EFFECT OF OLEIC ACID AND CHOLESTEROL ON THE ACTIVITY OF HEPATIC HYDROXYMETHYLGLUTARYL COENZYME A REDUCTASE

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1. Introduction

It was reported previously from this laboratory that oleic acid stimulated the net secretion of cholesterol by the isolated perfused rat liver and increased the biosynthesis of cholesterol as measured by the incorporation of tritium (^3H_2O) into cholesterol [1]. These observations led us to suggest that stimulation by oleate of secretion of the very low density lipoprotein (VLDL), which contains cholesterol as an obligatory moiety, may be an important stimulus for the biosynthesis of cholesterol by the liver. Since 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase; EC 1.1.1.34) is considered to be the rate-limiting enzyme in hepatic cholesterogenesis [2], it was of interest to determine whether stimulation of cholesterogenesis by oleic acid would also increase the activity of hepatic HMG-CoA reductase.

2. Materials and methods

Livers obtained from normal fed male rats were perfused in vitro with 65 ml of a medium containing 3 g bovine serum albumin and 100 mg glucose per 100 ml Krebs–Ringer bicarbonate buffer, pH 7.4, as described earlier [3]. A complex of albumin alone was added to the perfusate at a rate of 11.7 ml/h for 4 h. Appropriate complexes containing 10 g albumin, 1416 μmol oleic acid, and 472 μmol cholesterol/100 ml complex were prepared as reported [4]. Under these circumstances, a steady-state concentration of approx. 0.55 mM free fatty acid was maintained in the perfusate in the presence or absence of added cholesterol; the concentration of sterol in the medium in experiments with oleate plus free cholesterol was approx. 0.88 mM (34 mg%). At the termination of the experiment, the liver was perfused with a single passage of ice-cold 0.9% NaCl, and adherent nonhepatic tissues were removed. The liver was weighed and homogenized (1:6, w/v) in a buffer consisting of 30 mM EDTA, 250 mM NaCl, 1.0 mM dithiothreitol, and 50 mM potassium phosphate, pH 7.4 [5]. The hepatic microsomal pellet was isolated by centrifugation at 100 000 g for 60 min at 4°C and was then frozen with liquid nitrogen. The frozen pellets were stored at –20°C until analyzed. The activity of HMG-CoA reductase was estimated by the procedure described by Shapiro et al. [5] using ^{14}C-labelled HMG-CoA. Specific activity of the enzyme is expressed as nmoles mevalonic acid formed/min/mg protein. Microsomal protein was determined as by Lowry et al. [6] after precipitation with 5% trichloroacetic acid. Triglyceride was isolated from the perfusate and determined as described previously [7].

3. Results and discussion

It is well known that oleic acid and other free fatty acids stimulate the formation and secretion of triglyceride by the liver. The triglyceride is transported as the major component of the VLDL. The obligatory requirement of cholesterol as a component of the VLDL for transport of triglyceride may be an important stimulant to the biosynthesis of cholesterol

Table 1
Activity of microsomal HMG-CoA reductase and output of triglyceride by rat liver

Treatment	HMG-CoA reductase (nmoles/min/mg protein)	Output of triglyceride (μmoles/g liver/4 h)
(A) Non-perfused liver	0.49 ± 0.12 (6)	–
(B) Perfused, albumin alone	1.08 ± 0.11 (5)	2.44 ± 0.16 (5)
(C) Perfused, albumin + oleic acid	2.55 ± 0.33 (6)	6.33 ± 0.71 (6)
(D) Perfused, albumin + oleic acid + cholesterol	0.63 ± 0.03 (4)	± 4.36 ± 0.53 (5)
Statistical analysis:		
A vs B	< 0.005	–
B vs C	< 0.001	< 0.001
A vs D	< 0.20	–
B vs D	< 0.01	< 0.01
C vs D	< 0.005	< 0.10

Data presented are means ± standard error. The figures in parentheses indicate the number of experiments. Probability values are derived from a two-tailed table for Student's *t*-test. All livers were removed from the animals between 8:00 and 10:00 a.m. Non-perfused livers were isolated as for perfusion, but were then homogenized immediately. The fatty acid–albumin complex was prepared as reported previously [4]. To prepare the complex containing cholesterol, the sterol was added to the solution of oleic acid in 95% ethanol, and the complex with albumin was prepared as usual.

by the liver. It can be seen that perfusion of the liver with albumin alone increased the activity of microsomal HMG-CoA reductase in comparison to the nonperfused control (table 1). Addition of oleic acid to the medium stimulated the activity of the reductase and the output of triglyceride. Simultaneous infusion of cholesterol and oleic acid decreased the activity of the enzyme to levels observed in non-perfused liver, while the output of triglyceride remained elevated. Presumably, the exogenous free cholesterol antagonized the stimulatory effect of oleate on the biosynthesis of cholesterol, by providing cholesterol for the formation of the VLDL necessary for the transport of triglyceride. Whether the increased activity of HMG-CoA reductase resulted from synthesis of new enzyme or only changes in the activity of existing enzyme remains to be determined.

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