DIURNAL VARIATION AND CHOLESTEROL REGULATION OF HEPATIC HMG-CoA REDUCTASE ACTIVITY[1,2]

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SUMMARY

HMG-CoA reductase exhibits a diurnal rhythm. Cycloheximide injection completely prevents both the observed 9.5 fold rise in activity and the loss of activity during daylight. This is compatible with the existence of a specific, labile, degradative or inactivating protein for HMG-CoA reductase. Effects of cholesterol on reductase activity were also studied. K_M and V_{max} for reductase from normal- and cholesterol-fed rats are 8.1×10^{-5} M and 0.33 nmole/min/mg and 6.9×10^{-5} M and 0.0404 nmole/min/mg respectively. Reductase from both sources is substrate-inhibited by HMG-CoA. Mixing experiments suggest that a soluble inhibitor of HMG-CoA reductase does not cause the profound drop in activity observed in cholesterol-fed rats.

INTRODUCTION

Several laboratories have reported that cholesterol feeding virtually abolishes hepatic cholesterol synthesis (1,2). While indirect methods (3-5) localized the site of this control at HMG-CoA reductase (EC 1.1.1.34), the first enzyme unique to cholesterol biosynthesis, direct evidence for decreased HMG-CoA reductase activity following cholesterol feeding was first provided by Linn (6). Noradrenalin (7) and Triton (8) increase reductase activity *in vivo*. Bile acids, which inhibit incorporation of ^{14}C-acetate into mevalonate (9) and cholesterol (10), do not affect HMG-CoA reductase activity *in vitro* (10). Kandutsch and Saucier (8) observed a diurnal variation in mouse liver HMG-CoA reductase activity. More recently, Back *et al.* (11) and Hamprecht *et al.* (12) provided evidence for diurnal variations in hepatic cholesterol synthesis and HMG-CoA reductase activity. We have investigated both the cyclic rise and fall in rat liver HMG-CoA reductase activity and the effects of cholesterol on reductase

[1]Abbreviations used: HMG, 3-hydroxy-3-methylglutaric acid; MVA, mevalonic acid.

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activity with a view to establishing the molecular mechanisms which control HMG-CoA reductase activity.

METHODS

Female 125-150 g Wistar rats were sacrificed at noon, their livers excised into Linn's medium (13) (EDTA-NaCl-β-mercaptoethanol) at pH 6.0, minced in a tissue press, and homogenized in 3.5 volumes of Linn's medium for 40 seconds at full speed using a tight-fitting Teflon pestle. The crude homogenate was centrifuged twice for 15 min at 12,000 x g to completely remove mitochondria and their associated HMG-CoA lyase activity (EC 4.1.3.4). The supernatant liquid was centrifuged 1 hr at 48,000 x g and the heavy microsomal pellet resuspended in 1/10 the initial volume of Linn's medium at pH 6.8. Each 1 ml incubation contained: 30 μmoles glucose-6-phosphate, 3 μmoles NADP, 2 E.U. glucose-6-phosphate-dehydrogenase, 300 nmoles D,L-HMG-CoA-^{14}C (S.A. 1.9 x 10^5 cpm/μmole) and 0.5 ml resuspended microsomes. Triplicate incubations were at 37° for 30 min. Mevalonate was quantitated by the TLC method of Shapiro et al. (14) except that samples were lyophilized, then dissolved in 0.5 ml acetone:H_2O::9:1 for TLC. Under the conditions used, mevalonate formation is a linear function of protein concentration (to 10 mg) and of time (to 60 min).

RESULTS AND DISCUSSION

Double-reciprocal plots of HMG-CoA reductase activity from normal- and cholesterol-fed rats both show substrate inhibition by low concentrations of HMG-CoA (Fig. 1). Two separate determinations using normal rats give K_M = 8.1 x 10^{-5} and 7.2 x 10^{-5} M, and V_{max} = 0.329 and 0.306 nmole mevalonate formed/min/mg. For cholesterol-fed rats, K_M was 6.9 x 10^{-5} M and V_{max} was 0.0404 nmole mevalonate formed/min/mg. Siperstein and Fagan (15) have postulated that HMG-CoA reductase is an allosteric enzyme feedback inhibited by cholesterol or a derived metabolite. However, the similarity in K_M for reductase from normal- and cholesterol-fed rats indicates that hepatic HMG-CoA reductase is not a K-series allosteric enzyme.

Feeding cholesterol for 24 hr profoundly decreased HMG-CoA reductase activity,

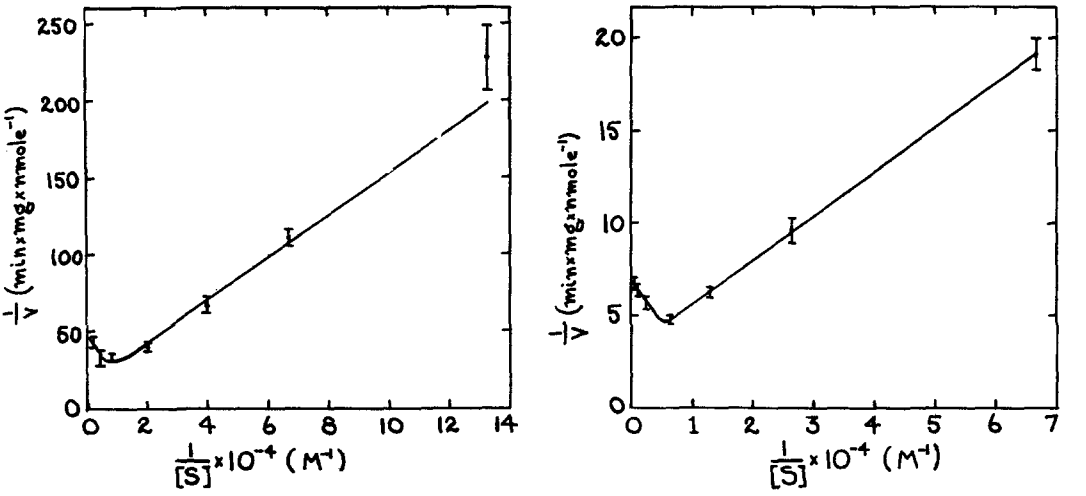

Fig. 1. Lineweaver-Burke plots for HMG-CoA reductase from normal- and cholesterol fed rats. K_M for the normal enzyme (left) was 8.1 x 10^{-5} M and V_{max} was 0.330 nmoles/min/mg. For a second determination (not shown) K_M = 7.2 x 10^{-5} M and V_{max} = 0.306 nmole/min/mg. For cholesterol-fed rats (right) K_M = 6.9 x 10^{-5} M and V_{max} = 0.0404 nmole/min/mg. Kinetic constants were from the slopes obtained by computer least-square fits using IBM 7094 and Control Data 6500 computers and our "Dataplot" program. Incubations were performed as described in the text using varying HMG-CoA concentrations. Vertical lines represent the standard error of the mean.

TABLE I. EFFECT OF DIETARY CHOLESTEROL AND PREINCUBATION ON REDUCTASE ACTIVITY

Microsomes from livers of rats fed the indicated diets were isolated both directly and following preincubation of homogenates by shaking 30 min at 37°. Incubation conditions are described im the text. Data are mean values ± the standard error of the mean for triplicate determinations.

Rat diet	Preincubated	Specific activity (nmole MVA/min/mg)x10^2	Fraction of control (%)
Normal	-	8.9±0.1	(100)
Normal	+	5.0±0.9	56
5% Cholesterol, 24 hr	-	0.73±0.1	8.2
5% Cholesterol, 24 hr	+	0.49±0.05	5.5
5% Cholesterol, 1 week	-	0.33±0.02	3.7

while continued feeding for 1 week further depressed activity (Table I). To test for the presence of an inhibitor of HMG-CoA reductase in livers of choles-

TABLE II. HMG-CoA REDUCTASE ACTIVITY OF MICROSOMES ISOLATED FROM MIXED HOMOGENATES OF LIVERS FROM NORMAL- AND CHOLESTEROL-FED RATS

Crude liver homogenates from normal-fed (NF) and cholesterol-fed (CF) rats, alone or mixed in the indicated proportions, were preincubated 30 min at 37°, with shaking, prior to isolation of microsomes. Incubation conditions are described in the text. Mean specific activity ± the standard error of the mean of mixed homogenates was computed per mg normal microsomes.

Source of homogenate for isolation of microsomes	Specific activity (nmole MVA/min/mg)x10^2	Fraction of control (%)
Normal-fed (NF)	13.0±0.8	(100)
Cholesterol-fed (CF)	0.16±0.002	12
3/4NF + 1/4CF	12.8±0.5	98
1/2NF + 1/2CF	13.3±0.5	103

terol-fed rats, crude homogenates from livers of normal- and cholesterol-fed rats were mixed, preincubated, and microsomes isolated (Table II). Since the activity of normal microsomes was not impaired by isolation from mixed homogenates it appears that the virtually complete loss of HMG-CoA reductase activity following cholesterol feeding is not due to feedback inhibition. Repression or accelerated degradation of HMG-CoA reductase may thus represent the mechanism of cholesterol action. We therefore investigated the diurnal variations in HMG-CoA reductase activity to determine the contributions made by alterations in rates of synthesis or degradation to this cyclic rhythm.

HMG-CoA reductase exhibits striking diurnal variations with peak activity at about 10:00 p.m. Between 4:00 and 8:00 p.m. reductase activity in normal animals rises from 0.037±0.03 to 0.351±0.013 nmole/min/mg, a 9.5-fold increase (Fig. 2). Cycloheximide injection completely prevents this increase, indicating that enzyme synthesis is responsible for the rise in activity. Reductase activity falls steadily from midnight to noon. The decay of enzyme activity is also prevented by cycloheximide. While the specific activity falls from 0.273±0.005 to 0.143±0.003 nmole/min/mg from 4:00 to 8:00 a.m., the activity in cycloheximide-injected animals remains at 0.290±0.019 nmole/min/mg (Fig. 2).

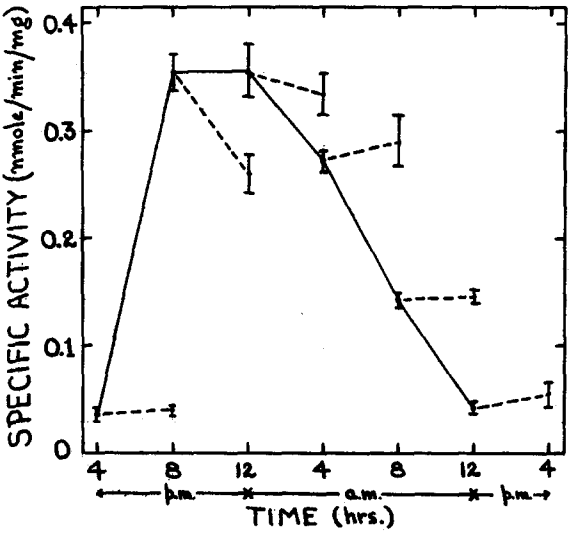

Fig. 2. Diurnal variation of HMG-CoA reductase. Rats fed a standard laboratory diet were kept in separate cages and acclimated for at least 48 hr. Artificial illumination from 7:00 a.m.-7:00 p.m. (EST) paralleled daylight. Cycloheximide (0.5 mg/100 g) was injected intraperitoneally into paired rats 4 hr prior to sacrifice. Two normal and two injected rats were sacrificed at each time. Paired livers were pooled and microsomes isolated. Incubations were as described in the text but for 15 min. Vertical lines represent the standard error of the mean. For the decay of activity $t_{\frac{1}{2}} = 3.3$ hr. Solid lines indicate the diurnal variation in normal enzyme activity. The activity following cycloheximide injection (dashed lines) may be compared to the changes in normal rats during the same time interval.

Similarly, from 8:00 a.m. to noon the normal activity falls from 0.143 ± 0.003 to 0.042 ± 0.003 nmole/min/mg while activity in cycloheximide-treated animals is 0.146 ± 0.009 nmole/min/mg (Fig. 2).

Kenney (16) and Levitan and Webb (17) have interpreted similar data from antibody experiments to favor the existence of a highly labile specific degradative enzyme for tyrosine transaminase. Schimke et al. (18) have shown that enzyme degradation is important in controlling the activity of tryptophan pyrrolase. Finally, the rate of catalase degradation in mice appears to be a genetically-controlled variable (19,20). Thus regulation of the rate of enzyme degradation may be an important mechanism for controlling the activity of mammalian enzymes.

The ability of cycloheximide to arrest cyclic loss of HMG-CoA reductase

activity is compatible with the existence of a labile degradative enzyme or inactivating protein. The activity of HMG-CoA reductase, which can vary over 100 fold, may be controlled by alterations both in its rate of synthesis and degradation. To our knowledge, this constitutes the first evidence for modulation of the activity of a mammalian biosynthetic enzyme at the levels of synthesis and degradation.

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Erratum

Volume 36, No. 6 (1969), in the Communication "DNA-Dependent RNA Polymerase from E. coli: Studies on the Role of σ in Chain Initiation" by John J. Dunn and Ekkehard K. F. Bautz, pp. 925-930, line 5 under the heading "Nucleoside triphosphate added" in Table I on p. 927 should read: "GTP+UTP" instead of CTP+UTP".