Regulation of hepatic level of fatty-acid-binding protein by hormones and clofibric acid in the rat

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Regulation of the hepatic level of fatty-acid-binding protein (FABP) by hormones and *p*-chlorophenoxyisobutyric acid (clofibric acid) was studied. The hepatic level of FABP, measured as the oleic acid-binding capacity of the cytosolic FABP fraction, was decreased in streptozotocin-diabetic rats. The level of FABP was markedly increased in adrenalectomized rats, and the elevation was prevented by the administration of dexamethasone. Hypothyroidism decreased the level of FABP and hyperthyroidism increased it. A high correlation between the incorporation of [¹⁴C]oleic acid *in vivo* into hepatic triacylglycerol

and the level of FABP was found for normal, diabetic and adrenalectomized rats. The level of FABP was increased by administration of clofibric acid to rats in any altered hormonal states, as was microsomal 1-acylglycerophosphocholine (1-acyl-GPC) acyltransferase, a peroxisome-proliferator-responsive parameter. These results suggest that the hepatic level of FABP is under regulation by multiple hormones and that clofibric acid induces FABP and 1-acyl-GPC acyltransferase by a mechanism which may be distinct from that by which hormones regulate the level of FABP.

INTRODUCTION

Fatty-acid-binding protein (FABP) is a cytosolic protein capable of binding a large number of hydrophobic ligands [1,2], including fatty acid [3], long-chain fatty acyl-CoA [4,5], eicosanoids [6–8] and metabolites of carcinogens [9,10]. This small polypeptide, with a molecular mass of 14 184 Da [11], is abundant, comprising 3–5% of liver cytosolic protein of physiologically normal rats [12–14]. Owing to its high affinity to long-chain fatty acid and long-chain fatty acyl-CoA, specific involvement of FABP has been demonstrated in numerous enzymic reactions in which fatty acids or acyl-CoAs are substrates or products [1,2,15]. The changes in hepatic level of FABP have been documented for a number of physiological conditions. Namely, the hepatic concentration of FABP increases with the development of rats [16–18], and sex-related differences in the level become evident in mature rats [13,19]. Diurnal variations in the hepatic level of FABP have been reported [20,21]. The administration of peroxisome proliferators to rats was shown to cause a striking increase in the level of FABP in livers or hepatocytes [12–14,19,22]. Interestingly, the level of FABP has been demonstrated to increase markedly in hepatocytes in a mitotic or hyperplastic stage caused by carcinogens such as 2-acetylaminofluorene, 3'-methyl-4-dimethylaminoazobenzene and ethionine [23,24]. These responses of FABP to the changes in physiological states of animals led us to suggest the possibility that the hepatic level of the polypeptide is under hormonal control. Less information, however, is available about hormonal stimuli that provoke the changes in hepatic level of FABP, except for gonadal hormones [25]. Moreover, information is lacking about whether peroxisome proliferators induce FABP through hormonal stimuli. In this context, we studied (i) effects of insulin, glucocorticoid and thyroid hormone on hepatic level of FABP and (ii) the response of the hepatic level of FABP under altered hormonal states to clofibric acid (*p*-chlorophenoxyisobutyric acid), a peroxisome proliferator.

EXPERIMENTAL

Materials

[1-¹⁴C]Oleic acid (57.0 Ci/mol) was obtained from New England Nuclear (Boston, MA, U.S.A.). Clofibric acid, oleoyl-CoA, palmitoyl-CoA, BSA and streptozotocin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); 1-acylglycerophosphocholine (1-acyl-GPC) and egg-yolk phosphatidylcholine were from Avanti Polar Lipids (Pelham, AL, U.S.A.); CoA and NAD⁺ were from Oriental Yeast Co. (Tokyo, Japan); protamine/zinc/insulin suspension was from Takeda Chemical Industries (Osaka, Japan); dexamethasone sodium phosphate was from Banyu Pharmaceutical Co. (Tokyo, Japan); 6-propyl-2-thiouracil and 3,3',5-tri-iodothyronine sodium salt were from Tokyo Kasei Co. (Tokyo, Japan); Sephadex G-50 was from Pharmacia Biotech (Tokyo, Japan). 2-Acyl-GPC was prepared from egg-yolk phosphatidylcholine as described previously [26].

Animals and treatments

Male rats (Wistar strain), weighing 160–180 g, were used. Rats were fed *ad libitum* on a standard diet or a diet that contained 0.5% (w/w) clofibric acid for 3 or 7 days.

Rats (initial body wt. approx. 90 g) were made diabetic by an intravenous injection of streptozotocin (dissolved in 50 mM citrate buffer, pH 5.4) at a dose of 65 mg/kg body wt. 21 days before being killed. Blood glucose was estimated enzymically with a glucose assay kit (Wako Pure Chemicals, Tokyo, Japan) and ranged from 400 to 500 mg/dl in diabetic rats. Some diabetic rats were given 2 units of insulin (as protamine/zinc/insulin suspension) subcutaneously every 12 h for 2 days before being killed.

Rats (initial body wt. approx. 150 g) were adrenalectomized under anaesthesia with diethyl ether 7 days before killing. The adrenalectomized rats were provided with 0.9% NaCl in their

Abbreviations used: FABP, fatty-acid-binding protein; GPC, glycerophosphocholine.

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drinking water. Some adrenalectomized rats were administered intraperitoneally dexamethasone sodium phosphate at a dose of 2 mg/kg body wt. once a day for 7 days.

Rats (initial body wt. approx. 90 g) received daily an intraperitoneal injection of propylthiouracil (10 mg/kg body wt.) to produce hypothyroidism, or one of tri-iodothyronine (150 µg/kg body wt.) to produce hyperthyroidism, for 17–19 days before being killed. These two compounds were dissolved in slightly alkaline 0.9 % NaCl.

Preparation of cytosol and microsomes

At the end of the treatments mentioned above, rats were decapitated and livers were isolated. Blood remaining in the liver was washed out with cold 0.9 % NaCl until the liver turned pale with no further colour change. Cytosol and microsomes were prepared by differential centrifugations as described previously [19,27]. Concentrations of protein were determined by the method of Lowry et al. [28], with BSA as standard.

Assay for FABP and acyltransferases

FABP-associated fatty-acid-binding receptors in cytosol were measured by means of binding of [14 C]oleic acid to the FABP fraction as described previously [19]. In brief, 7.5 mg of cytosolic protein was incubated with 120 nmol of [14 C]oleic acid (45 nCi) and 3 mg of Triton WR-1339 in 1.5 ml of 0.154 M KCl/0.01 M potassium phosphate buffer (pH 7.4) at 37 °C for 30 min. After cooling on ice, a sample of the incubated mixture was chromatographed on a Sephadex G-50 column, which was eluted with the same buffer at 4 °C. The radioactive material, which was eluted as a radioactive peak around a molecular mass of 14000 Da, was integrated as the amounts of oleic acid bound to FABP. 1-Acyl-GPC acyltransferase was assayed spectrophotometrically, with oleoyl-CoA and 1-acyl-GPC as substrates and microsomes as enzyme source, and microsomal 2-acyl-GPC acyltransferase was assayed with palmitoyl-CoA and 2-acyl-GPC, as described previously [26].

Incorporation of [14 C]oleic acid into hepatic lipid *in vivo*

The injection solution of [14 C]oleic acid was prepared by mixing labelled oleic acid with rat serum as previously described [29]. Then 0.3 ml of this solution, containing 1 µCi of [14 C]oleic acid, was injected into the tail vein of a rat. At 10 min after the injection, the rat was decapitated and the liver was isolated. The liver was perfused with cold 0.9 % NaCl to wash out blood. Lipid was extracted by the method of Bligh and Dyer [30]. Lipids were separated by t.l.c. on silica gel G plates (E. Merck, Darmstadt, Germany), which were developed with hexane/diethyl ether/acetic acid (80:30:1, by vol). The regions on each plate that corresponded to specific lipids were scraped off and transferred to vials. The silica gel was treated with 1 ml of water and then mixed with 10 ml of toluene/Triton X-100-based scintillator. Radioactivity was measured with a liquid-scintillation spectrometer.

Statistical analysis

Analysis of variance was used to test the significance of the difference between the means. Where the difference was significant, the statistical significance of the difference between any two

means was determined by using a Scheffé's multiple-range test. Statistical significance between non-treated and clofibric acid-treated groups was determined by Student's *t* test. Linear regression analyses were performed to evaluate the correlation between two parameters.

RESULTS

Effects of altered hormonal states on hepatic level of FABP

Altered hormonal states of three hormones, insulin, glucocorticoid and thyroid hormone, were produced experimentally, and the effects of the physiologically altered conditions on the hepatic level of FABP were examined (Table 1). The level of hepatic FABP was measured as the capacity of cytosolic FABP fraction to bind [14 C]oleic acid. In streptozotocin-diabetic rats, the level of hepatic FABP was lowered to 48 % of control, and the decreased level was not reversed by the treatment of diabetic rats with insulin. Adrenalectomy of rats led to a 2.8-fold increase in FABP level, and the elevation was prevented by administration of dexamethasone to adrenalectomized rats. The level of hepatic FABP was decreased slightly in the hypothyroid state produced by the propylthiouracil, but was increased considerably in tri-iodothyronine-induced hyperthyroid rats.

Induction of FABP by clofibric acid in altered hormonal states

Effects of the altered hormonal states on the response of hepatic level of FABP to clofibric acid were examined (Table 1). Administration of clofibric acid to diabetic rats caused a 4.2-fold increase in the level of hepatic FABP. Nevertheless, the elevated level was 57 % of that in normal rats that were treated with clofibric acid. Treatment of adrenalectomized rats with clofibric acid increased the level of FABP additionally by 42 %. Clofibric acid elevated the level of hepatic FABP of hypothyroid rats (3.6-fold) and, to a lesser extent, hyperthyroid rats (1.6-times). To confirm the response of livers in the altered hormonal states to

Table 1 Effects of altered hormonal states and administration of clofibric acid on hepatic level of FABP

Values represent the means $\pm$ S.D. for three or seven animals. Values within control groups with different states of a single hormone without a common superscript are significantly different by Scheffé's multiple range test at $P < 0.05$ (a versus c) or $P < 0.01$ (a versus b, b versus c). When compared within clofibric acid-treated groups with different states of a single hormone by Scheffé's multiple range test or Student's *t* test, a significant difference was found only in the diabetic group at $P < 0.01$ (†). Significantly different from corresponding control (non-treated with clofibric acid) by Student's *t* test at $P < 0.01$ (*).

Hormonal state	Oleic acid bound to FABP (nmol/g of liver)		
	Control	Clofibric acid (7 days)	Clofibric acid (3 days)
Normal	45.0 $\pm$ 5.6 ^a	160.5 $\pm$ 16.1*	
Diabetic	21.8 $\pm$ 7.3 ^b	91.7 $\pm$ 23.2*,†	
Diabetic + insulin	14.7 $\pm$ 3.6 ^b		
Normal	45.0 $\pm$ 5.6 ^a		166.0 $\pm$ 17.4*
Adrenalectomized	126.6 $\pm$ 26.0 ^b		179.4 $\pm$ 20.5*
Adrenalectomized + dexamethasone	24.3 $\pm$ 14.1 ^a		
Sham-operation	51.4 $\pm$ 13.4 ^a		
Normal	45.0 $\pm$ 5.6 ^a	160.5 $\pm$ 16.1*	
Hypothyroid	33.8 $\pm$ 5.7 ^c	121.8 $\pm$ 31.5*	
Hyperthyroid	72.1 $\pm$ 3.1 ^b	113.6 $\pm$ 16.8*	

Table 2 Effects of altered hormonal states on the induction of 1-acyl-GPC acyltransferase and 2-acyl-GPC acyltransferase in hepatic microsomes

Values represent means $\pm$ S.D. for three or six animals. When compared within control (non-treated with clofibrilic acid) groups, significantly different from control of normal rats by Scheffé's multiple range test at $P < 0.05$ (a) or $P < 0.01$ (b). Significantly different from corresponding control (non-treated with clofibrilic acid) by Student's t test at $P < 0.05$ (*) or $P < 0.01$ (**).

Hormonal state	Clofibrilic acid	Activity (nmol/min per mg of protein)	
		1-Acyl-GPC acyltransferase	2-Acyl-GPC acyltransferase
Normal	—	33.5 $\pm$ 1.9	36.3 $\pm$ 1.1
	+	149.0 $\pm$ 34.2**	30.6 $\pm$ 4.2
Diabetic	—	43.4 $\pm$ 5.8	60.0 $\pm$ 3.4 ^b
	+	109.0 $\pm$ 10.2**	36.6 $\pm$ 4.1**
Adrenalectomized	—	48.5 $\pm$ 8.8 ^a	43.0 $\pm$ 8.2
	+	111.3 $\pm$ 5.7**	25.2 $\pm$ 3.3*
Hypothyroid	—	26.2 $\pm$ 4.0	47.1 $\pm$ 3.1
	+	93.8 $\pm$ 6.5**	34.0 $\pm$ 2.6**
Hyperthyroid	—	32.0 $\pm$ 7.0	37.0 $\pm$ 7.9
	+	97.1 $\pm$ 9.2**	27.9 $\pm$ 2.9

clofibrilic acid, the effects of the drug on microsomal 1-acyl-GPC acyltransferase, a parameter responsive to peroxisome proliferators [26,31], and 2-acyl-GPC acyltransferase, a non-responsive parameter to peroxisome proliferators [26], were tested (Table 2). It has been known that, although 1-acyl-GPC acyltransferase is induced by peroxisome proliferators, basal activity of this enzyme is little affected by hormonal changes [31]. By contrast, the activity of peroxisomal β -oxidation is dependent on hormonal states [31]. Therefore, microsomal 1-acyl-GPC acyltransferase is considered to be more suitable as a parameter to detect the effect of peroxisome proliferators on hepatocytes, compared with peroxisomal β -oxidation. The administration of clofibrilic acid to rats caused inductions of 1-acyl-GPC acyltransferase regardless of the hormonal states examined. By contrast, the drug did not induce 2-acyl-GPC acyltransferase.

Incorporation of [14 C]oleic acid into hepatic triacylglycerol *in vivo*

To examine the relationship between the level of FABP and metabolism of fatty acid in liver, incorporation of [14 C]oleic acid into hepatic lipids *in vivo* was compared for control, diabetic and adrenalectomized rats (Table 3). The incorporation of [14 C]oleic acid into esterified lipids was conspicuously elevated in adrenalectomized rats and was slightly lowered in diabetic rats. Compared with normal and diabetic rats, the proportion of the incorporation of labelled oleic acid into phospholipid was higher in adrenalectomized rats. The elevated incorporation of [14 C]oleic acid into phospholipid in adrenalectomized rats was due to the higher incorporation (7.7% of total labelled oleic acid incorporated into hepatic lipid) into phosphatidylethanolamine compared with those in normal (5.5%) and diabetic (5.0%) rats (S. Nakagawa, Y. Kawashima and H. Kozuka, unpublished work). Linear regression analyses were performed between the level of hepatic FABP and the incorporation of [14 C]oleic acid into various lipids. A significant correlation was found between the amounts of [14 C]oleic acid bound to FABP and the amounts of labelled oleic acid incorporated into hepatic triacylglycerol

Table 3 Metabolism of [14 C]oleic acid *in vivo* in liver in altered hormonal states

Values are means $\pm$ S.D. for three or four animals. [14 C]Oleic acid (1 μ Ci) was injected intravenously into control, diabetic and adrenalectomized rats. At 10 min after the injections, livers were isolated and lipid was extracted. Lipids were analysed as described in the Experimental section.

Lipid	10 ⁻³ $\times$ Metabolism (d.p.m./g of liver)		
	Control	Diabetic	Adrenalectomized
Cholesterol ester	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2	0.7 $\pm$ 0.6
Triacylglycerol	37.5 $\pm$ 7.7	33.0 $\pm$ 7.4	62.2 $\pm$ 14.1
Non-esterified fatty acid	1.9 $\pm$ 0.7	1.3 $\pm$ 0.6	1.6 $\pm$ 0.2
Diacylglycerol	1.5 $\pm$ 1.1	1.4 $\pm$ 0.5	3.0 $\pm$ 0.3
Phospholipid	7.0 $\pm$ 0.7	5.8 $\pm$ 1.0	16.2 $\pm$ 2.6
Triacylglycerol (μ mol/g of liver)	3.9 $\pm$ 1.3	7.5 $\pm$ 1.6	2.7 $\pm$ 0.7
Triacylglycerol (d.p.m./ μ mol)	10.1 $\pm$ 2.4	4.6 $\pm$ 1.7	23.6 $\pm$ 6.3

Figure 1 Relationship between incorporation of [14 C]oleic acid into triacylglycerol and the FABP level in liver

Regression analysis was performed on three mean data from Tables 1 and 3. Each value represents mean $\pm$ S.D. $y = 25.86 + 0.285x$; $r = 0.998$; $P < 0.05$. $\circ$, Control; $\bullet$, diabetic; $\blacksquare$, adrenalectomized.

(Figure 1). A similar correlation was observed between the level of FABP and the specific radioactivity of triacylglycerol as well ($y = 1.347 + 0.117x$; $r = 0.997$; $P < 0.05$). No significant correlations were found between the level of FABP and the incorporation of [14 C]oleic acid into other esterified lipids.

DISCUSSION

In the present study, the influence of three hormones and clofibrilic acid on the hepatic level of FABP was investigated. In agreement with a previous study [32], the hepatic level of FABP was lowered in diabetic rats, although insulin failed to prevent the level of FABP falling, suggesting that FABP is not so sensitive to insulin level as an insulin-responsive parameter such as stearoyl-CoA desaturase [33]. The FABP level was confirmed to depend on the level of thyroid hormone and, by contrast, to be reciprocally dependent on the level of glucocorticoid. A previous study [25] demonstrated that gonadal hormones regulate the hepatic level of FABP through the changes in the level of mRNA specific for

hepatic FABP [13]. These findings, taken together, suggest that the hepatic level of FABP is under regulation by multiple hormones. Since hepatic FABP has been known to be induced by peroxisome proliferators [12–14,19,22], the present study examined the relationship between hormonal stimuli and clofibrate acid-caused induction affecting the FABP level. The present results showed that the level of FABP was increased by challenge with clofibrate acid in any altered hormonal states, although the extent of the increase varied with alterations in hormonal states. Microsomal 1-acyl-GPC acyltransferase, a peroxisome-proliferator-responsive parameter [31], was conspicuously induced by clofibrate acid in any altered hormonal state examined. Similarly to other peroxisome proliferator-responsive parameters [27,34,35], the levels of FABP and microsomal 1-acyl-GPC acyltransferase were increased by clofibrate acid in altered hormonal states in animals as well, indicating that the drug caused the inductions of these peroxisome-proliferator-responsive parameters by a common mechanism, which may be distinct from that by which hormones regulate the level of FABP. Although the mechanism by which the three hormones regulate the level of FABP remains to be elucidated, it is of interest to speculate that expression of the gene coding FABP and 1-acyl-GPC acyltransferase is mediated by a common peroxisome-proliferator-activated receptor [36], which was found recently for enzymes involved in peroxisomal β -oxidation [37–39].

A number of functions have been considered with regard to hepatic FABP [1,2,15]. Upon challenges with peroxisome proliferators, hepatocytes elevate the level of FABP [12–14,19,22], and the level is correlated with the activity of peroxisomal β -oxidation [12], but not with esterification of oleic acid [19]. By contrast, hepatic FABP was considered to direct the traffic of fatty acid toward triacylglycerol biosynthesis [25,40,41], when free from the influence of peroxisome proliferators. Accordingly, it may not be logical to postulate a single function for the fluctuation of FABP level. Although a physiological significance of the fluctuation of FABP level by hormones has not been elucidated in detail, a significant correlation, observed in the present study between the level of hepatic FABP and the incorporation of labelled oleic acid into triacylglycerol *in vivo* in the three different hormonal states, suggests the participation of these hormones in regulation of triacylglycerol formation through the changes in FABP level. Long-term exposure of rodents to peroxisome proliferators is known to induce hepatocellular carcinomas [42], and FABP has been shown to be involved in mitosis of hepatocytes [23,24] and to be a carrier for eicosanoids which regulate cell growth [8]. These findings might imply another physiological significance for the fluctuation of FABP level by hormones and peroxisome proliferators.

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