue). Insulin treatment for 10 h only normalized the PEPCK activity in the jejunum (Fig. 2). In the ileum, no significant difference in PEPCK activity was observed among the 3 conditions (~0.1 µmol · min^{-1} · g^{-1}).

The quantification by Northern blot analysis also revealed that PEPCK mRNA was increased ~17 times in the both the duodenum and jejunum and only 3 times in the ileum from 48 h–fasted rats compared with normal fed rats (Fig. 3). The

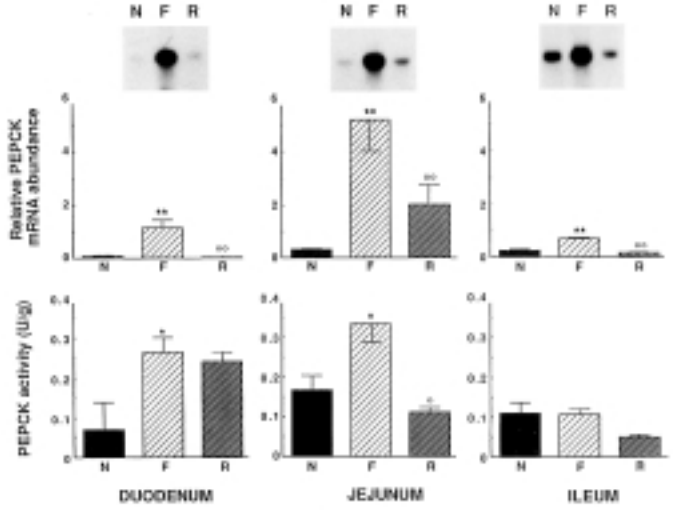

FIG. 3. Effect of fasting and refeeding on PEPCK mRNA abundance and enzyme activity in rat SI. Upper panels: Northern blot analysis of PEPCK mRNA abundance in normal fed (N), 48 h–fasted (F), and refed (R) rats. Middle panels: densitometric analysis of Northern blots obtained from 4 animals in each group (see Fig. 2 legend for quantitative analysis). Lower panels: specific PEPCK activity assayed in cytosolic tissue extracts. The results are expressed as mean of micromoles per minute per gram (unit per gram) of tissue ± SE. Significantly different from N value: *$P < 0.05$, **$P < 0.01$; significantly different from D value: °$P < 0.05$, °°$P < 0.01$.

abundance of PEPCK mRNA was normalized upon refeeding fasted rats for 7 h (Fig. 3). The PEPCK V_{max} was 0.26 ± 0.09 μmol · min^{-1} · g^{-1} in the duodenum and 0.33 ± 0.10 μmol · min^{-1} · g^{-1} in the jejunum of 48 h–fasted rats (Fig. 3). This result represented 270 and 100% increases compared with the respective tissues in normal fed rats. PEPCK activity was normalized upon refeeding for 7 h in the jejunum but not the duodenum (Fig. 3). In the ileum, PEPCK activity was not altered upon modification of nutritional status (~0.1 μmol · min^{-1} · g^{-1}).

DISCUSSION

The pattern of PEPCK expression has been the subject of considerable interest because it is a major enzyme of GNG and is also highly expressed in tissues without a clearly defined function until now (9). This enzyme is known to be present in high levels in tissues such as liver, kidney cortex, and white adipose tissue. In this study, PEPCK gene expression has been shown by RT-PCR in various additional tissues such as urinary bladders, adrenals, and brown adipose tissue and has also been shown in the duodenum, jejunum, ileum, and colon in normal adult rats. We also demonstrate that PEPCK mRNA is present at very significant levels in the SI in adult humans. PEPCK is expressed along the entire SI in both rat and human species. It should be mentioned that the SI PEPCK activity in normal fed rats is rather low and only represents ~10% of the liver activity on a weight basis, as previously reported (6). This result appeared to be the same for G6Pase, another key enzyme of GNG, which we have previously reported being expressed in the SI in human and rat (14). However, the fact that the SI has a large mass (~11 g in rats studied here) compared with the kidney, for example (~1 g each), must be taken into account when a quantitative estimation is drawn.

We have shown here that PEPCK mRNA and activity levels are dramatically increased in both the duodenum and jejunum in streptozotocin-induced diabetic rats. These levels of PEPCK mRNA are downregulated by insulin treatment for 10 h. As for G6Pase gene expression (14), this result strongly suggests that insulin exerts a suppressive effect on PEPCK gene expression in the SI in rats. Similarly, PEPCK activity and mRNA levels are increased in the SI in 48 h–fasted rats, and PEPCK mRNA abundance is further restored to fed values after refeeding for 7 h. Interestingly, as we have previously described for G6Pase, PEPCK activity is normalized in the jejunum but is not normalized in the duodenum of diabetic-treated and refed rats within the time of treatment or refeeding, although mRNA levels are similarly corrected in both tissues. This observation could be related to variable half-lives of both enzymes at the protein level, depending on the tissue.

Therefore, these results demonstrate striking similarities between PEPCK and G6Pase gene expressions in the duodenum and jejunum in diabetic and fasted rats (see above). However, the expressions of the 2 genes in the ileum present some differences. Hence, the PEPCK gene, but not the G6Pase gene, is expressed in the ileum of normal fed rats. In addition, G6Pase gene expression is strongly induced in the ileum in fasted rats (and rapidly restored to nil by refeeding), whereas this is not the case in the ileum in diabetic rats (14). This result suggests that the latter may be tightly controlled by nutritional mechanisms in this part of the SI. In contrast, in the ileum in diabetic and fasted rats, PEPCK activity seems only weakly regulated, if at all, because the variations in the mRNA abundance do not result in significant alterations in enzymatic activity. These last results suggest that the metabolic role of both enzymes could differ along the SI depending on the hormonal and/or nutritional situation. The fact that both PEPCK and G6Pase are found at high upregulated levels in the duodenum and jejunum in fasted and diabetic rats strongly suggests that these 2 parts of the SI play a significant role in GNG in insulinopenia. On the other hand, because G6Pase is not expressed in the ileum of fed rats (normal and diabetic), one may hypothesize a role in glyceroneogenesis for PEPCK in this part of the SI.

In strong agreement with the former proposal, we have recently observed, using methodology involving the combination of arterio-venous balance and isotopic dilution techniques, that rat SI, which does not release glucose in the normal fed state, contributes ~20–25% of the total endogenous glucose production under insulinopenic conditions (15). Most interestingly, ~60% of the glucose production in SI in these situations could be accounted for by GNG from glutamine, which is already known as a major substrate for SI (15). Because glutamine enters the Krebs cycle at the level of α-ketoglutarate, the further conversion of a significant part of oxaloacetate in phosphoenolpyruvate due to the dramatic induction of PEPCK gene expression is in agreement with the metabolic fluxes observed under insulinopenic conditions. PEPCK is already considered to be the rate-limiting enzyme for induction of GNG in both the liver and kidney cortex (9). The present study strongly suggests that it should play a similar crucial controlling role in GNG in SI.

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