Volume 318, number 3, 269–272 FEBS 12178 March 1993

An enhancer unit of L-type pyruvate kinase gene is responsible for transcriptional stimulation by dietary fructose as well as glucose in transgenic mice

Tamio Noguchi[a], Masaru Okabe[b], Ziyuan Wang[a], Kazuya Yamada[a], Enyu Imai[a] and Takehiko Tanaka[a]

[a]*Department of Nutrition and Physiological Chemistry, Osaka University Medical School, Osaka 565, Japan* and [b]*Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan*

Received 11 January 1993

We produced three lines of transgenic mice containing the 5' flanking region of the L-type pyruvate kinase gene from nucleotides −189 to +37, which includes an enhancer unit and TATA box as functional elements, linked to the chloramphenicol acetyltransferase gene. Since transgene expression was stimulated by both dietary fructose and glucose in a tissue-dependent manner, we suggest that this unit is responsive to both stimuli.

L-Type pyruvate kinase gene; Transgenic mouse; Carbohydrate response unit

1. INTRODUCTION

Intake of a high glucose or fructose diet modulates gene expression of enzymes involved in carbohydrate and lipid metabolisms at both transcriptional and post-transcriptional levels [1–3]. Although insulin mediates the effect of dietary glucose in many cases, it may exert its effect through stimulating glucose metabolism [1–5]. In this case, many authors have postulated that some metabolite of glucose could be involved in stimulation of gene expression. On the other hand, the insulin-secretory effect of dietary fructose is much weaker than that of dietary glucose [6], suggesting that fructose stimulates the gene expression by a mechanism, as yet unclarified, independent of insulin.

The L-type isozyme of pyruvate kinase (LPK) provides a good example of regulation of gene expression by dietary carbohydrates. This important glycolytic enzyme is present in the liver, kidney, small intestine and pancreatic B-cells [7,8]. The induction of LPK is caused by dietary glucose in the liver and small intestine at the transcriptional level, whereas dietary fructose induces the enzyme not only in these two tissues but also in the kidney at the transcriptional and post-transcriptional levels [9–12]. The transcriptional stimulation of the LPK gene by dietary glucose requires the presence of insulin and occurs much slower than that by dietary fructose. However, we suggested that metabolites of these carbohydrates are involved in the induction of LPK in both cases [12–14]. These metabolites could be identical or different. If the former is the case, the same regulatory DNA region should be involved in the induction of LPK by both glucose and fructose. Using transgenic mice, we found that the region of the LPK gene from about nucleotides −3000 to +37 (relative to the transcription start site) contains element(s) responsive to both dietary glucose and fructose [15]. Recently, the regulatory DNA region responsible for insulin/glucose was identified in the 5' flanking region of the LPK gene using in vitro transient expression assay [16,17]. This region is a part of an enhancer unit that we have previously identified [18]. This region is a part of an enhancer unit that we have previously identified [18]. The unit consists of three elements designated as PKL-I, PKL-II and PKL-III, and plays an important role in cell type-specific expression of the LPK gene. Therefore it is interesting to see whether this unit of the LPK gene also shows responsiveness to dietary fructose. Here we report that an enhancer unit of the LPK gene is responsive not only to dietary glucose, but also to dietary fructose in transgenic mice, suggesting that both carbohydrates share the common mechanism of transcriptionsl stimulation of the LPK gene.

Correspondence address: T. Noguchi, Department of Nutrition and Physiological Chemistry, Osaka University, Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan. Fax: (81) (6) 875-7426.

Abbreviations: LPK, L-type pyruvate kinase; CAT, chloramphenicol acetyltransferase.

2. MATERIALS AND METHODS

Fertilized eggs were collected from the oviduct of B6C3F1 females mated with B6C3F1 males. The DNA fragment of the LPK gene from nucleotides −189 to +37 linked to the chloramphenicol acetyltransferase (CAT) gene was isolated from pLcat189 [18] and injected into one of the two pronuclei. The injected eggs were transferred to female mice of *ddy* strain made pseudopregnant by mating with vasectomized males. Three weeks after birth, the offspring were examined for the

presence of the transgene by Southern blot analysis of DNA isolated from tail, using a ^{32}P-labeled CAT probe [19]. Positive founder mice were mated with C57BL/6 mice, and transgenic offspring were used for experiments at 6–8 weeks of age. The copy number of the integrated gene was estimated by dot blot analysis [19].

Transgenic mice were starved for 28 h and then given a synthetic diet containing 81% glucose or fructose [15]. Mice were killed 16 h after the start of feeding and control mice were killed at zero time. Total RNA was isolated from tissues by the acid guanidine–phenol–chloroform method [20] and used to purify poly(A)$^+$ RNA by oligo(dT)-cellulose column [19]. Electrophoresis of poly(A)$^+$ RNA (4 μg), subsequent transfer to a nylon filter and hybridization of the filter with labeled probes were carried out as described before [9,12]. The results were analyzed by densitometry of bands on autoradiograms. The probes used for LPK, CAT and β-actin were described previously [12,15,21]. They were labeled with [α-^{32}P]dATP using random oligonucleotide primers [22].

3. RESULTS AND DISCUSSION

We produced three lines of transgenic mice containing the 5' flanking region of the LPK gene from nucleotides −189 to +37 linked to the CAT gene. This upstream region included an enhancer unit from −170 to −76 and TATA box from −28 to −23 [18,23]. The copy numbers of the integrated transgene into these lines were 4, 20 and 1 for lines 6, 7 and 12, respectively. The heterozygous F_1 mice produced from these founders were starved and re-fed with a high glucose or fructose diet. Poly(A)$^+$ RNA from various tissues were analyzed by Northern blot hybridization. As observed previously [15], two bands of CAT mRNA of about 1.5 and 1.9 kb were found in several RNA samples and the major band was the smaller species. CAT mRNA was detected only in the tissues expressing LPK mRNA, such as liver, kidney and small intestine (Figs. 1–3), but not in other tissues such as brain, spleen and skeletal muscle of the three lines (data not shown).

Dietary carbohydrates caused an increase in the CAT mRNA levels in the liver, kidney and small intestine of transgenic mice, but its extent was dependent on both tissues and lines. In the liver of line 6, dietary glucose or fructose greatly stimulated expression of CAT mRNA (Fig. 1). In line 7, however, CAT transcripts were barely detectable despite the high copy number of the transgene. The mRNA was detected in each of three mice fed a glucose or fructose diet, but not in control mice (Fig. 2). The expression of the transgene in line 12 was as low as that in line 7, which agrees with its copy number. Its expression was stimulated by both carbohydrates (Fig. 3). These treatments markedly induced LPK mRNA in all lines.

Dietary fructose caused 6- to 9-fold increases in the LPK mRNA levels in the small intestine of all transgenic lines, whereas significant induction of the mRNA following glucose feeding was observed only in line 7 (2.2-fold). These results agree well with those in rat [11,12]. Dietary treatments caused changes in expression of the transgene comparable to those observed for the endogenous gene in all lines. The levels of CAT

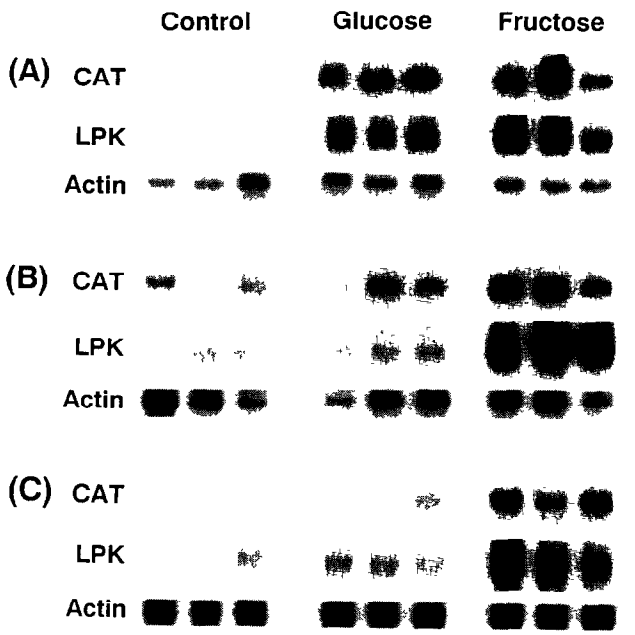

Fig. 1. Northern blot analysis of poly(A)$^+$ RNA isolated from liver (A), kidney (B) and small intestine (C) of transgenic line 6 given a glucose or fructose diet.

mRNA induced by fructose were about 2- and 8-fold higher in lines 6 and 7 than in line 12, respectively, which is roughly proportional to their copy number.

This was also the case in the kidney. The fructose-induced levels of CAT mRNA were about 3-fold higher in line 7 than in line 6 and this message was undetectable in line 12 (data not shown). For lines 6 and 7, fructose

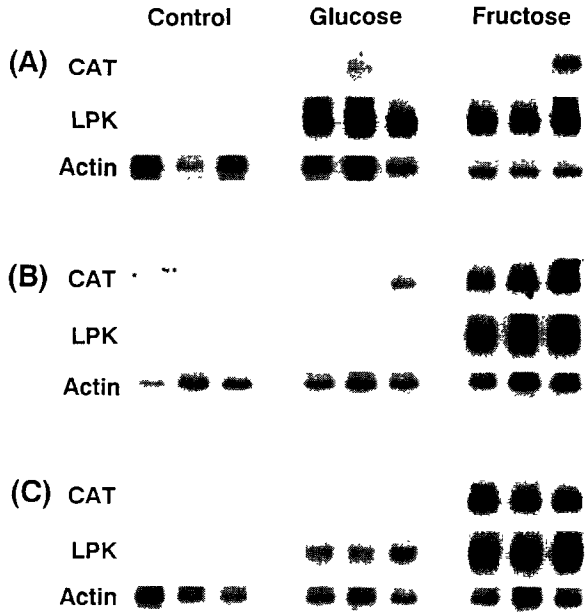

Fig. 2. Northern blot analysis of poly(A)$^+$ RNA isolated from liver (A), kidney (B) and small intestine (C) of transgenic line 7 given a glucose or fructose diet.

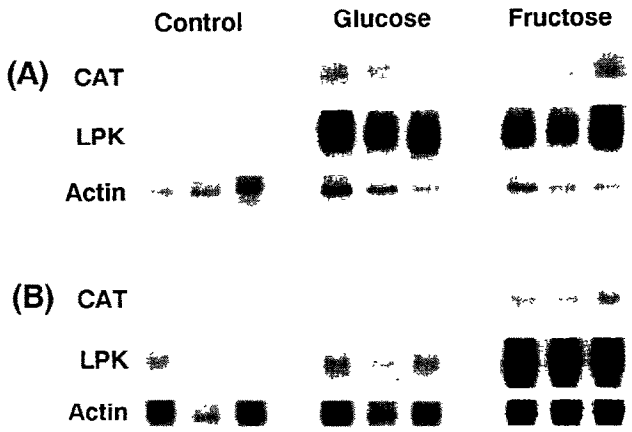

Fig. 3. Northern blot analysis of poly(A)$^+$ RNA isolated from liver (A) and small intestine (B) of transgenic line 12 given a glucose or fructose diet.

feeding resulted in about a 17-fold increase in the LPK mRNA levels, but only a 4- to 6-fold increase in the CAT mRNA levels. This is consistent with previous findings that dietary fructose acts at both transcriptional and post-transcriptional levels to induce LPK in the kidney [12]. In contrast, dietary glucose did not affect the expression of the transgene or of the endogenous gene in the kidney for these lines. None of the dietary treatments appreciably affected the β-actin mRNA levels in any tissues of any lines.

These results indicate that the 5' flanking region of the gene from nucleotides −189 to +37 contains regulatory sequences responsible for tissue-specific expression of LPK and that the region also contains the element responsive to dietary fructose as well as to dietary glucose. No other regulatory sequences may be involved in expression of the LPK gene in the kidney and small intestine, because the fructose-induced levels of the transgene in these tissues were roughly proportional to the copy number. However, this was not the case in the liver. In spite of the high copy number of the transgene in line 7, its expression was as low as that in line 12, which contained a single copy of the CAT gene. Thus, other elements modulating expression of the LPK gene in the liver may be present between about −3000 to −189, since the upstream region of the LPK gene up to −3000 contains all the information necessary for regulation of the gene expression [15]. Cuif et al. suggested recently that regulatory elements may be present in the region upstream from −183 [24]. In addition, they reported that the region from −183 to +11 of the LPK gene confers tissue specificity and glucose responsiveness to the CAT reporter gene. Their conclusion was based on analysis of various transgenic mice containing different combinations of various upstream regions of the LPK gene, although they did not directly analyze transgenic mice containing only the region from −183 to +11. The present study showed that this region was actually responsible for tissue-specific expression and glucose regulation of the LPK gene. Furthermore, we demonstrated that this region was also responsive to dietary fructose. Since the region from −189 to +37 contains only an enhancer unit besides the TATA box as functional elements [18], it is reasonable to conclude that this unit is responsible for transcriptional stimulation of the LPK gene by both carbohydrates in vivo. Using transient expression assay in hepatocytes, two groups recently reported that L4 box (which refers to PKL-III) is an element responsive to glucose/insulin [16,17]. Since PKL-III is part of the enhancer unit and since the effects of both glucose and fructose on LPK gene expression may involve their metabolites, we propose that an intermediate common to the metabolisms of both glucose and fructose somehow stimulates the gene transcription through PKL-III. The extent and rapidity of accumulation of this intermediate could depend on the tissues and also on the nature of the carbohydrates. As PKL-III alone does not have any enhancer activity and is only active as a unit together with PKL-I and PKL-II [18], this unit may be called a carbohydrate response unit.

Acknowledgements. This investigation was supported by grants from the Ministry of Education, Science and Culture of Japan and the Yamanouchi Foundation for Research on Metabolic Disorders.

REFERENCES

[1] Goodridge, A.G. (1987) Annu. Rev. Nutr. 7, 157–185.
[2] Noguchi, T., Iritani, N. and Tanaka, T. (1992) Proc. Soc. Exp. Biol. Med. 200, 206–209.
[3] Iritani, N. (1992) Eur. J. Biochem. 205, 433–442.
[4] Pilkis, S.J. and Granner, D.K. (1992) Annu. Rev. Physiol. 54, 885–909.
[5] O'brien, R.M. and Granner, D.K. (1991) Biochem. J. 278, 609–619.
[6] Hara, E. and Saito, M. (1981) Endocrinology 109, 966–970.
[7] Imamura, K. and Tanaka, T. (1972) J. Biochem. (Tokyo) 71, 1043–1051.
[8] Noguchi, T., Yamada, K., Yamagata, K., Takanaka, M., Nakajima, H., Imai, E., Wang, Z. and Tanaka, T. (1991) Biochem. Biophys. Res. Commun. 181, 259–264.
[9] Noguchi, T., Inoue, H. and Tanaka, T. (1985) J. Biol. Chem. 260, 14393–14397.
[10] Munnich, A., Lyonnet, S., Chauvet, D., Van Schaftingen, E. and Kahn, A. (1987) J. Biol. Chem. 262, 17065–17071.
[11] Ogier, H., Munnich, A., Lyonnet, S., Vaulont, S., Reach, G. and Kahn, A. (1987) Eur. J. Biochem. 166, 365–370.
[12] Matsuda, T., Noguchi, T., Takanaka, M., Yamada, K. and Tanaka, T. (1990) J. Biochem. (Tokyo) 107, 655–660.
[13] Inoue, H., Noguchi, T. and Tanaka, T. (1984) J. Biochem. (Tokyo) 96, 1457–1462.
[14] Matsuda, T., Noguchi, T., Yamada, K., Takanaka, M. and Tanaka, T. (1990) J. Biochem. (Tokyo) 108, 778–784.
[15] Yamada, K., Noguchi, T., Miyazaki, J., Matsuda, T., Takanaka, M., Yamamura, K and Tanaka, T. (1990) Biochem. Biophys. Res. Commun. 171, 243–249.
[16] Shih, H. and Towle, H.C. (1992) J. Biol. Chem. 267, 13222–13228.
[17] Bergot, M.O., Diaz-Guerra, M.M., Puzenat, N., Raymondjean, M. and Kahn, A. (1992) Nucleic Acids Res. 20, 1871–1878.

[18] Yamada, K., Noguchi, T., Matsuda, T., Takenaka, M., Monaci, P., Nicosia, A. and Tanaka, T. (1990) J. Biol. Chem. 265, 19885–19891.
[19] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[20] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[21] Hotta, K., Nakajima, H., Yamasaki, T., Hamaguchi, T., Kuwajima, M., Noguchi, T., Tanaka, T., Kono, N. and Tarui, S. (1991) Eur. J. Biochem. 202, 293–298.
[22] Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13.
[23] Noguchi, T., Yamada, K., Inoue, H., Matsuda, T. and Tanaka, T. (1987) J. Biol. Chem. 262, 14366–14371.
[24] Cuif, M., Cognet, M., Boquet, D., Tremp, G., Kahn, A. and Vaulont, S. (1992) Mol. Cell. Biol. 12, 4852–4861.