3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase and Mevalonate Kinase Activity in Turkey Liver and Kidney

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The diurnal variations of HMG-CoA reductase and MVA kinase activities in turkey liver and kidneys were investigated. Parallel diurnal variations for HMG-CoA reductase and MVA kinase, with maxima near 1000 hr, were demonstrated in liver; a lesser variation was observed in the kidneys. Livers and kidneys of turkeys fed a diet containing 1% cholesterol for two weeks had reduced HMG-CoA reductase (97%) and MVA kinase activity (53%). Animals taken off the cholesterol diet for two weeks showed an increase in MVA kinase activity but not to the control level. Turkeys fasted for 48 hr also had a decrease in MVA kinase activity. Cholestyramine elevated MVA kinase activity in liver from nonlaying (egg production) hens but had no such effect in laying hens. Nonlaying hens showed MVA kinase activity of 157 ± 12 nmoles/hr/mg of protein while those laying showed this activity to be 565 ± 53 nmoles/hr/mg of protein. These results are consistent with the possibility that enzymatic reactions other than HMG-CoA reductase play a role in the regulation of sterol synthesis.

INTRODUCTION

3-Hydroxy-3-methylglutaryl coenzyme A reductase (E.C. 1.1.1.34) is generally considered to catalyze the rate-limiting reaction in cholesterol biosynthesis. The enzyme catalyzes the formation of mevalonic acid. The hepatic enzyme is depressed by high-cholesterol diet (1). Cholesterol feeding lowers HMG-CoA reductase activity by rapid inactivation of preformed enzyme and longer-term reduction in enzyme synthesis (2). The activity of HMG-CoA reductase is decreased by phosphorylation, *in vitro* (3,4) and *in vivo* (5). Bucher *et al.* (6) have observed, however, that the incorporation of mevalonic acid into cholesterol is affected by factors that influence sterol synthesis. Thus, the enzymatic steps that follow mevalonate production may be subject to physiological regulation. Gould and Swyryd (7) found that the rate of conversion of mevalonate to farnesyl pyrophosphate in rat liver is decreased by cholesterol feeding. Faust *et al*. (8) reported that squalene synthetase activity is inhibited by low density lipoproteins in human fibroblast. Ramachandran and Shah (9) suggested that mevalonate pyrophosphate decarboxylase may play a role in regulation of sterol synthesis in developing rat brain. In a preliminary report Hunter and Mitchell (10) reported the suppression of HMG-CoA reductase and mevalonate kinase in tissues from cholesterol-fed turkeys. Mitchell and Avigan (11) reported that rats fed a 1% cholesterol diet for seven days had reduced mevalonate kinase and mevalonate pyrophosphate decarboxylase activities. The mevalonate kinase and mevalonate pyrophosphate decarboxylase activities in human skin fibroblasts grown in culture were increased when whole fetal calf serum in the incubation medium was replaced with lipid-deficient serum (11).

The present study describes some factors that affect the conversion of mevalonate to other sterol biosynthesis intermediates in turkey liver *in vivo*. A preliminary report has been published (10).

MATERIALS AND METHODS

Nicholas White poults, toms, and hen turkeys were fed controlled diets by the Oklahoma State University Poultry Department. Poults (1-25 days post hatching) were fed a control turkey starter (Tallow) diet. Mature turkey hens (12-16 weeks of age) were fed a control turkey breeder ration. Turkeys were kept in a room with lights on from 0600 hr to 1800 hr.

The animals were fed daily at 0800 hr during a two-week adjustment period. Experimental animals were then placed on specific rations, for two weeks. After two weeks of experimental diets the animals were returned to the control diet. Enzyme activities were determined at 4-hr intervals over a 24-hr period. Unless stated otherwise, three animals were used for each determination.

Radioactive compounds

D,L-Mevalonic-*2*-^{14}C acid (specific activity: 45.9 mCi/mmole), D,L-glutaryl-*3*-^{14}C-3-hydroxy-3-methylglutaryl coenzyme A (sp. act. 49.5 mCi/mmole), and D,L-5-^{3}H(N)-mevalonic acid (sp. act. 5 Ci/mmole) were purchased from New England Nuclear, Boston, MA. Mevalonic acid (^{14}C and ^{3}H) was obtained as the dibenzoylethylenediamine salt, and labeled HMG-CoA as an aqueous solution, pH 5.0. All compounds were routinely checked for purity by ion exchange and paper chromatography.

Liver preparations

Turkeys were sacrificed and livers were rapidly excised, transferred to chilled beakers, and weighed. Approximately 25-30 g of liver were homogenized in two volumes of Krebs-Ringer phosphate buffer (0.154 M NaCl, 0.154 M KCl, 0.11 M $CaCl_2$, 0.15 M $MgSO_4$, 5 mM dithiothreitol, 1 mM EDTA, and 0.1 M potassium phosphate at pH 7.4) in a loose-fitting Potter-Elvehjem-type glass homogenizer equipped with a motor-driven Teflon pestle. The homogenate was filtered through three layers of cheesecloth, centrifuged (5×10^3 *g*, 15 min) and the supernatant centrifuged (*A*) at 15×10^3 *g* for 15 min. The supernatant from *A* was centrifuged (*B*) at 105×10^3 *g* for 1 hr. The pellet material from *B* was resuspended and centrifuged (*C*) at 105×10^3 *g* for 1 hr. The pellet from *C* contained washed microsomes and HMG-CoA reductase, while the supernatant from *C* contained 85-93% of the mevalonate kinase activity.

Kidney preparation

Kidneys (10-15 g) were minced and pressed through a Harvard screw press before being homogenized in the Potter-Elvehjem homogenizer. Washed microsomes and supernatant from *C* were prepared as described for liver.

HMG-CoA reductase assay

Activity was measured as follows: The reaction mixture (1 mL) was 10 mM in glucose 6-phosphate, 1 mM in NADP, 1 mM in EDTA, 1 mM in dithiothreitol, 0.1 M in potassium phosphate pH 7.4, and contained glucose 6-phosphate dehydrogenase (2 units), 100 mg protein, and [3-^{14}C]HMG-CoA (7.9×10^4 dpm). The reactions were allowed to proceed at 37 °C for 1 hr. [^{3}H]-Mevalonate (2×10^5 dpm) was added as an internal standard after the reaction was stopped by quick freezing and the addition of 200 μL of H_2SO_4. Anhydrous sodium sulfate was added to the reaction mixture. After standing for 1 hr at room temperature, the samples were shaken with four portions of anhydrous diethyl ether. The combined extracts were evaporated to dryness, the residue was dissolved in 1.0 mL of water and placed on a Dowex-1-formate column (0.5 × 5 cm), and HMG-CoA reductase eluted with 5.0 mL of water. The eluate, which contained mevalonolactone, was collected in a scintillation vial and dissolved in 10.0 mL of Insta-gel. The radioactivity was then counted in a Packard 3320 scintillation spectrometer.

Mevalonate kinase

Mevalonate kinase activity was measured in a volume of 1.0 mL 5 mM in $MgCl_2$, 0.5 mM in dithiothreitol, 1.0 mM in EDTA, 15 mM in ATP, and 0.1 mM in potassium phosphate buffer pH 7.4, and contained [2-^{14}C]-MVA (1×10^6 dpm), and 100 mg of protein. After 1 hr at 37 °C, the reaction was terminated by heating in boiling water for 5 min. The products were separated on a Dowex-1-formate ion exchange column (0.5 × 5 cm) as previously reported (11).

Protein

Protein concentrations were determined as described by Hartree (12), bovine serum albumin being used as standard.

RESULTS

Diurnal variations

Figure 1 illustrates the diurnal variations in HMG-CoA reductase activity in microsomes from the liver and kidneys of turkey hens fed a normal ration. There is no HMG-CoA reductase activity in the supernatant from *C*. The hepatic enzyme increases sharply between 0400 and 1600 hr, while the kidney enzyme, which is highest at 0400 hr, drops in activity at 0800 hr and increases slightly to a plateau at 1200 to 1600 hr. (The rhythm of the kidney enzyme was much less pronounced.)

Figure 2 shows the diurnal variations in MVA kinase activity. Between 85 and 93% of the MVA kinase activity is present in the supernatant from *C*; the remainder is in the pellet. Thus, MVA kinase activity is found in both fractions while HMG-CoA reductase activity is detected only in the pellet from *C*. As with HMG-CoA reductase, the hepatic enzyme level shows a sharp rise at 0800 and 1200 hr while the kidney enzyme shows a slight rise at 1200 and 1600 hr. Extrapolation of Figs. 1 and 2 suggests a peak activity near 1000 hr for liver enzymes.

Effect of cholesterol

Adding 1% cholesterol to a single daily feeding resulted in a decrease in HMG-CoA reductase (90% of controls) and mevalonate kinase (50-70% of controls) activities in turkey liver and kidneys. HMG-CoA reductase activity was decreased over 93% while MVA kinase activity in cholesterol-fed animals was only 47% that in the control animals (Table 1). Mevalonate kinase activity in livers of animals on a cholesterol-unsupplemented diet for two weeks after cholesterol feeding was 74% of that in control animals (Fig 3).

Effect of cholestyramine

Hen turkeys in egg production (laying) and out of egg production (nonlaying) were fed cholestyramine (0.5 g/day) for seven days. Animals fed cholestyramine that were out of egg production showed an increase in MVA kinase activity while those in egg production were unaffected by cholestyramine treatment (Table 2). The effect on MVA kinase activity in nonlaying hens was still present two weeks after termination of treatment.

Effect of fasting

Ten-week-old turkey hens were fasted for 48 hr before MVA kinase activity from liver was determined. The activity in fed animals was 209 ± 19 nmoles/hr/mg of protein, and 171 ± 13 nmoles/hr/mg of protein in fasted animals. Refeeding fasted animals promptly restored MVA kinase activity to control levels.

Age and MVA kinase activity

Turkey hens were maintained on a simple ration for 18 months post hatch. MVA kinase

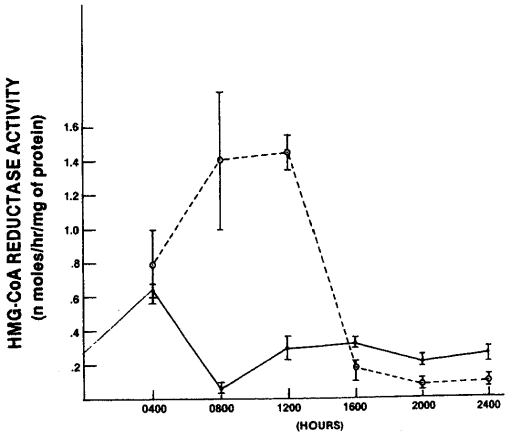

Figure 1. Diurnal variations in hepatic (o — o) and kidney (• — •) HMG-CoA reductase activity; mean ± S.E.M. in turkey hens fed a single daily meal at 0800 hr. Food was consumed between 0800 and 1000 hours. Three animals were used for each determination.

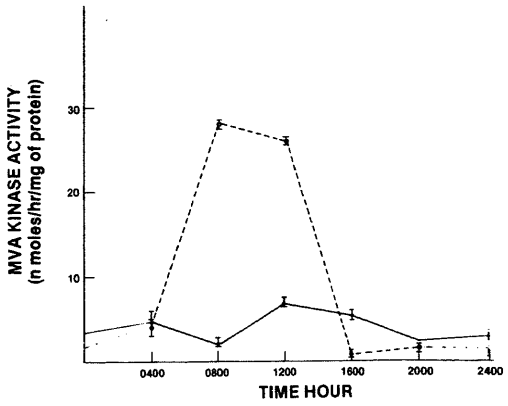

Figure 2. Diurnal variations in hepatic (o — o) and kidney (• — •) mevalonate kinase activity; mean ± S.E.M. in turkey hens fed a single daily meal at 0800 hr. Food was consumed between 0800 and 1000 hours. Three animals were used for each study.

activity in liver increased with age (Fig. 4); however, there was a very significant increase in MVA kinase activity during the period of egg production (laying) and a very significant drop in liver MVA kinase activity when the animal went out of production (nonlaying).

DISCUSSION

HMG-CoA reductase activity in turkey liver and kidney is subject to diurnal variations similar to those observed in rats (13), hamsters (14), and swine (15). This is the first demonstration of HMG-CoA reductase and MVA kinase diurnal activity in an avian. The diurnal variation in rat liver is associated with changes in the rate of synthesis of new enzyme (16); this has not been investigated in turkeys. Peak activity in the rat corresponds with the time of most rapid feeding (16,17). A similar connection has been made between feeding and the circadian rhythms in cholesterol biosynthesis in hamsters (18) and swine (14). Because the peak time of activity (5-6 hr after food consumption) is the same for rats (16,17), hamsters (14), and swine (15), it is likely that the cyclic rise is related to food ingestion rather than food

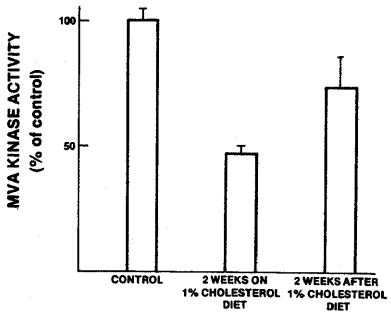

Figure 3. Effect of dietary cholesterol on MVA kinase activity from turkey liver. The activity of MVA kinase in liver tissue isolated from nonlaying hens (control) was 175 ± 38 nmoles/hr/mg of protein. Animals were fed a daily ration with 1% cholesterol for two weeks. Those animals fed a 1% cholesterol ration were taken off cholesterol and MVA kinase activity in livers was determined after two weeks.

TABLE 1. Effects of dietary cholesterol on HMG-CoA reductase and mevalonate kinase activities for turkey liver and kidneys

	Activity (Relative values)[a]			
	HMG-CoA reductase		MVA kinase	
Dietary additions	Liver	Kidney	Liver	Kidney
None	100	100	100	100
1% cholesterol	7	3	47	33

[a]The values obtained for HMG-CoA reductase in liver from turkeys without cholesterol was 2.1 ± 0.2 nmol/hr/mg of protein and in kidneys the value was 0.14 ± 0.01 nmol/hr/mg of protein. Mevalonate kinase activity in turkey liver without cholesterol diet was 405 ± 33 nmol/hr/mg protein and for kidneys was 150 ± 8 nmol/hr/mg of protein.

TABLE 2. Effect of cholestyramine on MVA kinase activity in turkey liver

Cholestyramine added	Mevalonate kinase activity (nmol/hr/mg of protein; relative activities)	
	Nonlaying[a]	Laying[a]
Part 1		
None	260 ± 20 (100%)	368 ± 63 (100%)
0.5 g/day for 7 days	304 ± 38 (116%)	365 ± 36 (99%)
Part II		
None	228 ± 9 (100%)	349 ± 32 (100%)
two weeks off additive	278 ± 8 (121%)	352 ± 50 (101%)

[a]Turkeys in egg production (laying) and out of egg production (nonlaying) were fed 0.5 g of cholestyramine for seven days. Data are reported as mean ± S.D. of 4 turkeys in each group.

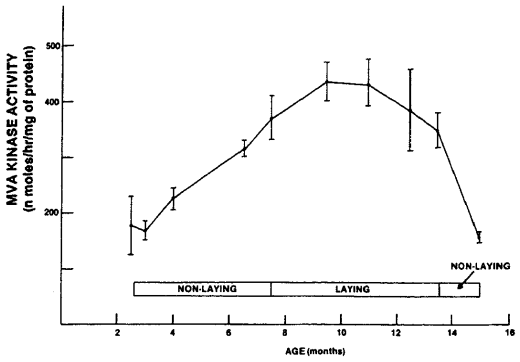

Figure 4. Effect of age on the specific activity of MVA kinase from turkey liver. Three hen turkeys used in each determination; mean ± SEM values are shown by the bars.

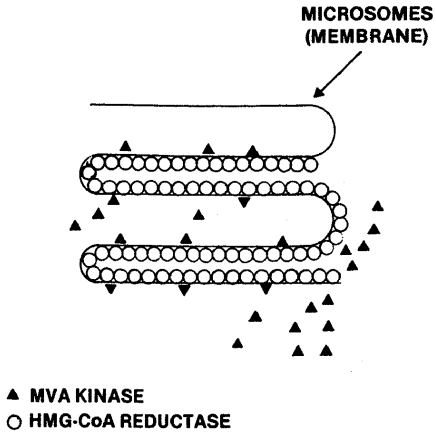

Figure 5. HMG-CoA reductase and MVA kinase attachment to membranes.

anticipation. HMG-CoA reductase activity in turkey livers showed peak activity at the time of greatest food ingestion. Avians do not eat in the dark and consumed the bulk of the meal between 0800-0900 hr; however, during the hours of light they continue to feed.

Diurnal variation of mevalonate kinase has not been previously reported. It is similar to that of HMG-CoA reductase (Fig 2). We decided to investigate MVA kinase more thoroughly since much less is known about this system than about HMG-CoA reductase.

It has been reported that mevalonate kinase and mevalonate 5-phosphate kinase (MVAP) activities in livers from rats fed 1 % cholesterol are 45-50 % lower than in controls (11). We observed a similar effect of dietary cholesterol on MVA kinase activity in turkey liver, whether or not the hens were laying. MVA kinase specific activity was significantly higher during egg production. Feeding cholestyramine to nonlaying animals increased MVA kinase activity, whereas animals in egg production (laying) were not thus affected. Cholestyramine greatly enhances the activity of HMG-CoA reductase in rat (19), guinea pig (20), dog (21), and swine (15). Even though the effect of cholestyramine on MVA kinase is not as pronounced as that on HMG-CoA reductase, it is conceivable that the small additive effects on other enzymes will have a more pronounced effect on sterol synthesis.

Early studies by Bucher et al. (6) showed that the conversion of mevalonic acid and squalene to cholesterol is reduced by fasting and by feeding cholesterol (however, to a lesser extent than the conversion of acetate). This suggests a secondary regulatory mechanism which may be very important in the overall control of the flux of intermediates through the sterol pathway. Slakey *et al.* (22) suggested that secondary regulatory processes exist between mevalonic acid and squalene, although their experiments did not show a direct effect on MVA kinase activity. Mitchell and Avigan (11) showed a direct effect of dietary cholesterol on MVA kinase and MVAP kinase activities in rat liver. Slakey *et al.* (22) used a coupled spectrophotometric assay for MVA kinase, whereas Mitchell and Avigan (11) and the work here reported used a simpler procedure. The method for quantitative isolation of phosphorylated metabolites of mevalonic acid provides a convenient assay for MVA kinase, MVAP kinase, and mevalonate-5-pyrophosphate (MVAPP) decarboxylase, the three enzymes that produce sterol precursors. Our results show a very direct effect of cholesterol and cholestyramine on MVA kinase activity.

An interesting relationship between HMG-CoA reductase and MVA kinase refers to their solubility. HMG-CoA reductase is a membranous enzyme located only in the microsomes. MVA kinase is regarded as a soluble enzyme; however, 9-15% of the total MVA kinase activity was located in washed microsomes. Of the total MVA kinase activity, 85-93% was in the supernatant from *C* and 7-15% in the pellet from *C*. Figure 5 shows a diagram demonstrating the tight binding of

HMG-CoA reductase and the looser binding of MVA kinase.

In conclusion, we reaffirm, more directly, the conclusions that secondary regulatory processes occur in the cholesterol biosynthetic pathway and that although HMG-CoA reductase is the major regulatory site, MVA kinase and other enzymes may be depressed or activated together. Until the K_m concentration of substrates and the roles of individual enzyme reactions are determined it cannot be concluded that only a few steps beyond HMG-CoA reductase may be significant. There may be a concerted modulation of the activity of all the enzymes involved in a sterol synthesis.

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