A DIRECT RELATIONSHIP BETWEEN THE AMOUNT OF STEROL LOST FROM RAT HEPATOCYTES AND THE INCREASE IN ACTIVITY OF HMG-CoA REDUCTASE

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SUMMARY. Incubation of rat hepatocytes in a sterol-free medium containing 1.5% albumin resulted in loss of cholesterol from the cells and increased activity of HMG-CoA reductase. Addition of egg-lecithin dispersions to the hepatocytes resulted in increased rates of sterol efflux and increased levels of reductase. The increase in enzyme activity after three hours incubation was directly proportional to the amount of cholesterol lost by the cells to the medium during the first 45 min of incubation. Sterol loss preceded the increase in enzyme activity. The data support the view that one mode of regulation of hepatic HMG-CoA reductase is dependent on the relative rates of movement of cholesterol into and out of cells.

It is generally accepted that HMG[1]-CoA reductase (E.C. 1.1.1.34) is the rate-limiting enzyme for hepatic cholesterogenesis from acetate under most physiological conditions (1,2). In the rat the hepatic enzyme exhibits a circadian rhythm of activity with a five to ten-fold amplitude (3,4).

It was reported recently that in isolated rat hepatocytes the activity of HMG-CoA reductase was regulated by hormones (5) and by plasma lipoproteins (6). Reductase levels were increased when the medium contained HDL or lecithin dispersions; lecithin also promoted the efflux of endogenously synthesized ^{14}C-labelled non-saponifiable lipids from the hepatocytes into the medium (6). Jakoi and Quarfordt have reported that a 4-hour infusion of lecithin dispersions into rats resulted in a decrease in the cholesterol content of hepatic microsomes and a concomitant increase in the activity of HMG-CoA reductase (7).

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[1]Abbreviations: HMG, 3-hydroxy-3-methylglutaryl; PC, phosphotidylcholine or lecithin; HDL, high density lipoprotein.

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Other studies have demonstrated an inverse relation between the rate of cholesterogenesis, or reductase activity, and the concentration of cholesterol in the liver (8) or of cholesterol esters in hepatic microsomes (9,10). It is possible that these phenomena were determined by the relative rates of flux of cholesterol into and out of cells. The data reported here demonstrate that in isolated rat hepatocytes the activity of HMG-CoA reductase is determined by the amount of cholesterol lost by the cells to the medium.

Methods

Hepatocytes were isolated from rat liver as previously described (5) except that the modified Swims S-77 medium used during the perfusion of the liver also contained sodium bicarbonate (15 mM). The cell preparations were made at a time when reductase activity was basal, i.e. at 10 a.m. (5,6). HMG-CoA reductase was assayed and the dispersions of pure egg lecithin were made as described (6). Reductase activities are given as nmols of mevalonate formed per min/mg microsomal protein. The specific activity of the [3-^{14}C]HMG-CoA used in the reductase assay was 0.34 Ci/mol. Approximately 180 mg (wet weight) of cells containing 260 μg of total cholesterol were incubated in 10 ml of the modified Swims S-77 medium (5) with or without added lecithin. At the end of the incubation the cells were sedimented at 50xg for 2.5 min. The medium was recentrifuged at 10,000xg for 15 min and an aliquot of the supernatant added to an equal volume of 20% trichloroacetic acid containing 7.64 μg coprostanol. The cholesterol content of the media was determined, after saponification, by the method of Bates and Rothblat (11) in a Varian Aerograph series 2700 gas-liquid chromatograph on a six-foot column packed with 100-120 mesh Gas-Chrom-Q, coated with 3% OV-17 and at 250°.

Results and Discussion

Incubation of rat hepatocytes in a medium containing amino acids, salts and 1.5% BSA resulted in a loss of cell cholesterol to the medium (Fig. 1A). The increase in cholesterol concentration in the medium was linear for at least three hours (Fig. 1A). Lecithin dispersions increased the rate of loss of cellular cholesterol into the medium and the rate increased with increasing concentrations of lecithin (Fig. 1A). In agreement with a previous report (6) HMG-CoA reductase activity increased in cells incubated for 3 hours even under standard conditions (without lecithin). Activities were elevated further when lecithin was included in the medium (Fig. 1B). The increase in reductase activity during the 3 hour incubation was proportional to the amount

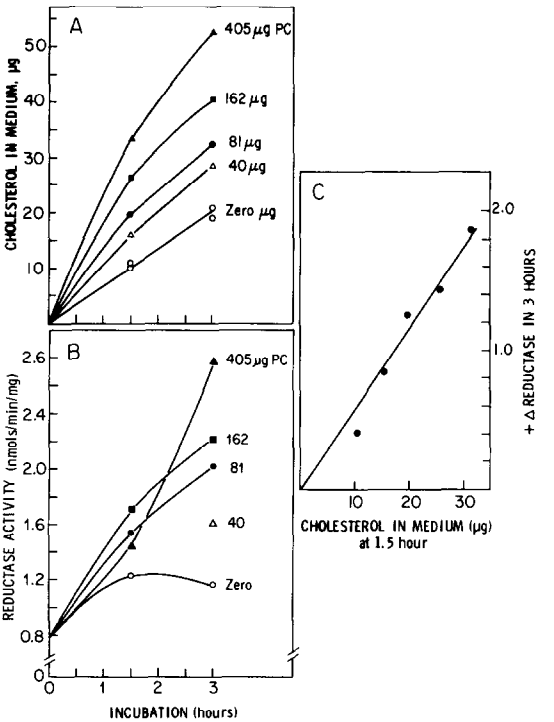

Figure 1. Stimulation of cholesterol efflux and the activity of HMG-CoA reductase by lecithin dispersions. Rat hepatocytes were incubated with various concentrations of lecithin (PC) dispersions: (A), the total amount of cholesterol present in the medium and (B), the activity of HMG-CoA reductase at different times of incubation are shown. In (C) the data from the same experiment are plotted to show the relationship between the cholesterol in the medium at 1.5 hours and the increase in reductase activity after the 3 hour incubation.

of cholesterol which had entered the medium by 1.5 hour (Fig. 1C), but there was no correlation between the increase in reductase activity at 1.5 hours and the sterol in the medium at 1.5 hours.

In other experiments hepatocytes were incubated for 45 min with various concentrations of lecithin dispersions. The cells were then washed and resuspended in fresh medium, containing 1.5% BSA but no lecithin, and were incubated for a further 2.25 hours. During the first 45 min of incubation the rates of cholesterol efflux were directly proportional to the lecithin concentrations (Fig. 2A). However, the rates of sterol efflux during the subsequent 2.25 hours of incubation in the absence of lecithin were independent of the initial treatment of the cells (Fig. 2B). Reductase levels increased during the 3 hour

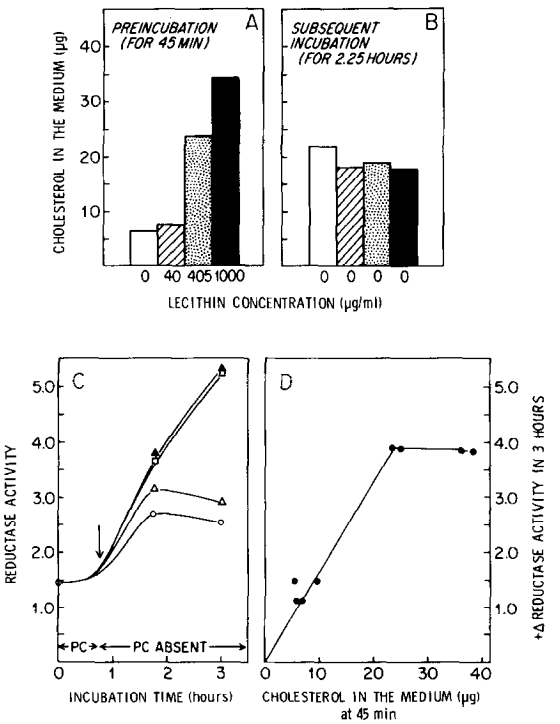

Figure 2. Hepatocytes were pre-incubated for 45 min with lecithin dispersions at the stated concentrations. The cells were then washed and resuspended in fresh standard medium, containing 1.5% BSA but no lecithin, and incubated for a further 2.25 hours. The cholesterol content of the medium (A) at 45 min and (B) at the end of the 2.25 hour incubation are given. The shading indicates the treatment during the first 45 min. The reductase activity of the cells is shown in (C). The arrow indicates the time cells were removed from the lecithin-containing medium. Lecithin-concentration during the first 45 min: None (0); 40 µg/ml (△); 405 µg/ml (▲); 1,000 µg/ml (□). (D), Plot of the increase in reductase levels after the 3 hour incubation versus the cholesterol in the medium at 45 min.

incubation (Fig. 2C) and the increase was directly proportional to the amount of sterol lost from the cells during the initial 45 min (Fig. 2D). These data show that the enzyme levels observed at 3 hours were predetermined by the amount of sterol lost from the cells during the first 45 min.

Stimulation of the reductase was maximal at a lecithin concentration of approximately 405 µg/ml (Fig. 2C). Higher lecithin concentrations did not result in further increases in reductase levels although both the rate and the amount of cellular sterol loss were enhanced (Fig. 2D). Presumably under these

conditions other factors (e.g. the rate of synthesis of new HMG-CoA reductase) became rate limiting so that the amount of sterol loss no longer was the primary determinant of enzyme activity.

The data demonstrate clearly that the activity of hepatic HMG-CoA reductase depended on the amount of cholesterol leaving the cell. The rapid response of the cells to even small losses of cholesterol by induction of HMG-CoA reductase is, presumably, a mechanism for the maintenance of their cholesterol content at a constant level. In the intact animal hepatic sterol efflux could be affected by changes in the rate of lipoprotein or bile acid secretion, or by some other specific loss of cholesterol from the cells. Catabolism of liver cholesterol to bile acids was found to undergo a circadian rhythm with peak catabolism at 8 p.m. while cholesterogenesis was reported to be maximal between 8 p.m. and midnight (14). However, it remains to be determined whether such a circadian loss of liver cholesterol to bile acids predetermines the activity of hepatic HMG-CoA reductase.

It is possible that regulation of reductase activity in other cell types is also controlled by the relative rates of flux of cholesterol in and out of cells. For example, Fogelman et al. (15) have reported that when human leukocytes were incubated in a lipid free medium the loss of radioactive sterol from the cells preceded the induction of HMG-CoA reductase. Furthermore, leukocytes from persons heterozygous for familial hypercholesterolemia lost a greater percent of their radioactive cholesterol into the lipid free medium and demonstrated greater reductase activity but no greater sterol content than normals. Kachadurian (16) and Brown et al. (17) have demonstrated that the sterol content of fibroblasts taken from homozygote familial hypercholesterolemics and grown in the presence of fetal calf serum, had a sterol content no greater than normal fibroblasts despite the fact that their reductase activity was many times higher than normal. Perhaps, the increased activity was a compensatory mechanism for greater than normal sterol loss. Further work is required to determine whether sterol flux is the primary determinant of reductase activity of various cell types under physiological conditions.

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