The Role of Fatty Acid Binding Protein on the Metabolism of Fatty Acids in Isolated Rat Hepatocytes

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Received June 1,1976

SUMMARY. In isolated rat hepatocytes flavaspidic acid, a competitor with free fatty acids for the fatty-acid-binding-protein, decreased the uptake of oleic acid and triglyceride synthesis but stimulated the formation of CO_2 and ketone bodies from oleic acid. Flavaspidic acid had no effect on the utilization of octanoic acid. Stimulation of the microsomal fatty-acid-activating enzyme by the fatty-acid-binding protein was reversed by flavaspidic acid. In contrast, the binding protein inhibited the mitochondrial fatty-acid-activating enzyme. Flavaspidic acid not only prevented this inhibition but actually stimulated the enzyme activity. The results indicate that the cytosol fatty-acid-binding protein directs the metabolism of long chain fatty acids toward esterification as well as enhancing their cellular uptake.

The hepatic uptake of plasma FFA which is extremely rapid involves their transfer from albumin across the plasma membrane into the tissue (1,2). The rate of FFA uptake is dependent upon its plasma concentration with maximal uptake occurring at concentrations approaching 3 mM (3). Until the recent discovery of a low molecular weight (4,5) binding protein for long chain fatty acids in the cytosol of liver and intestine as well as in other tissues, little was known regarding the mechanics of the transfer of the hydrophobic FFA into the aqueous environment of the cytosol. The role of the Z-protein or FABP in subsequent hepatic fatty acid metabolism is unclear. The concentration of intestinal FABP is increased when animals are fed a high fat diet (6) which suggests that the FABP may be involved in the

ABBREVIATIONS: FFA, free fatty acids; FABP, fatty-acid-binding protein

partition of cellular FFA towards esterification thereby preventing their accumulation within the cell.

Flavaspidic acid, known to compete with bilirubin and sulfobromophthalein for binding to Z-protein, has recently been shown to also compete with FFA for binding to FABP (7) which is strong evidence that the two proteins are quite similar if not identical. In this report flavaspidic acid is shown to decrease the esterification and increase the oxidation of oleic acid in isolated hepatocytes. Metabolism of octanoate which precedes only through mitochondrial β-oxidation was not affected by flavaspidic acid.

Since long-chain fatty-acid activating enzymes are distributed between the microsomes and the outer mitochondrial membrane, an association of the FABP with these membrane-activating enzymes might be anticipated. The influences of FABP and flavaspidic acid on the microsomal and mitochondrial fatty acid activating enzyes were therefore also investigated. FABP was shown to stimulate microsomal fatty acid activation and to inhibit mitochondrial fatty-acid activation, while flavaspidic acid reversed these effects of FABP.

MATERIALS AND METHODS

Male rats (250 g Holtzman) fed *ad libidum* were used for these studies. Hepatocytes were isolated from liver perfused with collagenase according to the method of Berry and Friend (8) as modified by Zahlten *et al.* (9). The cells were suspended in a calcium-free Krebs bicarbonate buffer, pH 7.4, with 5 mM glucose and 1 ml suspensions of the cells were preincubated in rubber capped 2.5 x 5 cm vials at 37° on a Dubnoff shaker (100 cycles/min) under an atmosphere of 95% O_2-5% CO_2. The reactions were initiated with the addition of 1 ml 3% albumin with 3 mM [1-^{14}C]oleate or 3 mM [1-^{14}C]octanoate. After 17 min, 1 ml of the reaction mixture was extracted with 5 ml solvents according to Dole (10). Aliquots of the upper phase were washed twice with 50% ethanolic KOH (11), mixed with Bray's solution (12) and the radioactivity in the esterified fatty-acid fraction was determined. In agreement with other reports (e.g. 13), no esterified fatty acids were present in the incubation medium after the cells were removed by centrifugation. Residual free fatty acids in the

incubation medium were isolated according to Dole (10). Water-soluble material (ketone bodies) were measured after protein precipitation by the addition of 1 ml 30% perchloric acid to the medium (13). The pH was adjusted to 8.5 with 20% KOH and after centrifugation, an aliquot of the supernatant fluid was added to Bray's solution (12) and radioactivity determined.

At the end of the incubation period, CO_2 was displaced from the cells and medium by the addition of 0.5 ml 6 N H_2SO_4. The radioactive CO_2 was collected in 0.2 ml hyamine hydroxide injected into a plastic cup suspended through the rubber cap of the vial. After shaking for 1 hr the cup was placed in scintillation vials containing Bray's solution (12) and radioactivity was determined.

FABP was prepared from rat liver as described by Ockner et al. (4). Mitochondrial and microsomal fractions were obtained by differential centrifugation (14) of rat liver homogenates.

The assay for the acyl CoA synthetase was a modification of the method of Samuel and Ailhaud (15), which is based on the insolubility of acyl CoA in diethyl ether. The standard system contained the following constituents which were added in the order: 15 μM [1-^{14}C] palmitate, 0.4 mg of aqueous solution of Triton WR-1339, 350 mM Tris-HCl (pH 7.4), 10 mM ATP, 4 mM $MgSO_4$, 1.2 mM CoA, 5 mM dithiothreitol and water in a final volume of 0.4 ml. Control experiments without CoA and ATP were systematically included in each series of incubations. Reactions started by the addition of the enzyme were continued for 5 min at 37°. Reactions were terminated by the addition of 250 μl of 0.5 M H_2SO_4 and 250 μl of water was added to dissolve any K_2SO_4 formed at the termination of the reaction. The remaining free fatty acids were extracted four times with 5 ml diethyl ether. The aqueous phase was then mixed with Bray's solution (12) and directly transferred to counting vials. Radioactivity was measured in a Packard Tricarb scintillation spectrometer and all measurements were corrected by use of internal standards.

Protein was determined by the Lowry procedure (16).

Collagenase (CLS II) was purchased from Worthington Biochemical Corporation; β-flavaspidic acid-n-methyl glucaminate was generously provided by Dr. Esa Aho, Turku, Finland; [1-^{14}C] octanoic, [1-^{14}C] pamitic acid and [1-^{14}C] oleic acid were purchased from New England Nuclear, and unlabeled fatty acids were obtained from Sigma Chemical Company.

RESULTS AND DISCUSSION

Hepatocytes, isolated from fed rats were incubated with oleic acid under the conditions shown on Table 1. The flow of fatty acids was towards esterification; 57.7% of the radioactive label taken up by control cells was incorporated into the cellular lipid fraction. The addition of 1 mM flavaspidic acid elicited a 13.1% decrease in the cellular uptake of the albumin bound oleic acid. Within the cell, the incorporation of label into the ester fraction was decreased slightly with a concomitant

Table 1

Effects of Flavaspidic Acid on Uptake and Metabolism of [1-^{14}C] Oleate in Isolated Liver Cells

Supensions containing 1.0 ml liver cells and 1.0 ml of 3% albumin - 3 mM [1-^{14}C] oleate (839810 dpm) were incubated for 17 min at 37°. Each vial contained 24 mg protein. Values presented are the averages of duplicate assays.

	Control	Flavaspidic Acid 1 mM	Flavaspidic Acid 10 mM
Total Uptake			
% added dose	66.4	57.7	40.3
% of control[1]		-13.1	-30.3
Conversion to Water-Soluble Products			
% uptake	33.2	34.7	47.0
% control[2]		+ 4.5	+41.6
Incorporation as Esterified Fatty Acids			
% uptake	59.7	54.2	43.0
% control[2]		- 9.2	-27.9
Oxidation to CO_2			
% uptake	3.8	6.2	18.9
% control[2]		+64.0	+397.0

[1] $\frac{\%\text{ of added dose, Experimental} - \%\text{ of added dose, Control}}{\%\text{ of added dose, Control}} \times 100$

[2] $\frac{\%\text{ of uptake, Experimental} - \%\text{ of uptake, Control}}{\%\text{ of uptake of control}} \times 100$

Table 2

Effects of Flavaspidic Acid on the Uptake and Metabolism of [1-^{14}C]Octanoate in Isolated Liver Cells

Incubations and calculations were carried out as discussed in Table 1. Radioactivity added was 800,000 dpm. Each value is the average of duplicate vials.

	Uptake % of added dose	% of Uptake: Water soluble products	% of Uptake: CO_2	% of Uptake: Esterified Fatty Acids
Control	25	81	17	1
Flavaspidic Acid (1 mM)	25	78	18	1
Flavaspidic Acid (10 mM)	24	77	19	2

increase in the oxidation fatty acids to CO_2. The uptake of the fatty acid was inhibited by 30.3% in the presence of 10 mM flavaspidic acid, and of the radioactivity in the cell, only 43.0% of the label appeared in the ester fraction with the remainder in CO_2 and water soluble products (ketone bodies). To determine the stimulatory and inhibitory effects of flavaspidic acid, ratios of the % distributions were calculated. A four-fold increase in CO_2 production and 41.6% increase in ketone body formation were observed while esterification was inhibited by 27.9% when 10 mM flavaspidic acid was added. At a lower concentration of oleic acid (0.75 mM), flavaspidic acid did not significantly influence the partitioning of FFA between the microsomal and mitochondrial fractions.

Table 3

Effect of Flavaspidic Acid and FABP on

Microsomal Palmitate Activating Enzyme(s)

The radioactive assay procedure as described in Methods was employed with 3 μg protein, 15 μM [^{14}C] potassium palmitate (2472 dpm/nmole). The results represent the mean ± S.E.M. of three experiments.

Additions	Flavaspidic Acid (1 mM)	Enzyme Activity nmole/min/mg microsomal protein	% of control
None	-	102.60 ± 3.10	100.0
None	+	26.31 ± 0.43	25.6
FABP (1.2 nmole)	-	119.7 ± 3.3	116
FABP (4.0 nmole)	-	140.2 ± 1.13	136
FABP (1.2 nmole)	+	90.27 ± 0.93	88.0
FABP (4.0 nmole)	+	95.6 ± 2.85	93.2
Albumin (4.0 nmole)	-	103.9 ± 0.95	101
Albumin (4.0 nmole)	+	90.3 ± 1.17	88

The more soluble short chain fatty acids are completely oxidized and not usually esterified to form triglycerides. In contrast to its effect in oleic metabolism, flavaspidic acid was without effect on octanoate metabolism (Table 2).

Fatty acid activation by hepatic microsomes from fed rats was stimulated 16 and 36% by the respective additions 1.2 and 4.0 nmoles FABP (Table 3). Albumin, added at equimolar concentrations elicited no effect which suggests that the FABP also serves functions other than the binding of FFA. Flavaspidic acid, believed to compete with FFA for binding sites on FABP was a most effective inhibitor of the microsomal fatty acid

Table 4

Effects of Flavaspidic Acid and FABP on

Mitochondrial Palmitate Activating Enzyme(s)

The radioactive assay procedure as described in Methods was employed with 3 μg protein, 15 μM [1-^{14}C] potassium palmitate (2978 dpm/nmole). The results represent the mean ± S.E.M. of three experiments.

Additions	Flavaspidic Acid (1 mM)	Enzyme Activity nmole/min/mg	% of control
None	-	80.0 ± 1.21	100
None	+	105.3 ± 4.06	131
FABP (1.2 nmole)	-	69.3 ± 3.4	86
FABP (4.0 nmole)	-	49.3 ± 0.76	61.0
FABP (4.0 nmole)	+	102.1 ± 2.7	127
Albumin (4 nmole)	-	77.3 ± 8	96
Albumin (4 nmole)	+	74.0 ± 1.5	93

activating enzyme in the absence of FABP (Table 3). In the presence of either FABP or albumin, flavaspidic acid was not an effective inhibitor. Thus, the binding sites of the enzyme, FABP and albumin all accommodate flavaspidic acid.

In contrast to results obtained from experiments involving isolated microsomes, FABP was shown to effectively inhibit mitochondrial fatty acid activation (Table 4) even at levels as low as 1.2 nmoles. In this case flavaspidic acid acted in a reverse fashion by increasing the activation. FABP, even at 4.0 nmoles failed to offset the stimulatory effect of flavaspidic acid. Albumin did not exert an effect on the enzyme.

These data are interpreted to indicate that FABP increases

the uptake of FFA and specifically enhances the flow of long chain fatty acids toward microsomal esterification and subsequent triglyceride formation. When the binding sites on FABP are filled, fatty acids are directed principally toward mitochondrial oxidation.

ACKNOWLEDGEMENTS. This work was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by NIH Grant AM-15893.

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