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Regulation of Acetyl-CoA Carboxylase Gene Expression by Hormones and Nutrients

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Abstract

This study was investigated to identify the regulatory mechanism of ACC gene expression by hormones and nutrition. The fragment of ACC promoter I (PI) -220 bp region was recombined to pGL3-Basic vector with luciferase as a reporter gene. The primary hepatocyte from the rat was used to investigate the regulation of ACC PI activity. ACC PI (-220 bp)/luciferase chimeric plasmid was transfected into primary rat hepatocyte by using lipofectin. ACC PI activity was shown by measuring luciferase activity. The addition of insulin, dexamethasone, and triiodothyronine to the culture medium increased the activity of ACC PI by 2.5-, 2.3- and 1.8-fold, respectively. In the presence of 1 μM dexamethasone, the effects of insulin was amplified about 1.2-fold showing the additional effects of dexamethasone. Moreover the activity of luciferase was increased by insulin, dexamethasone, and triiodothyronine treatment approximately 4-fold. These results indicated that insulin, dexamethasone and thyroid hormone coordinately regulate ACC gene expression *via* regulation of promoter I activity. On the -220 to +21 region of ACC PI, the addition of the glucose to the culture medium increased the activity of ACC PI. With 25 mM glucose, luciferase activity increased by 7-fold. On the other hand, on the -220 bp region, ACC PI activity was not changed by polyunsaturated fatty acids. Therefore, it can be postulated that there are response elements for insulin, triiodothyronine, dexamethasone, and glucose, but not PUFAs on the -220 bp region of ACC PI.

Key words: acetyl-CoA carboxylase, gene expression, promotor, primary hepatocytes, hormone, nutrition

INTRODUCTION

Acetyl-CoA carboxylase (ACC) enzyme (EC 6,4,1,2) catalyzes the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA. This is the first committed step in fatty acid biosynthesis and thus this enzyme represents an important control point for intermediary metabolism. There are two ACC genes, α and β. ACC-β is the major isoform observed in heart and skeletal muscle. The β form is thought to play a specific role in producing malonyl-CoA for the purposes of regulating fatty acid oxidation and comparatively little is know of its regulation (1). ACC-α is the principal isoform expressed in tissues that exhibit high rates of fatty acid synthesis such as liver, adipose tissue, and mammary gland. ACC-α is regulated by nutrients and hormones at both short-term (enzyme activity) and long-term (enzyme synthesis) levels in a tissue-specific manner. Short-term control of ACC-α involves covalent modification and allosteric regulation (2,3). Long-term regulation involves multiple hormones and dietary factors that interact with each other, to inhibit or amplify ACC-α gene expression (1). For example, food deprivation for 48 h reduced the levels of ACC mRNA by 50 ~ 85% in rat liver and adipose tissue; refeeding the high carbohydrate diet after food deprivation dramatically stimulated the expression of ACC mRNA 20-fold compared with the 48 h starved animals (4).

The ACC-α gene is transcribed from two promoters, designed as promotor I (PI) and promotor II (PII) (5). Studies on the physiological regulation of these two promoters indicate that the promoter II is fairly uniformly expressed in all tissues and does not respond to dietary changes (6). The PI promoter is expressed predominantly in adipose and liver and its utilization is markedly elevated in response to diets inducing lipogenesis (6,7). Hence, the 20-fold increase in ACC mRNA in livers of fasted rats that have been shifted to a high carbohydrate diet (4) is largely a consequence of PI promoter induction.

However, only limited information on the regulatory mechanism for gene expression of the enzyme is available at present (8,9). Previously, we reported that activity of ACC PI was regulated on -1.2 kb region of ACC PI

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by insulin, dexamethasone, and thyroid hormone (10). It was postulated that 1) these hormones coordinately regulate the acetyl-CoA caroxylase gene expression *via* regulation of promoter activity, 2) the -1.2-kb region of ACC promoter I seemed to have the response element sequences for insulin, dexamethasone, and thyroid hormone. The major signal controlling ACC gene expression was reported as glucose (11). Although an intrinsic repressor element has been described (1), the role of this element in regulation of the PI promoter is unknown. In this study, we examined the effects of hormones and nutritions such as insulin, thyroid hormone, dexamethasone, glucose and PUFAs on ACC PI (-220 bp) which requires identification of the regulation mechanism of ACC PI in further studies.

MATERIALS AND METHODS

Plasmid constructs

A rat genomic library was screened by Genome Systems (St. Louis, MO) using primers recognizing ACC exon I. A single positive 85 kb clone was obtained. Digestion with BamH I yielded a 12 kb clone including exon 1 and the PI promoter region. The PI promoter fragment corresponding to -220 to +21 was inserted into the modified pGL3 basic vector (11). The modified pGL3 basic vector was kindly gifted by Dr. Towle (Univ. Minnesota).

Primary hepatocyte culture

Primary hepatocytes were isolated from male Harlan Sprague-Dawley rats (200 ~ 250 g) maintained individually in stainless steel wire mesh cages on a 12/12 hour light/dark cycle at 20 ~ 22°C, using the collagenase perfusion method (12,13). The animals were given free access to rodent laboratory chow (Purina Mills, St. Louis, MO). Before plating, cells were judged to be greater than 85% viable using 5% trypan blue exclusion (13). Cells were plated at a density of approx. 1.2×10^6 cells per 30 mm tissue culture dish (Becion Dickinson, Franklin Lakes, NJ) and suspended in 3 ml culture media (Williams E media with supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbnd, California), 23 mM HEPES, 26 mM $NaHCO_3$, 10 nM dexamethasone (Dex), 0.1 unit/mL insulin (Sigma, ST. LOUIS, MI), 1 unit/mL penicillin, 1 µg/mL streptomycin) but without Matrigel. The cells were incubated at 37°C in an atmosphere of 5% CO_2 in air with 95% humidity.

Transfection of hepatocytes

After a 6 h attachment period, cells were transfected with lipofectin (Invitrogen, Carlsbnd, California) in serum-free culture media for 14 h. In order to observe the effects of hormones, cells were then incubated in serum-free, hormone-free culture media containing with or without hormones. In order to observe the effects of glucose concentration, cells were treated in serum-free culture media with different concentrations of glucose in the presence of 0.1 µM insulin, 1 µM dexamethasone (Dex), and 5 µM triiodothyronine (T_3). In order to observe the effects of polyunsaturated fatty acids (PUFA), cells were treated in serum-free culture media with different kinds of 300 µM PUFA in the presence of 0.1 µM insulin, 1 µM Dex, 5 µM T_3, and 25 mM glucose.

Before experiments, non-esterified fatty acids (Sigma, ST. Louis, MI) were diluted in ethanol (95%) (14). Fatty acids were combined to 7.5% BSA (1.11 mM) by stirring for 1 h at 37°C. 0.1% butylated hydroxytoluene and 20 µM-tocopherol were added to fatty acid (4 mM)-albumin (1 mM) stock solution to minimize the oxidation of the fatty acid. These antioxidants were dissolved in DMSO.

Just before use, the fatty acid stock solutions were diluted into serum-free culture media. The fatty acids used in the present study included oleic acid (OA, C18:1, n-9), linolenic acid (LNA, C18:3, n-6), docosahexaenoic acid (DHA, C22:4, n-3), arachidonic acid (AA, C20:4, n-6), and eicosapentaenoic acid (EPA, C20:5, n-3).

Luciferase activity and protein assay

After 48 h, treated cells were collected, resuspended in 400 µL luciferase lysis buffer (Promega, madison, WI), and incubated 10 min at room temperature (15). After centrifugation 2 min at 500 *g*, supernatants (50 µL) were incubated 5 sec in the presence of 100 µL luciferase assay buffer including luciferin (0.2 mM). The luciferase activity was determined using a luminometer (TD 20/20, Promega, madison, WI), and expressed as relative light units (RLU) measured per microgram of protein.

Protein was measured by a method of Bradford (16). The experiments were repeated at least three times with triplicate samples for each point.

Statistics assay

One way analysis of variance (ANOVA) were used to test for differences in experimental groups. Duncan's multiple range test was employed to examine differences between individual treatment conditions.

RESULTS AND DISCUSSION

Effects of insulin, dexamethasone, and thyroid hormone on acetyl-CoA carboxylase promoter I

Acetyl-CoA carboxylase (ACC) is enzyme that control *no devo* fatty acid biosynthesis, and this enzyme catalyzes the carboxylation pathway of acetyl-CoA to malonyl-CoA. Acetyl-CoA carboxylase gene expression was reg-

ulated by nutritional and hormonal status. The present study was performed to identify the regulation mechanism of ACC gene promoter I. In previous study, -1.2-kb region of ACC promoter I seemed to have the response element sequences for insulin, dexamethasone, and T3.

To investigate the regulatory mechanism of acetyl-CoA carboxylase (ACC) gene expression, we digested -220 bp of the flanking region of ACC promoter I (PI) using restriction enzymes; BamH I and Xho I on their 5' and 3'-ends. After primary hepatocytes were isolated from rats, the ACC PI -220 bp fragment was transfected. The hepatocytes then were treated under different conditions such as; 0.1 μM insulin, 1 μM Dex, 5 μM T_3, 0.1 μM insulin plus 1 μM Dex, 0.1 μM insulin plus5 μM T_3, 1 μM Dex plus 5 μM T_3, 0.1 μM insulin plus 1 μM Dex plus 5 μM T_3.

Effects of various hormones on the -220 bp region of ACC PI are shown in Fig. 1. Luciferase activity was not significantly changed by various hormones in the cells which were transfected with pGL3-Basic vector without promoter (data is not shown). But, ACC PI activity was changed by various hormones in the cells which were transfected with pGL3-Basic vector containing ACC PI (-220 bp) fragment. Insulin treatment increased ACC PI activity by 2.5-fold compared to the control. These results showed similar pattern of Fukuda's reports (17) that ACC mRNA was increased about 4-fold by insulin treatment in rat primary hepatocytes. Although increase of mRNA was not exactly same with increase of ACC promotor activity, the increase of ACC mRNA by insulin may be

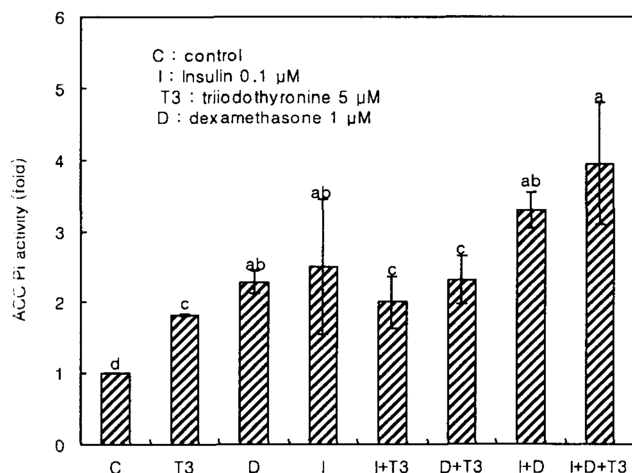

Fig. 1. ACC PI activity by hormones in primary rat hepatocyts. Hepatocytes were incubated for 48 hours in serum free medium containing various hormones at the concentrations given in parentheses after transfection with ACC PI (-220 bp)/pGL-Basic vector plasmid. After hepatocytes were lysed, assayed for luciferase activity and measured protein concentration. Values are expressed as luciferase activity/mg protein in hepatocytes. Means ± SD. Means with different letters are significant at $p<0.05$.

due to activation of transcription resulted from activation of ACC promoter. On the recent study, glucose-response element was identified (-126/-102) on the ACC PI promoter, homologous to those previously described in other responsive genes, incuding L-type pyruvate kinase, S_{14} and fatty acid synthase (15). Thus, it can be postulated that the insulin response element (IRE) involved in the regulation of ACC gene expression is present in -220 bp of ACC promotor I.

Treated with Dex and T3, ACC PI activity increased by 2.3- and 1.8-fold, respectively. Also, Dex addition potentiated the action of insulin on ACC PI activation. These results were similar to Fukuda's studies (17) which Dex addition greatly increased the ACC mRNA induction by insulin. In the previously report, Dex enhanced effect of insulin on fatty acid synthase promotor activity which has a similar mechanism of acetyl-CoA carboxylase gene expression (17). Travers (18) also reported that Dex acts to potentiate the action of insulin on PI transcript abundance in ovine adipose tissue. In the ovine adipose tissue, ACC-α transcripts with insulin alone for 72 h increased the abundance of those approximately 2-fold compared with culture with no added hormones and culture with insulin plus Dex resulted in an approximately 4.5-fold increase (18). Lu et al. (19) reported that effect of Dex on FAS expression are mediated by the DNA sequences in the promoter region of fatty acid synthase. Therefore, we expect that the effect of Dex on ACC gene expression is mediated by DNA sequences in the promotor region (-220 bp) of ACC. T_3 alone slightly increased ACC promoter I activity, and T_3 did not appear to amplify the effects of insulin. Fukuda et al. (17) reported that insulin addition increased the mRNA concentrations of ACC by 5.5-fold above the control values for hepatocytes cultured without any hormones. Also, they reported that with insulin plus Dex and insulin plus T_3 treatment, ACC mRNA was increased by 8.5- and 6-fold, respectively. The ACC mRNA was increased with insulin plus T_3 plus dexamethasone by 10-fold. These results indicate that insulin, Dex and T_3 synergically activated ACC gene expression. In the present study, insulin plus Dex plus T_3 addition increased the ACC promoter I activity by 4-fold. Our result implied that response elements of insulin, Dex, and T_3 may locate on -220 bp of ACC PI.

On the other hand, Zhang et al. (20) reported that transcription of ACC was increased approximate 7-fold by T_3 in chick embryo hepatocyte cultures, but they examined ACC PII instead of ACC PI. Therefore, the ACC PII, which is known not to be changed by hormones or nutritional status (6), was regulated by T_3 on transcription step. These results supported that regulatory mechanism of ACC gene expression in hepatocytes differ between

avian and mammary systems.

Effects of glucose on ACC carboxylase promoter I

A segment of the ACC PI from -220 to +21 bp was fused to a luciferase reporter gene and the resulting construct was introduced into primary hepatocytes. Cells were subsequently maintained 0, 5, or 25 mM glucose. The level of reporter gene activity increased by 2- and 7-fold in 5 and 25 mM glucose concentrations, respectively (Table 1). But the level of reporter gene activity was not significantly changed by glucose in the cells which were transfected with pGL3-Basic vector without promoter. These results implied that response elements of glucose may locate on -220 bp of ACC PI. Recently, O'Callaghan et al. (15) reported that the glucose response element located at -126/-102 bp on the ACC PI. Also, they reported much higher increase in ACC PI promoter activity by glucose treatment compared with our results (15). It may be possible that the Martrigel which they used to increase the effect of glucose may amplify the effect of glucose.

Effects of polyunsaturated fatty acids on ACC carboxylase promoter I

In order to analyze the effects of various polyunsaturated fatty acids (PUFAs) on ACC gene expression, the hepatocytes were cultured in the presence of 25 mM glucose, 0.1 μM insulin, 1 μM dexamethasone, 5 μM T_3, and 300 μM of various fatty acids combined to albumin for 48 h to induce expression of ACC gene. Effects of various fatty acids on the -220 bp region of ACC PI are shown in Table 2.

Fukuda et al. (17) reported that the treatment of PUFAs greatly decreased the ACC mRNA induction in rat primary hepatocytes. However, in the present study, the ACC PI (-220 bp) activity did not change by fatty acids treatment. These result implied that response elements of PUFAs on ACC promoter I may not locate on -220 bp of ACC PI.

Table 1. Glucose stimulated ACC PI activity in primary rat hepatocytes

Glucose concentration (mM)	Luciferase activity/mg protein (fold)	
	PGL-3 basic	ACC (-220/+21)
0	1.00±0.00	1.00±0.00[c]
5	1.26±0.72	2.83±0.48[b]
25	0.95±0.51	7.35±0.01[a]

Primary hepatocytes were transfected with luciferase reporter vector containing 5'-flanking regions of the ACC PI (-220/+21 bp). Cells were cultured for 48 h in 0, 5, or 25 mM glucose in the presence of 0.1 μM insulin, 1 μM Dex., and 5 μM T_3. Cells were harvested and cell extracts assayed for luciferase activity and protein concentration as described under Materials and Methods. The experiments were repeated at least three times with triplicate samples for each point and showed identical response patterns. Values are expressed as means±SD. Means with different superscript letters are significant at $p<0.05$.

Table 2. Effects of various fatty acids on ACC PI activity in primary rat hepatocytes

Fatty acids (300 μM)	Luciferase activity/mg protein (fold) ACC (-220/+21)
Control	1.00±0.00
Oleic acid (C18:1)	1.31±0.40
Linolenic acid (C18:3)	1.10±0.00
Archidonic acid (C20:4)	1.05±0.03
DHA (C22:4)	1.34±0.12
EPA (C20:5)	0.98±0.82

Primary hepatocytes were transfected with luciferase reporter vector containing 5'-flanking regions of the ACC PI (-220/+21). Fatty acids (OA, LNA, AA, EPA, and DHA) were combined with 1 mM albumin. Cells were cultured in the presence of 0.1 μM insulin, 1 μM Dex., 5 μM T_3, 25 mM glucose, and 300 μM fatty acids for 48 h. Cells were harvested and assayed for luciferase activity and protein concentration as described under Materials and Methods. The experiments were repeated at least three times with triplicate samples for each point and showed identical response patterns. Values are expressed as means±SD.

In summary, the response elements of insulin, Dex, T_3, and, glucose may be located on -220 bp region of ACC PI, but not PUFAs. From these results, it is postulated that the gene expression of acetyl-CoA carboxylase, which is rate-limiting enzyme in lipogenesis, is thought to be regulated by hormones and glucose at transcription level. Further research is required to identify the location of elements to demonstrate the regulatory mechanism of ACC gene expression by hormones and nutrients.

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