Molecular Mechanism of Induction of Key Enzymes Related to Lipogenesis (43419)

TAMIO NOGUCHI,*[1] NOBUKO IRITANI,† AND TAKEHIKO TANAKA*
Department of Nutrition and Physiological Chemistry, Osaka University Medical School, Suita, Osaka 565, and Tezukayama Gakuin College,† Sakai, Osaka 590-01, Japan*

Abstract. **Key enzymes related to lipogenesis in the liver are induced by a high glucose diet or insulin and suppressed by starvation, diabetes, or glucagon. Most of these enzymes are also induced by dietary fructose, even in diabetic liver. This regulation occurs at the posttranscriptional level as well as at the transcriptional level. We studied extensively the molecular mechanism of induction of L-type pyruvate kinase (LPK). The transcription of the LPK gene in the liver was stimulated by insulin and inhibited by glucagon. This insulin action required ongoing protein synthesis and metabolism of glucose and was enhanced by glucocorticoid. On the other hand, the mechanism of induction of the LPK by dietary fructose depended on plasma insulin levels. Dietary fructose stimulated transcription of the LPK gene in normal rats, whereas it acted mainly at the posttranscriptional level in diabetic rats. These fructose effects were attributable to a common metabolite of fructose and glycerol. The induction of LPK mRNA by dietary glucose was impaired in the liver of Wistar fatty rats, a model of obese non-insulin-dependent diabetes mellitus, but fructose-induced accumulation of the mRNA was not. Studies on transgenic mice indicated that the 5′-flanking region up to −3 kb of the LPK gene contained all *cis*-acting elements necessary for tissue-specific expression of LPK and its stimulation by diets and insulin. Further analysis using a transient expression assay revealed the presence of three *cis*-acting elements necessary for expression of LPK in hepatocytes in the region up to −170 kb. However, these elements alone were not sufficient for dietary and hormonal regulation of this enzyme when analyzed in transgenic mice.** [P.S.E.B.M. 1992, Vol 200]

Gene expression of key enzymes related to lipogenesis in the liver is regulated by hormones and diets (1). These enzymes include glucokinase, L-type pyruvate kinase, glucose-6-phosphate dehydrogenase, malic enzyme, acetyl-CoA carboxylase, and fatty acid synthase. A high glucose diet or insulin induces these enzymes, leading to a stimulation of lipogenesis, whereas starvation, diabetes, or glucagon suppresses them. Dietary fructose, which is known to stimulate lipogenesis more than dietary glucose, also induces these enzymes, except glucokinase. This induction occurs even in diabetic liver, in contrast with the effect of dietary glucose (2, 3). Since fructokinase, which catalyzes the first step of fructose metabolism, is not regulated by hormones or diets (4), dietary fructose can stimulate lipogenesis in diabetic liver.

[1] To whom requests for reprints should be addressed at Department of Nutrition and Physiological Chemistry, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan.

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We have studied the molecular mechanism of induction of these enzymes by insulin and dietary fructose (5–15) and found that this induction occurs at the posttranscriptional level as well as at the transcriptional level. In this paper, we describe our recent studies on L-type pyruvate kinase (LPK), since it has been most extensively investigated (7–11).

Regulation of LPK Gene Expression in Normal and Diabetic Rats

Previously, using streptozotocin-treated diabetic rats, we showed that insulin induced hepatic LPK mRNA by stimulating the rate of transcription of this gene (6). On the other hand, dietary fructose acted mainly at the posttranscriptional level, probably stabilizing nuclear RNA species of LPK (5, 6). Dietary glycerol was also a potent inducer of LPK mRNA in diabetic liver, suggesting that the effect of fructose is attributable to an intermediate common to the metabolism of both fructose and glycerol (6). Besides the liver, LPK is expressed in the kidney and small intestine. The major sites for metabolism of fructose are known to be

the liver, kidney, and small intestine (16), whereas glycerol can be appreciably metabolized only in the liver and kidney (17). Therefore, we examined the effects of various carbohydrates on the levels of LPK mRNA in the kidney and small intestine of normal rats. Dietary fructose increased the levels of LPK mRNA in these tissues. Glycerol feeding also increased the mRNA level in the kidney after 6 hr, but did not affect the mRNA level in the small intestine. Glucose induced a slight but significant increase in the level of LPK mRNA in the small intestine, but not in the kidney. No other carbohydrates (including galactose, sorbitol, xylose, and xylitol) were effective inducers of LPK mRNA in the two tissues and in the liver of diabetic rats. Thus, the sites where fructose and glycerol induce LPK mRNA are closely related to the sites of their metabolism, supporting the metabolite hypothesis. The candidate molecule may not accumulate significantly during metabolism of other carbohydrates except glucose.

Recently, Munnich *et al.* (18) reported that dietary fructose stimulated transcription of the LPK gene in the liver of normal rats. As indicated above, we reported the different mechanism of fructose induction of LPK in diabetic rat liver as mentioned above. This difference could be attributable to the difference in the condition of the animals used, that is diabetic versus normal. We tested this possibility. Feeding fructose for 4 hr resulted in only a 2.4-fold stimulation of transcription of the LPK gene in diabetic rats, but about a 27-fold increase in transcription in normal rats. In contrast, the increases in the LPK mRNA levels were similar under these two conditions. To determine whether this difference in the response of gene transcription to fructose was due to insulin, we injected insulin 30 min after the start of fructose or glucose feeding. Insulin treatment resulted in a further 4.7-fold increase in transcription in fructose-fed diabetic rats, but no change in transcription in glucose-fed diabetic rats was observed at this time point, which is consistent with our previous results (6). On the contrary, insulin did not increase the mRNA level further in fructose-fed rats. These results indicate that the mechanism of fructose induction of LPK in the liver is dependent on plasma insulin levels.

We also determined the rate of transcription of the LPK gene in the kidney of normal and diabetic rats given a high fructose diet for 4 hr. Dietary fructose stimulated transcription only 1.8-fold and 2.2-fold in normal and diabetic kidney, respectively. However, the magnitudes of these increases were much lower than those of the mRNA. Thus, dietary fructose acts mainly at a posttranscriptional level in both normal and diabetic kidney, as in diabetic liver. These results are understandable, since dietary glucose and insulin have no effect on expression of the LPK gene in the kidney.

The Wistar fatty rat develops both obesity and diabetes as genetically determined traits (19). Therefore, this strain is a good model for human non-insulin-dependent diabetes mellitus with obesity. We sought to determine whether the dietary induction of LPK mRNA is impaired in the liver of this model. The magnitude of induction of the mRNA by dietary glucose in Wistar fatty rats was about 60% of that in Wistar lean rats. On the other hand, similar increases in the LPK mRNA levels were observed in fatty and lean rats given a fructose diet. These results are consistent with those with streptozotocin-induced diabetic rats.

Regulation of the LPK Gene Expression in Primary Cultures of Rat Hepatocytes

We further investigated the mechanisms of actions of insulin and carbohydrates in cultured hepatocytes. Insulin caused time- and dose-dependent increases in the amount of LPK mRNA in cultured hepatocytes. The maximal insulin response was obtained at a concentration of 10 n*M* after 24–36 hr. The effect of insulin was dependent on not only the types of carbohydrates, but also on their quantity in the medium. The addition of glucose caused a marked dose-dependent increase in the induction of LPK mRNA by insulin. The additions of fructose and glycerol also enhanced the induction by insulin, but the maximal enhancement was less than 20% of that with glucose. No induction of LPK mRNA by insulin was observed in hepatocytes cultured in the presence of pyruvate. On the other hand, none of these carbohydrates induced LPK mRNA in the absence of insulin. This was unexpected, because fructose and glycerol can induce the mRNA even in diabetic liver. The reason for this discrepancy is not known at the present times.

The time course of insulin induction of LPK mRNA in the presence of fructose was similar to that in the presence of glucose. In the presence of glycerol, the mRNA increased in a diphasic manner: The first increase reached a maximum after 3 hr, whereas the second increase corresponded to the increase in the presence of glucose. The early effect of insulin-glycerol was inhibited by actinomycin D. Thus, this effect can be regarded as a partial reproduction of the fructose effect in normal liver, which occurred much faster than the insulin effect in diabetic liver.

These results suggest that the metabolism of glucose is required for the induction of LPK mRNA by insulin. If so, the effect of insulin should not be observed in the presence of 2-deoxyglucose, a nonmetabolizable glucose analog. This was the case: The effect of insulin was not observed in medium containing 2-deoxyglucose. Thus, we suggest that some metabolite accumulating during the metabolism of glucose is necessary for the induction of LPK mRNA by insulin and that this metabolite can be produced from fructose and glycerol

to some extent, but not from pyruvate, in cultured hepatocytes. However, the role of insulin in the induction remains to be determined.

Cycloheximide inhibited the insulin induction of LPK mRNA, suggesting that ongoing protein synthesis is required for the effect of insulin on LPK. The addition of H-7, an inhibitor of protein kinase C, caused a dose-dependent inhibition of the induction of LPK mRNA by insulin. However, phorbol 12-myristate 13-acetate did not increase the level of the mRNA. Thus, the effect of H-7 may not involve protein kinase C, although the possibility cannot be ruled out that protein kinase C may be involved in the action of insulin, but not sufficiently so to induce LPK mRNA by itself. Dexamethasone alone decreased the LPK mRNA level, but greatly enhanced the induction of the mRNA by insulin when added with insulin. 3,5,3′-Triiodothyronine had no effect either on the LPK mRNA level by itself or together with dexamethasone or on the induction of the mRNA by insulin-dexamethasone. 8-CPT-cAMP, a stable analog of cAMP, inhibited the insulin induction of the mRNA.

The *in vivo* and *in vitro* studies indicate that the mechanism of regulation of the LPK gene expression by insulin and dietary fructose is apparently very complicated. Figure 1 shows a proposed model of this regulation. Insulin binding to receptor activates receptor tyrosine kinase, which somehow transduces the signal to insulin-responsive protein. Insulin-responsive protein then interacts with insulin-responsive element located in the upstream of the LPK gene to stimulate transcription. Protein synthesis and glucose metabolism are required for this process. Insulin may simply increase the concentration of this metabolite by stimulating glucose metabolism or, in addition to this effect, another signal from insulin may also be necessary. On the other hand, in the presence of normal levels of insulin, fructose mainly stimulates transcription probably through some metabolite common to both fructose and glycerol. This metabolite may be identical to that involved in the insulin effect or different from that. In the latter case, different *cis*- and *trans*-acting factors

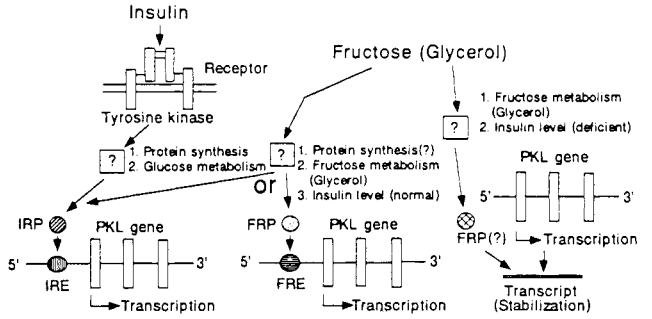

Figure 1. Proposed model of actions of insulin and dietary fructose on expression of the LPK gene.

(fructose-responsive element and fructose-responsive protein, respectively) are probably involved in the induction. In an insulin-deficient state, fructose somehow stabilizes the transcript of the LPK gene. A metabolite common to fructose and glycerol may also be involved in this effect, but its metabolite should be different from that involved in transcription.

Identification of *cis*-Acting Regulatory Regions of the LPK Gene

To test this model, we produced transgenic mice carrying the 5′-flanking region of the LPK gene from nucleotide about −3000 to +37 linked to the chloramphenicol acetyltransferase (CAT) structural gene. CAT activity was detected in the liver, kidney, and small intestine, but not in other tissues. The tissues expressing CAT activity coincided with those expressing the endogenous LPK. Dietary glucose or insulin induced similar increases in the levels of CAT and LPK mRNA in the liver. Dietary fructose also increased the level of CAT mRNA, although its induced level was significantly lower than that of LPK mRNA. Thus, the sequence of about 3 kb upstream of the LPK gene contains all the *cis*-acting elements responsible for tissue-specific expression of LPK and its transcriptional stimulation by dietary carbohydrates and insulin. Dietary fructose, but not glucose, also induced a marked increase in the LPK mRNA level in the kidney, but only a marginal increase in the level of CAT mRNA was observed in this tissue. These results are consistent with our finding mentioned above that posttranscriptional regulation is a major factor in fructose induction of LPK mRNA in the kidney.

To further analyze the *cis*-acting element, we introduced plasmid DNA containing 3.2 kb of the 5′-flanking region of LPK gene linked to the CAT gene into adult rat hepatocytes by electroporation, and the transient CAT expression was determined. Although hepatocytes transfected with this plasmid showed high CAT activity, the addition of insulin did not affect expression of the CAT gene. This contrasts with the results obtained in transgenic mice. The reason for this discrepancy remains to be determined. In any case, this indicates that strong promoter activity is present in the 3.2-kb upstream region. Therefore, we carried out functional analysis of a series of 5′ and internal deletion constructions of the CAT fusion genes. Consequently, we identified three positive regulatory regions required for expression of LPK in hepatocytes. These regions, designated as PKL-I, PKL-II, and PKL-III, were located between nucleotides −76 and −94, −126 and −149, and −150 and −170, respectively. PKL-I showed enhancer-like activity alone, whereas PKL-II and PKL-III did not have any independent effect. Combinations of L-I + L-II and L-II + L-III, but not of L-I + L-III, showed synergistic enhancer activities when oriented in

the same direction. The inclusion of all three elements oriented in the same direction had the maximum synergistic effect, indicating that the three elements function as a unit. This unit enhanced expression from heterologous as well as homologous promoters in a manner that was independent of its orientation and position relative to the cap site. The activity of the unit was not detected in HeLa cells or K562 erythroleukemia cells. PKL-I contains a sequence homologous to the LF-B1 (HNF1)-binding site. PKL-II and PKL-III include sequences homologous to the binding sites of LF-A1 and the adenovirus major late transcription factor, respectively. Gel retardation assays indicated that the different *trans*-acting factors interacted with the three elements and that the *trans*-acting protein bound to PKL-I was in fact LF-B1. However, the *trans*-acting factors bound to PKL-II and PKL-III were different from LF-A1 and major late transcription factor, respectively. Thus, we suggest that the three *cis*-acting elements are very important for cell-specific expression of the LPK gene and that LF-B1 and two unknown proteins bound to these elements interact with each other to cause a synergistic effect.

An important question concerns whether these *cis*- and *trans*-acting factors are responsible for transcriptional stimulation of the LPK gene by insulin and dietary fructose. Since this could not be answered in our transient expression system, we produced transgenic mice carrying the 5′-deletion LPK-CAT fusion gene up to nucleotide −610. Expression of the CAT gene showed tissue specificity similar to that of the endogenous LPK gene. This strongly supports the findings that the three *cis*-acting elements play an important role in cell-specific expression of the LPK gene. However, dietary glucose and fructose caused only a marginal change in the cAT mRNA level, although they greatly increased the LPK mRNA level. Thus, the three elements alone were not sufficient to confer insulin and fructose responsiveness to a linked reporter gene. The DNA region important for this regulation may be located between −3000 and −610 of the LPK gene.

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