In vivo modulation of rat liver acyl-coenzyme A:cholesterol acyltransferase by phosphorylation and substrate supply

Keith E. Suckling*, Eduard F. Stange and John M. Dietschy

Department of Internal Medicine, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235, USA

Received 17 June 1983

Rats were infused intravenously with a bolus of mevalonolactone or cholesterol-rich chylomicron and microsomes prepared from the livers at times up to 2 h after the infusion. Acyl-CoA cholesterol acyltransferase (ACAT) activity was measured in the microsomes under conditions that define the activity state of the enzyme which recent evidence suggests may depend on the degree of phosphorylation of the enzyme. ACAT activity was increased by both treatments. Some of the observed increase could be attributed to an increase in the supply of substrate cholesterol to the enzyme. The remaining change in activity was consistent with a change in the activity state of ACAT occurring in vivo in response to the influx of cholesterol to the enzyme

Acyl-CoA:cholesterol acyltransferase *Rat liver* *Phosphorylation*

1. INTRODUCTION

Three enzymes have attracted attention in recent years in studies on the regulation of hepatic cholesterol metabolism. Most studies have concentrated on 3-hydroxy-3-methylglutaryl–coenzyme A reductase (HMG–CoA reductase) as the rate-limiting step of cholesterol biosynthesis [1]. Cholesterol-7α-hydroxylase has been examined as the regulatory enzyme in the conversion of cholesterol into bile acids [2] and more recently the role of acyl-CoA–cholesterol acyltransferase (ACAT) in regulating cholesterol esterification has been studied [3]. In vitro experiments for all 3 enzymes have been reported which lead to the view that they may all be regulated by a short-term phosphorylation–dephosphorylation mechanism which results in inhibition of HMG–CoA reductase by phosphorylation and activation of the other two enzymes [1,4–10]. It has been suggested that such a mechanism may operate in vivo to coordinately control hepatic cholesterol metabolism [6,7]. However, only in the case of HMG–CoA reductase is there any evidence to suggest that changes in the activity state of the enzyme in parallel with its degree of phosphorylation may occur in vivo. Infusion of mevalonolactone into rats intragastrically decreases the activity of hepatic HMG–CoA reductase after 20 min by enzyme phosphorylation without altering the amount of enzyme present [1,8]. Short-term (60 min) cholesterol feeding has a comparable effect on the enzyme [1,9]. Treatment of isolated rat hepatocytes with glucagon has also been shown to alter the activity state of the reductase [10].

We have reported that the apparent activity of ACAT in vitro in rat liver and intestine could be modulated both by altering the supply of substrate cholesterol and by a mechanism which was shown to be dependent on ATP/$MgCl_2$ and 100000 × g supernatant protein in the presence of NaF [6]. The activity of ACAT in freshly prepared

Abbreviations: ACAT, acyl-CoA–cholesterol acyltransferase; HMG, 3-hydroxy-3-methylglutaryl

* Present address: Department of Biochemistry, University of Edinburgh Medical School, Hugh Robson Building, George Square, Edinburgh, EH8 9XD, Scotland

Published by Elsevier Science Publishers B.V.

microsomes was found to be very close to its minimum level. We here report experiments which were designed to investigate whether the activity state of rat liver microsomes towards cholesterol esterification could be altered in vivo by intravenous infusion of mevalonolactone and also of cholesterol-rich chylomicrons over short periods of time of up to 2 h. The rate of cholesteryl ester formation in liver microsomal fractions from experimental and control animals, which were treated in a variety of ways to define the activity state [6], was measured using [1-^{14}C]oleoyl–CoA as substrate. The results show that an increase in the rate of cholesteryl ester formation could be achieved by both in vivo treatments and that this increase could be ascribed partly to a greater supply of substrate cholesterol due to the infusions and partly to a change in the activity state of ACAT as assessed in vitro.

2. MATERIAL AND METHODS

Female Sprague-Dawley rats (CD®(SD)BR), Charles River Breeding Labs. (Wilmington MA) weighing about 150 g were fitted with femoral vein catheters and placed in restraining cages. A solution containing 0.9% (w/v) NaCl, 4.5 mM KCl and 20% (w/v) glucose was infused into the femoral vein overnight at a rate of 1 ml/min. A bolus of mevalonolactone (200 mg) or cholesterol-rich chylomicrons (10.5 mg cholesterol) was administered to each group at the start of the experiment, and the animals were then left for the required time (up to 2 h). Control animals received a bolus of 0.9% saline. At the end of the experimental period the rats were anaesthetised with diethyl ester and the livers rapidly perfused with cold 154 mM KCl and removed. The microsomal fractions from the control and experimental animals and the 100000 × *g* supernatant fraction from control animals were prepared as in [6]. The apparent ACAT activity in the various microsomal preparations was determined as in [6].

Donor rats for the preparation of cholesterol-rich chylomicrons were fitted with an intraduodenal catheter and the intestinal lymph duct was cannulated [11]. A medium consisting of the NaCl–KCl–glucose solution (described above) also containing 10 mM sodium taurocholate, 2 mM cholesterol and 3% (w/v) fatty acid-free bovine serum albumin was infused intraduodenally (2.4 ml/h). The cholesterol was added to the other components of the medium in solution in hot ethanol and the mixture was then sonicated for 5 min. The cholesterol-rich chylomicrons were prepared from the intestinal lymph by ultracentrifugation and dialysis as in [12].

3. RESULTS AND DISCUSSION

Rat liver microsomal ACAT activity is known to be increased by intragastric administration of mevalonolactone [13,14] and similar effects can also be observed in cultured hepatocytes [15]. In the experiments described in table 1 rats were infused intravenously with a bolus of mevalonolactone and the liver microsomal fraction was isolated after various periods of time. The ACAT activity of the untreated microsomes (column 1) increased significantly with time after mevalonolactone administration. Preincubation of these microsomes with the pooled 100000 × *g* supernatant fraction obtained from the livers of control animals (under conditions which have been shown to reverse the ATP-dependent activation in vitro [6]) nearly abolished this observed rise in basal ACAT activity (column 2). The inhibition was virtually complete at 20 min, but the activity of the microsomes remained somewhat higher at 60 min. This residual activity difference may indicate an increase in the supply of the substrate cholesterol to the acyltransferase from sterol newly-synthesized from the mevalonate during the 60-min interval.

All the microsomal fractions could be activated to the same level by preincubation with ATP, $MgCl_2$, NaF and 100000 × *g* supernatant. This shows that, whilst the activity of the untreated microsomes differed with time (column 1), the same maximum activity could be obtained with each microsomal group (column 3) after this treatment.

Preincubations were also carried out in the presence of cholesterol-rich liposomes [6] to demonstrate further that substrate supply also was important in regulating the amounts of cholesteryl ester formed by the different microsomal preparations. The data in columns 4 and 5 of table 1 show that although the relative increase varied (compared to column 1), identical maximal levels of activity were obtained for all 3 groups of animals.

Table 1

Effect of intravenously administered mevalonolactone on rat liver microsomal ACAT activity (pmol cholesteryl oleate formed.min^{-1}.mg protein^{-1})

	1	2	3	4	5
0 min	44.8 ± 6	54.0 ± 5	272.4 ± 15	102.0 ± 14	524.0 ± 62
20 min	79.3 ± 5[a]	50.8 ± 5	221.9 ± 49	103.0 ± 8	407.0 ± 65
60 min	132.5 ± 10[a]	73.2 ± 4[a]	224.0 ± 34	132.0 ± 9	504.0 ± 22

Results are expressed as the mean ± 1 SEM for 4 animals in each experimental group. Differences between groups were tested for significance using the unpaired Student's *t*-test

[a] Value is significantly different at the $p < 0.05$ level as compared with the control group (0-time group)

Rats fitted with femoral vein catheters were injected with a bolus of 200 mg mevalonolactone in a volume of 2 ml and groups were killed immediately or 20 min and 60 min later: Column: (1) ACAT activity of untreated microsomes in these 3 groups; (2) activity measured after 30 min preincubation of microsomes with 100000 × *g* supernatant and 2 mM $MgCl_2$; (3) activity after incubation of microsomes for 90 min with 100000 × *g* supernatant, 4 mM ATP, 4 mM $MgCl_2$ and 40 mM NaF; (4) activity after a 90 min preincubation of the microsomes with cholesterol-rich liposomes; (5) microsomes incubated as in column 3 with the addition of cholesterol-rich liposomes.

An identical group of experiments was performed in which the rats were infused with cholesterol-rich chylomicrons for periods of 1 and 2 h (table 2). Table 2 shows that no significant differences were observed over the 0-time animals with the 1 h infusion. After 2 h, however, a significant increase was observed in the activity of the microsomes, and this could be partially prevented by preincubation with the 100000 × *g* supernatant (column 2).

The most direct interpretation of these results is that the infusion of cholesterol-rich chylomicrons and mevalonolactone caused an increase in the supply of substrate-cholesterol to the endoplasmic reticulum of the liver and that this resulted in the increases in the amounts of cholesteryl ester formed. This is particularly apparent 60 min after the administration of mevalonolactone and 2 h, after the chylomicron infusion, since the relative increase in ACAT activity through enrichment of the cholesterol substrate supply from liposomes (columns 4 in table 1,2) was much less pronounced in these groups (1.0–1.3-fold) that in the 0-time groups (2.4-fold).

However, incubation of the microsomes under conditions that inhibit ACAT activity through dephosphorylation [6] completely reversed the ac-

Table 2

Effect of intravenously administered cholesterol-rich chylomicrons on rat liver microsomal ACAT activity (pmol cholesteryl oleate formed.min^{-1}.mg protein^{-1})

	1	2	3	4	5
0 h	20.3 ± 2.4	25.1 ± 1.3	74.3 ± 4.5	52.3 ± 3.1	172.7 ± 9
1 h	23.5 ± 2.3	25.5 ± 3.0	64.6 ± 7.1	52.4 ± 5.3	174.3 ± 15.9
2 h	57.3 ± 5.4[a]	38.2 ± 5.2[a]	116.0 ± 19	76.4 ± 8[a]	210.8 ± 32

[a]See table 1

Rats were injected with a bolus of cholesterol-rich chylomicrons containing 10.3 mg cholesterol in 2 ml. The microsomal treatments are as described in table 1

tivation observed in the 20-min mevalonolactone group (column 2, table 1) and largely reversed the effect of both mevalonolactone and chylomicrons at 60 min and 2 h, respectively. A similar inactivation was recently observed in hepatic microsomes after in vitro activation through ATP-dependent phosphorylation [6]. It seems likely, therefore, that the majority of the activation after both short-term treatments is due to a similar process occurring in vivo. Notably, this ACAT activation through apparent phosphorylation follows a time course similar to the inactivation of HMG–CoA reductase by its kinase [1,8].

It should also be noted that the activities measured after full activation through both increased substrate supply and phosphorylation (columns 5, table 1, 2) were not significantly different at the different time intervals in both treatment groups. The data, therefore, are consistent with a short term, in vivo regulation of hepatic ACAT activity both through phosphorylation and substrate supply, rather than a change in enzyme synthesis, and support our suggestion [6] of a coordinate control with HMG–CoA reductase under the conditions studied [1,8,9].

ACKNOWLEDGEMENTS

This work was supported by US Public Health Service grants HL-09610 and AM-19329 and by a grant from the Moss Heart Fund. E.F.S. acknowledges support from the Deutsche Forschungsgemeinschaft (STA 172/2-1). We thank Nancy Hammack for excellent technical assistance.

REFERENCES

[1] Beg, Z.H. and Brewer, H.B. (1981) Curr. Top. Cell. Regul. 20, 140–184.
[2] Myant, N.B. and Mitropoulos, K.A. (1977) J. Lipid Res. 18, 135–153.
[3] Spector, A.A., Mathur, S.N. and Kaduce, T.L. (1979) Prog. Lip. Res. 18, 31–53.
[4] Sanghvi, A., Grassi, G., Warty, V., Diven, W., Wight, O. and Lester, R. (1982) Biochem. Biophys. Res. Commun. 103, 886–892.
[5] Goodwin, C.D., Cooper, B.W. and Margolis, S. (1982) J. Biol. Chem. 257, 4469–4472.
[6] Suckling, K.E., Stange, E.F. and Dietschy, J.M. (1983) FEBS Lett. 151, 111–116.
[7] Scallen, T.J. and Sanghvi, A. (1982) Circulation 66, Suppl. II, II–281.
[8] Arebalo, R.E., Hardgrave, J.E., Noland, B.J. and Scallen, T.J. (1980) Proc. Natl. Acad. Sci. USA 77, 6429–6433.
[9] Arebalo, R.G., Hardgrave, J.E. and Scallen, T.J. (1981) J. Biol. Chem. 256, 571–574.
[10] Ingebritsen, T.S., Geelen, M.J.H., Parker, R.A., Everson, K.J. and Gibson, D.M. (1979) J. Biol. Chem. 254, 9986–9989.
[11] Weis, H.J. and Dietschy, J.M. (1969) J. Clin. Invest. 48, 2398–2408.
[12] Nervi, F.O., Weis, H.J. and Dietschy, J.M. (1975) J. Biol. Chem. 250, 4145–4151.
[13] Erickson, S.K., Shrewsbury, M.A., Brooks, O. and Meyer, D.J. (1980) J. Lipid Res. 21, 930–941.
[14] Mitropoulos, K.A., Balasubramaniam, S., Venkatesan, B. and Reeves, B.E.A. (1978) Biochim. Biophys. Acta 530, 99–111.
[15] Drevon, O.A., Weinstein, D.B. and Steinberg, D. (1980) J. Biol. Chem. 255, 9128–9137.