REGULATION OF STEROL SYNTHESIS IN ADRENAL GLAND OF THE RAT BY BOTH HIGH AND LOW DENSITY HUMAN PLASMA LIPOPROTEINS

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SUMMARY: In the intact rat the rate of sterol synthesis in the adrenal gland is normally suppressed to very low levels. However, following inhibition of hepatic lipoprotein production by administration of 4-aminopyrazolopyrimidine to the animals the mean serum cholesterol level decreased from 53 to 6 $mg \cdot dl^{-1}$, and, coincident with this change, the rate of adrenal sterol synthesis increased 51 fold. The infusion of purified high and low density lipoprotein fractions from the plasma of man inhibited sterol synthesis in such derepressed adrenal glands in proportion to the amount of cholesterol infused into the animal in each of these fractions. At any given plasma cholesterol level, however, the degree of inhibition was significantly greater following the infusion of HDL than after administration of LDL.

Introduction

The rate of cholesterol synthesis in liver and intestine is subject to control by a number of different physiological mechanisms such as light cycling, stress, fasting and the size of the circulating bile acid pool (1-3). Sterol synthesis in most of the other tissues that have been examined occurs at relatively low rates, and these rates are usually not altered by the same manipulations that influence hepatic and intestinal cholesterogenesis (4-6). Recently, however, it has been found that administration of the adenine analogue 4-aminopyrazolopyrimidine (APP)* apparently inhibits the release of lipoproteins from the liver (7-9) and causes a marked decrease in the level of circulating triglycerides and cholesterol (8). Coincident with this decrease in circulating lipid levels it has also been found that the rate of sterol synthesis increases in at least seven extrahepatic tissues (10), and

*ABBREVIATIONS: APP, 4-aminopyrazolopyrimidine; HDL, high density lipoproteins; LDL, low density lipoproteins; DPS, digitonin precipitable sterols; HMG CoA reductase, β-hydroxy-β-methylglutaryl Co-Enzyme A reductase (E.C. 1.1.1.34); ACTH, adrenocorticotropic hormone.

in the kidney and lung this increase is associated with enhanced activity of the rate limiting enzyme in the synthetic pathway, i.e., HMG CoA reductase (11). Of the various tissues examined, the greatest increase in synthetic activity, assayed by measuring the incorporation rate of [^{3}H]water into digitonin precipitable sterols (DPS), is seen in adrenal gland (10). The present study was undertaken, therefore, to examine the critical question of whether sterol synthesis in adrenal tissue is under direct feed-back regulation by one or more of the serum lipoprotein fractions.

Methods and Materials

LDL and HDL fractions were obtained from human plasma. The LDL fraction was obtained from a density cut between 1.006 and 1.050 while the HDL fraction utilized was in the density range of 1.080 to 1.215. After isolation, each lipoprotein fraction was dialyzed against 154 mM NaCl solution prior to administration to the test animals. Each of these fractions ran as a single band with the appropriate Rf value on agarose gel electrophoresis (12), and even after heavily loading the gels there was no detectable HDL in the LDL fractions or LDL contamination in the HDL fractions, as judged by this technique.

Female, Sprague-Dawley derived rats (Charles River Breeding Labs, Inc., Wilmington, Mass.) weighing 190-220 g were housed in an animal room with alternating 12 hr periods of light (1500-0300 hr) and dark (0300-1500 hr) and allowed free access to water and Formulab chow (Ralston Purina Co., St. Louis, Mo.). At the mid-dark point of the light cycle (0900 hr) a group of animals was placed in individual metabolic cages and injected intraperitoneally with an appropriate volume of a buffer solution containing Na phosphate (25 mM), APP (2 mg·ml^{-1}) and NaCl (154 mM) at a pH of 4.0 to give each animal a dose of APP equal to 2.0 mg per 100 g body weight. A control group of animals was similarly injected with an equal volume of the buffer solution alone. These injections were repeated each day at 0900 hr throughout the 72 hr experiments. During the treatment period all animals were deprived of food but allowed free access to 77 mM NaCl solution. After 36 hr of APP administration the animals were removed from the individual cages, catheters (PE 10) were placed in their tail veins under ether anesthesia, and the rats were then placed in cylindrical restraining cages with access to water. While in the restraining cages the rats were infused through the catheters with a solution containing NaCl (67 mM), K_2HPO_4 (8.3 mM), KCl (16.7 mM), glucose (370 mM) and regular insulin (10u·l^{-1}) at a rate of 1.5 ml·hr^{-1} for the final 36 hr of the study. In particular experiments HDL and LDL were added to this infusate.

At the completion of the 72 hr experimental period the animals were killed by decapitation at 0900 hr, the mid-dark point of the light cycle. The adrenal glands were removed, chilled, and cut by hand into slices approximately 1 mm thick. Adrenal glands from one animal were combined and placed in a 25 ml center-well flask containing 5 ml of oxygenated Krebs' bicarbonate buffer (pH 7.4), 40 μmol of Na acetate and 4 μC of [1-^{14}C]acetate. These flasks were incubated for 90 min at 37° C in a metabolic shaker set at 120 oscillations per min. At the completion of the incubation period radiolabelled CO_2 was collected as previously described (13). The contents of each flask were saponified and the sterols were extracted and precipitated as the

digitonide (13). The sterol synthetic rate was calculated from the nmol of acetate incorporated into digitonin precipitable sterols per g wet weight of tissue per hr ($nmol \cdot g^{-1} \cdot hr^{-1}$). Serum cholesterol determinations were carried out by previously described methods (13).

Results and Discussion

In order to circumvent the precursor pool dilution problem encountered when utilizing radiolabelled acetate (14), we used [^{3}H]water as the radiolabelled precursor in our initial survey of sterol synthetic rates in extrahepatic tissues of APP treated rats (10). However, in the current studies [1-^{14}C]acetate was used as the precursor since the large amounts of [^{3}H]water

TABLE 1 Inhibition of Sterol Synthesis in Adrenal Gland By Human Low and High Density Lipoproteins

Experimental Group	Total Cholesterol Infused	Final Plasma Cholesterol Concentration	[1-^{14}C]Acetate Incorporation Into	
			DPS	CO_2
	(mg)	($mg \cdot dl^{-1}$)	($nmol \cdot g^{-1} \cdot hr^{-1}$)	($\mu mol \cdot g^{-1} \cdot hr^{-1}$)
A. Buffer Control	0	52.9 ±7.8	1.4 ±0.4	4.6 ±1.2
B. APP Control	0	6.4 ±1.8	71.2 ±33.2	2.1 ±0.3
C. HDL Infused	16.1 ±1.2	56.2 ±8.7	13.1 ±3.3	5.0 ±1.5
D. LDL Infused	16.5 ±1.0	56.9 ±13.1	24.5 ±7.9	2.2 ±1.2

As outlined in the method section, the animals in Group A were injected intraperitoneally with only buffer solution while those in Groups B, C and D were injected with APP through the 72 hr experiment. During the final 36 hr each animal was also infused intravenously with a solution containing glucose, insulin and electrolytes alone (Groups A and B) or this solution with added HDL (Group C) or LDL (Group D). At the termination of the experiment the animals were killed, and the adrenal glands were assayed for sterol synthetic activity. The first column gives the amount of cholesterol carried in either HDL or LDL that was infused into each group of experimental animals while the second column shows the plasma cholesterol levels present at the time the animals were killed. The third and fourth columns, respectively, show the rates of [1-^{14}C]acetate incorporation into digitonin precipitable sterols and into CO_2 by the adrenal slices. Each value represents the mean ±1 S.E. for three animals in each group.

needed for the assays makes it impractical for routine use. As seen in Table 1 our previous results using [^{3}H]water were confirmed using [1-^{14}C] acetate. After 72 hr of APP treatment the plasma cholesterol concentration was 6.4 mg·dl^{-1} (line B) compared with 52.9 mg·dl^{-1} in the buffer injected control group (line A). Coincident with this fall in plasma cholesterol levels, the rate of sterol synthesis increased from 1.4 nmol·g^{-1}·hr^{-1} to 71.2 nmol·g^{-1}·hr^{-1} (group B). The data presented in the fourth column demonstrate that this marked change in sterol production occurred under circumstances where there was no significant change in the rate of incorporation of [1-^{14}C]acetate into CO_2 .

To test the hypothesis that sterol synthesis in the adrenal gland might normally be suppressed by one of the circulating plasma lipoprotein fractions, human HDL and LDL fractions were infused at a constant rate for 36 hr into rats treated with APP (lines C and D of Table 1). The mean plasma cholesterol level found when the animals were killed was very similar to the mean cholesterol level of the control group injected with buffer. Under these circumstances both HDL and LDL significantly suppressed the rate of [1-^{14}C]acetate incorporation into DPS, although HDL caused greater inhibition than an equivalent amount of cholesterol carried in LDL.

This apparent difference in inhibitory capacity was further explored by infusing varying amounts of human HDL and LDL into a series of rats and plotting the rate of DPS synthesis in the adrenal gland against the final plasma cholesterol concentration achieved (Fig 1.). From these curves it is apparent that HDL was significantly more effective than LDL in suppressing adrenal DPS synthesis. Indeed, following HDL infusion 50% suppression was achieved at a plasma cholesterol concentration of about 20 mg·dl^{-1} and nearly complete suppression occurred at a plasma cholesterol concentration in the range of 60 mg·dl^{-1}. In contrast, infusions of LDL caused 50% inhibition at plasma cholesterol concentrations in the range of 40-50 mg·dl^{-1}, while suppression to levels seen in the buffer-injected control group was achieved only at

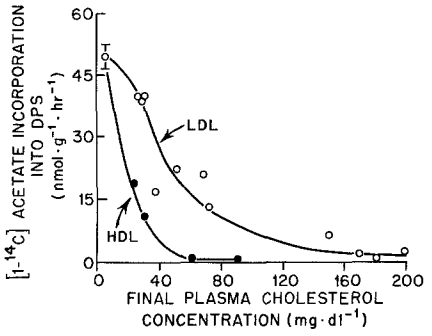

Fig.1 Relationship of the rate of sterol synthesis in the adrenal gland to the final plasma cholesterol concentration. As described in the method section, animals injected with APP were infused intravenously for 36 hr with solutions containing varying amounts of cholesterol carried in either human HDL or LDL. At the end of the experiments the animals were killed, and the rates of sterol synthesis were determined in adrenal slices. In this diagram, the plasma cholesterol level found in each animal at the time it was killed is plotted against the rate of adrenal sterol synthesis found in the same animal. Each point represents the result obtained in a single rat.

plasma cholesterol concentrations of 160 to 200 $mg \cdot dl^{-1}$.

Taken together these data suggest the possibility that in the intact rat the synthesis of digitonin precipitable sterols normally is suppressed to very low levels by circulating HDL. LDL also may suppress sterol synthesis; however, in view of the fact that rat plasma contains only low levels of LDL it seems likely that HDL is the more important inhibitor under physiological circumstances. This finding is consistent with the preliminary report of Gwynne, et al, that cholesterol uptake by the adrenal gland from HDL, but not from LDL, is stimulated by the administration of ACTH (15). Presumably, under normal circumstances the adrenal gland takes up and utilizes cholesterol principally from HDL as the major precursor from which adrenal steroids are synthesized. As long as HDL cholesterol is available, cholesterogenesis in the adrenal gland is suppressed. When the circulating levels of HDL are markedly reduced, however, as occurs following administration of APP, cholesterol synthesis in the adrenal cell can increase as much as 50 fold and so supply a continuous source of cholesterol for the synthesis of adrenal hormones.

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