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Biochemistry

Regulation of cholesterol synthesis in rat adrenal gland through coordinate control of 3-hydroxy-3-methylglutaryl coenzyme A synthase and reductase activities

[4-aminopyrazolopyrimidine/acetyl CoA acetyltransferase (acetoacetyl CoA thiolase)/mevalonate kinase/low density lipoprotein/diurnal variation]

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ABSTRACT **The activities of cytosolic 3-hydroxy-3-methylglutaryl coenzyme A synthase [3-hydroxy-3-methylglutaryl-CoA acetoacetyl-CoA-lyase (CoA-acetylating), EC 4.1.3.5] and microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase [mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34], two sequential enzymes in the cholesterol biosynthetic pathway, were shown to be regulated coordinately in the adrenal gland of the rat. When the plasma cholesterol level was lowered by administration of 4-aminopyrazolopyrimidine, a treatment known to enhance cholesterol synthesis in the adrenal, synthase activity in the gland rose by 14- to 29-fold and reductase activity rose by 50- to 100-fold. The subsequent intravenous infusion of low density lipoprotein restored the plasma cholesterol level and suppressed synthase and reductase activities in parallel. The activities of adrenal 3-hydroxy-3-methylglutaryl coenzyme A synthase and reductase were also shown to exhibit a coordinate pattern of diurnal variation with peaks in both enzymes achieved at the mid-point of the dark cycle. The activity of adrenal acetoacetyl coenzyme A thiolase (acetyl CoA acetyltransferase; acetyl-CoA:acetyl-CoA *C*-acetyltransferase, EC 2.3.1.9), the enzyme preceding the synthase in the cholesterol biosynthetic pathway, and the activity of adrenal mevalonate kinase (ATP:mevalonate 5-phosphotransferase, EC 2.7.1.36), the enzyme following the reductase, were not enhanced by cholesterol deprivation, and neither exhibited a pattern of diurnal variation. The coordinate control of 3-hydroxy-3-methylglutaryl CoA synthase and reductase in rat adrenal gland provides a model system to study the biochemical mechanism for the regulation of cholesterol synthesis in a tissue that uses cholesterol for the synthesis of steroid hormones.**

Recent studies have shown that cholesterol synthesis is subject to an extraordinary degree of regulation in the adrenal gland of the rat (1, 2). When plasma cholesterol levels are normal, the adrenal gland satisfies its large requirement for cholesterol as a precursor for steroid hormone synthesis by utilizing the cholesterol contained in plasma lipoproteins (1–5). However, when the availability of plasma cholesterol is diminished, such as when hepatic lipoprotein secretion is blocked by administration of the drug 4-aminopyrazolopyrimidine (4-APP) (6–9), cholesterol synthesis in the adrenal gland rises by as much as 30-fold (1, 2). This increase is associated with a 50- to 200-fold rise in the activity of the microsomal enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase [HMG CoA reductase; mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] (2). These findings are consistent with the view that in rat adrenal gland as in rat liver (10) HMG CoA reductase constitutes a major rate-controlling step in the cholesterol biosynthetic pathway.

Lane and coworkers have recently demonstrated by cholesterol feeding experiments that in rat and chicken liver a second site of regulation of cholesterol synthesis exists. This site involves the reaction catalyzed by HMG CoA synthase [3-hydroxy-3-methylglutaryl-CoA acetoacetyl-CoA-lyase (CoA-acetylating), EC 4.1.3.5], the enzyme that immediately precedes HMG CoA reductase in the cholesterol biosynthetic pathway (11, 12) (Fig. 1). The situation in liver is complex, however, because HMG CoA is synthesized at two sites within this tissue (13–15). The vast bulk of the HMG CoA is synthesized within the mitochondria by a specific intramitochondrial HMG CoA synthase (13) and is cleaved within the mitochondria by HMG CoA lyase (EC 4.1.3.4) to produce acetoacetate (16). A much smaller amount of hepatic HMG CoA is synthesized by a distinct HMG CoA synthase within the cytosol (13). This cytosolic HMG CoA is reduced by microsomal HMG CoA reductase to form mevalonate, which is subsequently converted to cholesterol (Fig. 1). The experiments of Lane and co-workers have shown that in liver the cytosolic but not the mitochondrial HMG CoA synthase is suppressed by cholesterol feeding (11, 12).

The magnitude of the rise in the activity of HMG CoA reductase in the adrenal gland of the 4-APP-treated rat suggested the use of this tissue as a model system to determine whether a similar degree of regulation exists at the level of HMG CoA synthase. This model is particularly useful because the adrenal gland is not known to produce large amounts of ketone bodies and hence should not have large amounts of intramitochondrial HMG CoA synthase. The current results indicate that cytosolic HMG CoA synthase activity in rat adrenal gland is regulated coordinately with microsomal HMG CoA reductase activity and that these two enzymes function in concert to supply substrate for cholesterol biosynthesis during conditions of cholesterol deprivation.

MATERIALS AND METHODS

Materials. [*1*-^{14}C]Acetyl CoA (54 mCi/mmol) and DL-3-hydroxy-3-methyl[3-^{14}C]glutaryl CoA (26.2 mCi/mmol) were obtained from New England Nuclear Corp. DL-[*2*-^{14}C]Mevalonic acid lactone (13 mCi/mmol) was purchased from Amersham/Searle.

Animals. Male Sprague-Dawley rats weighing between 300 and 400 g were obtained from Charles River Laboratories. Unless otherwise indicated, the rats were exposed to a light–dark cycle consisting of 12 hr of light (0700–1900 hr) and 12 hr of darkness for at least 2 weeks prior to use. Throughout these periods of adaptation, the rats had full access to water and formula chow (Wayne's Laboratory, Chicago).

In experiments in which 4-APP-treated rats were used, the animals were given daily injections of 4-APP (40 mg/kg of body weight intraperitoneally in 10 mM sodium phosphate, pH 3)

Abbreviations: 4-APP, 4-aminopyrazolopyrimidine; CoA, coenzyme A; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein.

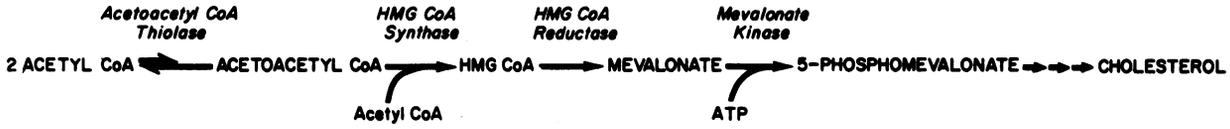

FIG. 1. Early steps in the cholesterol biosynthetic pathway. This sequence of reactions emerged from the experiments of Rudney, Lynen, Gould, Popjak, Siperstein, Lane, and others (reviewed in refs. 12 and 13).

for 3 days and were killed on the fourth day (2, 8). For intravenous injection of low density lipoprotein, a polyethylene cannula was introduced into the tail vein while the animal was under light anesthesia. Control animals received appropriate volumes of either 10 mM sodium phosphate, pH 3, intraperitoneally for the 4-APP experiments or saline intravenously for the lipoprotein experiments.

Lipoproteins. Human low density lipoprotein (LDL) (density, 1.019–1.063 g/ml) was obtained from the plasma of healthy subjects as described previously (17).

Preparation of Adrenal Cytosolic and Microsomal Fractions. Rats were routinely killed at 0900 hr by decapitation, and blood was collected by drainage from the neck into a tube containing EDTA. All subsequent steps were carried out at 4°. The adrenal glands were removed immediately, placed into ice-cold medium containing 0.3 M sucrose, 25 mM 2-mercaptoethanol, and 10 mM EDTA, pH 7, and homogenized as previously described (2). The homogenate was subjected to centrifugation at 10,000 × *g* for 10 min and the resulting supernatant was spun at 100,000 × *g* for 1 hr. The pellet from this latter centrifugation (microsomal fraction) was washed once in the homogenizing buffer, frozen, and stored at −60°. The supernatant from the 100,000 × *g* centrifugation (cytosolic fraction) was dialyzed for 24 hr at 4° against buffer containing 20 mM potassium phosphate at pH 7.2, 0.1 mM EDTA, and 0.5 mM dithiothreitol, after which it was frozen and stored at −60°.

Assay of Cytosolic HMG CoA Synthase. The radiochemical method of Clinkenbeard *et al.* was used to measure the synthesis of [^{14}C]HMG CoA from [^{14}C]acetyl CoA (13). The standard reaction contained the following components in a final volume of 0.2 ml: 0.1 M Tris chloride, pH 8; 10 μM acetoacetyl CoA; 0.1 mM EDTA; 20 mM $MgCl_2$; 5–60 μg of cytosolic protein, and 0.6 mM [*1*-^{14}C]acetyl CoA (12,000 cpm/nmol). The reaction mixture was preincubated for 2 min at 30°, after which the reaction was started by addition of the [*1*-^{14}C]acetyl CoA. Aliquots (50 μl) were removed at intervals of 0, 5, and 10 min and added to a scintillation counting vial that contained 0.3 ml of 6 M HCl. Each counting vial was then heated in an oven at 95° for 2 hr and the nonvolatile radioactivity was counted to determine the amount of [^{14}C]HMG CoA formed. The value for nonvolatile ^{14}C radioactivity present at 0 time was subtracted from the values at 5 and 10 min.

To confirm the identity of the reaction product, the nonvolatile ^{14}C radioactivity was subjected to ascending paper chromatography using butanol/acetic acid/water (5:2:3, vol/vol) as solvent. Authentic [^{14}C]HMG CoA was also heated with 0.3 ml of 6 M HCl at 95° for 2 hr and subjected to chromatography simultaneously with the ^{14}C-labeled reaction product. In both samples, two radioactive peaks were observed: a major peak with an R_F of 0.8, which corresponded to HMG, and a minor peak with an R_F of 0.6, whose identity was not determined. The relative proportions of the minor peak (35%) and the major peak (65%) were similar for the heated ^{14}C-labeled reaction product and the heated authentic [^{14}C]HMG CoA.

The identity of the ^{14}C-labeled reaction product was further confirmed by the demonstration that it could be converted to [^{14}C]mevalonate in the presence of NADPH and microsomal HMG CoA reductase. For this experiment, a scaled-up HMG CoA synthase reaction was conducted, and the reaction was stopped by heating for 15 min at 50° to inactivate the HMG CoA synthase. One aliquot of this mixture was added to 6 M HCl and heated to determine the amount of nonvolatile ^{14}C radioactivity. Another aliquot was added to a solution containing the reaction components for the HMG CoA reductase assay supplemented with adrenal microsomes from a 4-APP-treated rat. (The microsomes had been subjected to prior heating at 50° for 30 min to inactivate traces of adherent HMG CoA synthase.) The mixture was incubated at 37° and aliquots were removed at different times to determine the amount of [^{14}C]mevalonate formed (2). Under these conditions, the maximal amount of [^{14}C]mevalonate formed was equal to 80–90% of the value expected from the determination of nonvolatile ^{14}C radioactivity.

Assay of Microsomal HMG CoA Reductase. The conversion of [^{14}C]HMG CoA to [^{14}C]mevalonate in adrenal microsomes was measured using thin-layer chromatography as previously described (2).

Assay of Cytosolic Acetoacetyl CoA Thiolase (acetyl-CoA acetyltransferase; acetyl-CoA:acetyl-CoA *C*-acetyltransferase, EC 2.3.1.9). CoA-dependent cleavage of acetoacetyl CoA was determined spectrophotometrically by measuring the rate of decrease in absorbance at 300 nm (18). The standard reaction contained the following components in a final volume of 1 ml: 0.1 M Tris chloride, at pH 8; 0.1 mM EDTA; 0.12 mM acetoacetyl CoA; 90 μM CoA; and 10–60 μg of cytosolic protein. The reaction mixture was preincubated for 3 min at 25°, after which the reaction was started by addition of CoA. The amount of acetoacetyl CoA cleaved was linear with time up to 10 min and proportional to the concentration of cytosolic protein up to at least 60 μg. The concentration of CoA giving half-maximal enzyme activity was 38 μM.

Assay of Cytosolic Mevalonate Kinase (ATP:mevalonate 5-phosphotransferase, EC 2.7.1.36). The rate of formation of 5-phospho-[^{14}C]mevalonate from [^{14}C]mevalonate was measured by a modification of the method of Popjak (19). The standard reaction contained the following components in a final volume of 0.2 ml: 0.1 M Tris chloride at pH 7.5; 5 mM ATP; 7.5 mM $MgCl_2$; 1 mM dithiothreitol; 10–60 μg of cytosolic protein; and 0.2 mM DL-[*2*-^{14}C]mevalonic acid (8000 cpm/nmol). The reaction mixture was incubated at 30° and aliquots (25 μl) were removed at 0, 5, and 10 min and spotted directly on plastic-backed silica gel G thin-layer sheets that were developed with 1-propanol/concentrated ammonia/1% EDTA (6:3:1, vol/vol). The 5-phospho-[^{14}C]mevalonate formed (R_F = 0.13) was well separated from the starting [^{14}C]mevalonate (R_F = 0.6). The reaction was linear with time up to 10 min and proportional to the protein concentration up to at least 60 μg.

Other Assays. The protein content of cytosolic and microsomal fractions was determined by a modification of the method of Lowry *et al.* (20), with bovine serum albumin as a standard. The total cholesterol concentration in plasma was assayed by the cholesterol oxidase method (Boehringer-Mannheim Cholesterol Test Combination). The concentration

Table 1. Effect of 4-APP on enzymes of the cholesterol biosynthetic pathway in rat adrenal gland

Treatment of rats	Cytosolic acetoacetyl CoA thiolase (nmol·min^{-1})		Cytosolic HMG CoA synthase (nmol·min^{-1})		Microsomal HMG CoA reductase (nmol·min^{-1})		Cytosolic mevalonate kinase (nmol·min^{-1})	
	Per mg protein	Per gland	Per mg protein	Per gland	Per mg protein	Per gland	Per mg protein	Per gland
(*a*) Control	310	270	0.29	0.25	0.12	0.017	3.3	2.9
(*b*) 4-APP	240	420	4.1	7.2	6.1	1.7	2.3	4.0
(*b*)/(*a*)	0.8	1.6	14	29	51	100	0.7	1.4

Rats were treated as indicated, adrenal glands from four rats were pooled, cytosolic and microsomal fractions were prepared, and enzyme activities were determined as described under *Materials and Methods*. Each value represents the average of duplicate assays. The total recovery of protein in the cytosolic and microsomal fractions was about 2-fold higher in the 4-APP-treated rats as compared to the control rats.

of corticosterone in rat plasma was measured by direct radioimmunoassay (21).

RESULTS

To establish optimal conditions for the assay for HMG CoA synthase activity, cytosolic extracts were prepared from the adrenal glands of rats that had been treated for 3 days with 4-APP. When these extracts were assayed immediately after preparation, the rate of [^{14}C]HMG CoA formation was linear for less than 5 min and proportional to the amount of extract only when the total amount of protein in the assay was less than 30 μg. However, when enzyme activity was assayed after the extracts had been dialyzed against phosphate buffer for 24 hr, the reaction was linear with time up to 15 min (Fig. 2*A*) and with amounts of enzyme up to at least 60 μg of protein (Fig. 2*B*). A similar effect of dialysis has been observed by Clinkenbeard *et al.* for HMG CoA synthase in crude extracts of chicken liver and this has been attributed to the inactivation of contaminating HMG CoA lyase during the dialysis (12). In preliminary experiments, when care was taken to ensure that only initial reaction rates were measured, it was possible to show that the actual amount of adrenal HMG CoA synthase activity was not changed by the dialysis, irrespective of whether the extracts were prepared from control or 4-APP-treated rats. In all subsequent experiments, therefore, only dialyzed extracts were used.

With regard to [^{14}C]acetyl CoA, saturation of adrenal HMG CoA synthase activity was observed at a concentration of about 0.5 mM (Fig. 2*C*). Although the formation of [^{14}C]HMG CoA from [^{14}C]acetyl CoA by purified HMG CoA synthase requires acetoacetyl CoA (12), in the crude adrenal extracts no dependence on exogenous acetoacetyl CoA could be demonstrated (Fig. 2*C*). This is presumably because the large amounts of acetoacetyl CoA thiolase in these adrenal extracts (see below) are able to form sufficient acetoacetyl CoA from the acetyl CoA added to the reaction. Attempts were made to demonstrate acetoacetyl CoA dependence by selectively inactivating acetoacetyl CoA thiolase through heat inactivation or by iodoacetamide treatment of the extracts. However, in both cases, HMG CoA synthase appeared to be more sensitive to the treatments than was the thiolase.

The activity of HMG CoA synthase was not affected by omission of Mg^{2+} from the assay. More than 90% of the total HMG CoA synthase activity in the adrenal gland of 4-APP-

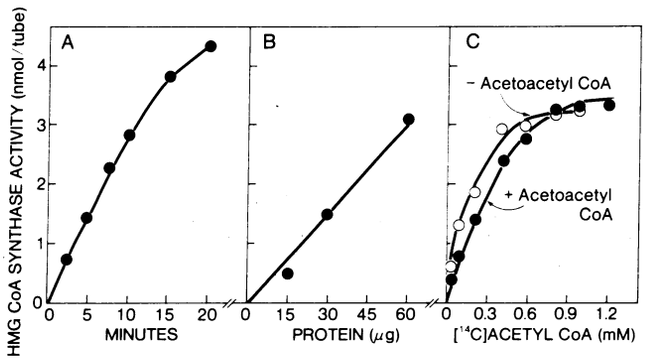

FIG. 2. Determination of optimal conditions for assay of HMG CoA synthase activity in rat adrenal cytosol. Rats were treated with 4-APP and adrenal cytosol was prepared as described under *Materials and Methods*. Aliquots of cytosol (60 μg of protein) were incubated for 10 min at 30° under standard assay conditions as described under *Materials and Methods* with the following exceptions: (*A*) time of incubation was varied; (*B*) amount of cytosolic protein was varied; (*C*) amount of [^{14}C]acetyl CoA was varied in the presence (●) or absence (○) of 50 μM acetoacetyl CoA. The amount of [^{14}C]acetyl CoA incorporated into [^{14}C]HMG CoA was quantified as described under *Materials and Methods*. Each value represents the average of duplicate assays.

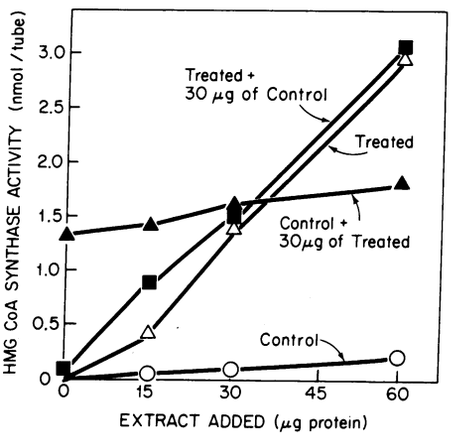

FIG. 3. Activity of HMG CoA synthase in mixtures of cytosolic fractions from the adrenal glands of control and 4-APP-treated rats. Cytosolic fractions were prepared from pooled adrenal glands obtained from four control rats and from four rats that had been treated with 4-APP as described under *Materials and Methods*. Various amounts of cytosolic protein from control rats (○) or 4-APP-treated rats (△) were incubated under standard assay conditions as described under *Materials and Methods*. In addition, a constant amount of cytosolic protein (30 μg) from either control rats (■) or 4-APP-treated rats (▲) was assayed with various amounts of cytosolic protein from the oppositely treated group. The amount of [^{14}C]acetyl CoA incorporated into [^{14}C]HMG CoA was quantified as described under *Materials and Methods*. Each value represents the average of duplicate assays.

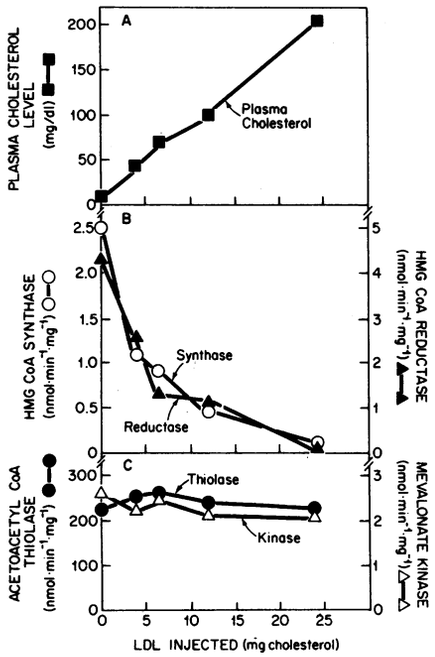

FIG. 4. Effect of various doses of human LDL on the plasma cholesterol level (*A*), adrenal HMG CoA synthase and reductase activities (*B*), and adrenal acetoacetyl CoA thiolase and mevalonate kinase activities (*C*) in rats previously treated with 4-APP. Five groups of rats (three rats per group) were treated with 4-APP for 3 days. Twelve hours after the last dose of 4-APP, four groups of rats received the indicated amount of human LDL intravenously as a bolus. The control group received an intravenous injection of 0.15 M NaCl. All the animals were killed 12 hr later (on the fourth day). Blood was collected, and the adrenal glands from the three rats in each group were excised and pooled for homogenization. All measurements were made as described under *Materials and Methods*. All assays were performed in duplicate.

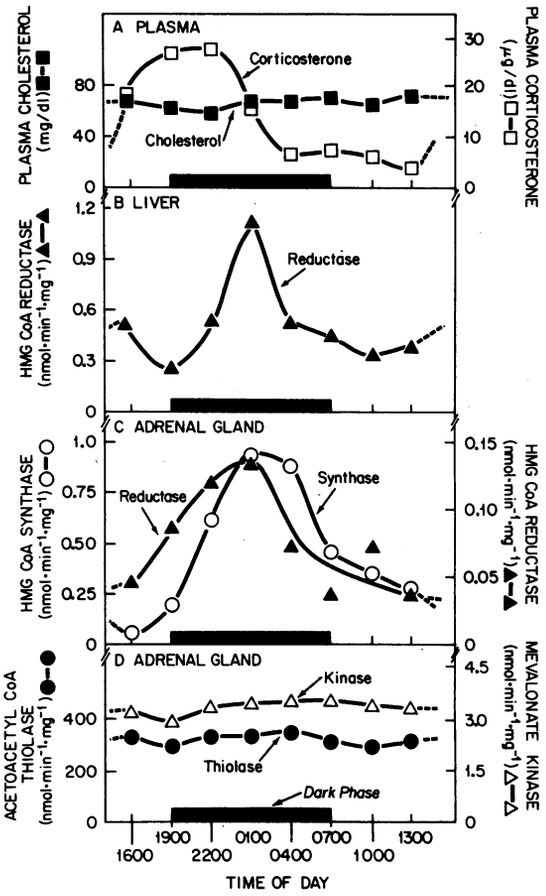

FIG. 5. Diurnal variations in the level of plasma corticosterone (*A*) and in the activities of hepatic HMG CoA reductase (*B*) and adrenal HMG CoA synthase and reductase (*C*) in the rat. Twenty-four rats were housed in cages in which lighting was supplied between the hours of 0700 and 1900 daily. After 3 weeks of such treatment, groups of three animals were killed at intervals of 3 hr as indicated. Blood was collected, and the adrenal glands from the three animals were excised and pooled for homogenization. One gram portions of liver were also removed from each animal, pooled, and homogenized, and microsomal HMG CoA reductase was assayed as described previously (24). Plasma cholesterol and corticosterone and adrenal enzyme activities were measured as described under *Materials and Methods*. All assays were performed in duplicate.

treated rats was found in the cytosolic fraction. No synthase activity could be detected in the mitochondrial fraction (10,000 × *g* pellet) even after a 24 hr dialysis of detergent-disrupted mitochondria (12). In the same extracts, more than 90% of the marker enzyme glutamate dehydrogenase was found in the mitochondrial fraction.

When adrenal extracts were prepared from control rats, HMG CoA synthase activity was barely detectable (Fig. 3). On the other hand, when rats had been treated for 3 days with 4-APP, a condition known to cause a marked decrease in plasma and adrenal cholesterol levels and a marked increase in adrenal cholesterol synthesis (1, 2), the activity of HMG CoA synthase was increased by more than 15-fold. The data in Fig. 3 show that mixing of the two types of cytosol preparations gave only additive activities. Hence, the difference in the enzyme activity between the 4-APP-treated and control rats was not due to the presence of enzyme inhibitors or activators.

Table 1 compares the activities of the first four enzymes in the cholesterol biosynthetic pathway in adrenal extracts from control and 4-APP-treated rats. The activities of cytosolic acetoacetyl CoA thiolase and mevalonate kinase were relatively high in the control animals and were not appreciably affected by the 4-APP treatment. In contrast, both cytosolic HMG CoA synthase and microsomal HMG CoA reductase activities were low in the adrenal gland of untreated rats and both enzymes showed large increases in activity after 4-APP treatment.

The coordinate regulation of adrenal HMG CoA synthase and reductase was also evident when human LDL was infused into 4-APP-treated rats, a treatment previously demonstrated to restore the content of adrenal cholesteryl esters and to suppress adrenal HMG CoA reductase activity and cholesterol synthesis (1, 2). The data in Fig. 4 show that with increasing doses of LDL the relative degrees of suppression of HMG CoA synthase and HMG CoA reductase were remarkably similar. On the other hand, the activities of acetoacetyl CoA thiolase and mevalonate kinase were unaffected by the LDL infusion.

Because of the known circadian rhythm in corticosterone secretion by the rat adrenal gland (22), we sought to determine whether a diurnal variation could be demonstrated in adrenal HMG CoA synthase and reductase activities. Accordingly, rats were exposed to a repetitive light–dark cycle for 3 weeks, and then groups of animals were killed at 3 hr intervals throughout a 24 hr cycle (Fig. 5). Both adrenal HMG CoA synthase and reductase activities showed a diurnal rhythm with peak activities at about the mid-point of the dark cycle (Fig. 5*C*). The rise in reductase appeared to slightly precede the rise in synthase activity. The peak in adrenal HMG CoA reductase activity coincided with the peak in liver HMG CoA reductase activity (Fig. 5*B*). Interestingly, in the adrenal gland the peaks in syn-

thase and reductase activities both occurred after the peak in plasma corticosterone levels had been reached (Fig. 5*A*). No significant diurnal variation was observed in the activities of acetoacetyl CoA thiolase and mevalonate kinase in the adrenal gland (Fig. 5*D*).

DISCUSSION

The striking increase previously observed in HMG CoA reductase activity in the adrenal gland of the 4-APP-treated rat (2) raised the question as to the source of the HMG CoA substrate for this enzyme. The present data demonstrate that this HMG CoA is derived through a parallel enhancement in the activity of cytosolic HMG CoA synthase. The substrate for the synthase in turn is provided through the action of acetoacetyl CoA thiolase, an enzyme that appears to be present in vast excess and that does not vary under the conditions studied. By the same token, mevalonate kinase, the enzyme that follows HMG CoA reductase in the cholesterol biosynthetic pathway, is also present in excess under all tested conditions and likewise does not show apparent regulation in the rat adrenal gland.

The coordinate control of cytosolic HMG CoA synthase and microsomal HMG CoA reductase is somehow linked to a specific intracellular pool of cholesterol. Thus, when the plasma cholesterol level is lowered with 4-APP, the content of adrenal cholesteryl esters drops (2) and there is a subsequent increase in the activity of both HMG CoA synthase and reductase. Moreover, both enzyme activities are suppressed in parallel when cholesteryl ester levels are restored in the adrenal gland of 4-APP-treated rats by the intravenous administration of plasma lipoproteins. It is not yet known whether the cholesterol-mediated regulation of these two adrenal enzymes involves some coordinate change in the rates of enzyme synthesis or perhaps some type of coupled posttranslational enzyme modification. The mixing experiments in Fig. 3 relating to HMG CoA synthase, as well as similar experiments performed with HMG CoA reductase (2), make unlikely the possibility that the coordinate control is simply due to enzyme inhibitors or activators.

The observation in the current studies of a diurnal variation in both HMG CoA synthase and reductase activities in adrenal gland provides additional evidence that these two enzymes are coordinately controlled. The peak activity of both enzymes occurred after the peak in plasma corticosterone levels. These data are consistent with the generally accepted view that the action of adrenocorticotropic hormone causes a cleavage of stored adrenal cholesteryl esters with rapid conversion of the liberated cholesterol to corticosterone (23). Thus, the rise in activities of HMG CoA synthase and reductase that follows the adrenocorticotropic hormone-mediated burst of corticosterone secretion during the early dark phase of the diurnal cycle may represent a mechanism by which the adrenal gland replaces the cellular cholesterol that has been consumed for hormone synthesis. The maximal levels of HMG CoA synthase and reductase activities achieved during the diurnal cycle are much lower than the levels observed when the level of plasma cholesterol is reduced by 4-APP treatment. This suggests that during the dark phase the adrenal gland not only synthesizes cholesterol but also takes up cholesterol from plasma lipoproteins, an action that keeps the activities of HMG CoA synthase and reductase partially suppressed. Consistent with this hypothesis was the observation that when 4-APP-treated rats were killed at 2100 hr, adrenal HMG CoA reductase activity was 4.6 nmol·min^{-1}·mg^{-1} as compared with 0.25 nmol·min^{-1}·mg^{-1} in control animals killed at the same time. In the same experiment, the activities of adrenal HMG CoA reductase in 4-APP-treated and control rats killed at 0800 hr were 3.9 and 0.03 nmol·min^{-1}·mg^{-1}, respectively (data not shown).

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