The Effects of Hypocholesterolemic Agents on Cholesterol Esterification *in Vitro* (35285)

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The hypocholesterolemic agents ethyl 2-(*p*-chlorophenoxy)-2-methylpropionate (Atromid-S, or CPIB[1] and sodium D-3,5,3′,5′-tetraiodothyronine (Choloxin, or D-thyroxine)[2] have recently come into use in treating hyperlipoproteinemia. Atromid-S is reported to decrease the endogenous synthesis of cholesterol from acetate. Synthesis from mevalonate was not decreased (1). The mechanism by which Choloxin effects a reduction of serum cholesterol is still incompletely understood. At the present time, it is believed that Choloxin (2) stimulates the liver to increase the oxidative catabolism of serum cholesterol as well as the excretion of cholesterol into the bile and feces.

Recently, two other hypocholesterolemic agents have come into experimental clinical use. They are SU-13437,[3] Melipan, 2-methyl-2-[*p*-(*p*-chlorophenyl) phenoxy] propionate, and SaH-2348,[4] 1-methyl-4-piperidyl bis(*p*-chlorophenoxyacetate). SU-13437 is believed to act on cholesterol synthesis by inhibiting acetyl coenzyme A carboxylase (3). SaH-2348 (4) does not affect hepatic cholesterol synthesis but rather increases the amount of bile acids excreted.

Cholesterol esters comprise the major part of total blood cholesterol in the human and rat. The ester form of cholesterol may represent a transport or sequestered phase, being part of the lipoprotein complex. Hypocholesterolemic drugs have a beneficial lipid lowering effect by one of several mechanisms—reducing cholesterol biosynthesis, increasing cholesterol oxidation and excretion as bile acids, or modifying the physicochemical state of the lipoprotein. Cholesterol esterifying enzymes are present in intestinal mucosa, pancreas, liver, and plasma (5). An active microsomal cholesterol esterifying enzyme has been extensively characterized by Goodman (5). Glomset (6) identified an active plasma acyltransferase (LCAT) which transfers the fatty acid from lecithin to free cholesterol. The relative contribution of these two enzymes to plasma and tissue cholesterol esters remains unclear; however, high density lipoprotein is the preferred substrate for lecithin cholesterol acyltransferase (LCAT).

In the present investigation, it was considered appropriate to investigate the effect of various hypocholesterolemic agents on liver microsomal cholesterol ester synthesase, which we have previously shown can be modified by hormonal agents (7). This enzyme may be one of the prime determinants in the disposition of cholesterol into the free or esterified state.

Materials and Methods. Livers from male Sprague-Dawley rats, 150–200 g, were homogenized in 0.1 *M* potassium phosphate buffer, pH 7.4, containing 0.25 moles of sucrose/1000 ml of buffer. A microsomal enzyme fraction was prepared as described by Goodman *et al.* (8). One μmole of cholesterol 7α-^{3}H (5.6 μCi) dissolved in 0.1 ml of propylene glycol and 0.3 μmoles each of potassium oleate, palmitate, and linoleate were placed in a 10-ml Erlenmeyer flask. The appropriate quantities of the hypocholesterolemic agents SU-13437, Clofibrate, and SaH-2348 were added in 0.1 ml of propylene glycol–acetone (4:1; v/v). Choloxin and L-thyroxine as the sodium salts were added, dis-

[1] Ayerst Laboratories, New York, N.Y.

[2] Flint-Eaton & Company, Morton Grove, Illinois.

[3] Ciba Pharmaceutical Company, Summit, New Jersey.

[4] Sandoz Pharmaceutical Company, Hanover, New Jersey.

solved in 0.2 ml of incubation buffer. The control incubations received the identical quantities of buffer or propylene glycol–acetone without the drugs. After the addition of 1 μmole of coenzyme A, 9 mg of defatted human serum albumin, 18 μmoles of ATP and 2.5 mg of microsomal protein (determined by Lowry reaction) dissolved in 0.1 *M* potassium phosphate buffer, pH 7.4, to each flask, the incubations were performed in a Dubnoff metabolic incubator at 37° for 2 hr with air as gas phase. The final total incubation volume was 3 ml in each flask. The incubations were terminated by freezing in a Dry Ice–acetone mixture (—60°) and stored at —20° for 3 days. After the addition of 15,000 dpm (300 μg) each of cholesteryl-4-^{14}C oleate, palmitate, and linoleate as carrier, the incubations were extracted with ethyl ether. Purification and separation of the individual cholesterol-^{3}H-^{14}C esters was done by column chromatography on alumina and thin-layer chromatography on silica gel G impregnated with $AgNO_3$ as described previously (9). A constant $^{3}H/^{14}C$ ratio of the isolated cholesterol ester was accepted as a criterion for its radiochemical purity. Based on the recovery of ^{14}C tracer, the losses of ^{3}H-labeled cholesterol ester incurred during the extraction and chromatographic procedures could be calculated. The data listed in Table I are correspondingly corrected.

Results and Discussion. In repeated experiments with the microsomal preparation as the enzyme source and without drug addition, cholesteryl palmitate and oleate are synthesized at about the same rate. The relative rates of cholesterol ester formation are in agreement with the results obtained by Goodman *et al.* (8, 10). Atromid-S had a potentiating effect at higher concentrations, particularly on the formation of cholesteryl oleate and linoleate. Choloxin had a marked stimulating effect on the *in vitro* synthesis of cholesteryl oleate. The effects of Choloxin on cholesteryl palmitate and linoleate formation were less pronounced. SU-13437 stimulated the formation of all cholesterol esters. SaH-2348 increased the rate of formation of cholesteryl oleate and linoleate. Interestingly, the active thyroid hormone *l*-thyroxine did not differ appreciably from its isomer *d*-thyroxine.

TABLE I. The Effect of Hypocholesterolemic Drugs on Cholesterol Esterification by Rat Liver Microsomes.

Conc of drugs in moles (*M*)	mμmoles formed		
	Cholesteryl palmitate	Cholesteryl oleate	Cholesteryl linoleate
Control[a]	11.6 ± 1.5[b]	12.6 ± 0.9	8.3 ± 0.9
Atromid-S			
13.7×10^{-7}	12.2 ± 1.9	12.9 ± 2.0	9.7 ± 0.6
13.7×10^{-6}	10.6 ± 1.3	19.7 ± 1.7	10.8 ± 0.7
13.7×10^{-5}	36.8 ± 2.9	40.3 ± 3.8	20.3 ± 2.1
SU-13437			
3.5×10^{-7}	11.5 ± 1.3	12.7 ± 1.1	9.5 ± 0.8
3.5×10^{-6}	12.7 ± 2.1	12.9 ± 1.9	10.3 ± 1.5
3.5×10^{-5}	26.9 ± 2.8	31.1 ± 3.0	25.3 ± 3.1
SaH-2348			
1.8×10^{-7}	18.3 ± 1.4	12.6 ± 1.3	12.8 ± 0.9
1.8×10^{-6}	19.5 ± 1.9	16.7 ± 1.4	14.9 ± 1.5
1.8×10^{-5}	21.8 ± 2.2	22.0 ± 1.9	23.8 ± 2.7
Control[c]	11.2 ± 1.8	13.0 ± 1.9	8.5 ± 0.8
Choloxin			
10.5×10^{-7}	11.9 ± 0.9	12.9 ± 1.3	10.7 ± 1.0
10.5×10^{-6}	20.3 ± 1.5	29.7 ± 2.0	19.9 ± 1.5
10.5×10^{-5}	25.7 ± 1.9	36.5 ± 2.8	20.7 ± 1.6
L-Thyroxine			
10.5×10^{-7}	11.5 ± 1.3	12.7 ± 2.0	9.1 ± 0.9
10.5×10^{-6}	21.5 ± 2.1	28.5 ± 3.1	26.7 ± 2.5
10.5×10^{-5}	27.6 ± 3.0	31.4 ± 2.8	25.3 ± 2.6

[a] 0.1 ml of propylene glycol–acetone (4:1; v/v) added without drugs.

[b] Values represent the mean ± standard error of four experiments.

[c] 0.1 ml of buffer added without Choloxin or L-thyroxine.

The conversion of free cholesterol to a cholesterol ester may be of physiological significance because of the fact that free and esterified cholesterol have markedly different turnover times (5) and possibly may have differences in their ability to dissociate from the lipoprotein complex. The esters are complexed to protein within the liver compartment and then circulated as lipoproteins. Lecithin cholesterol acyltransferase acts upon free cholesterol in the plasma compartment. The deposition of lipoprotein on intima or penetration of the ester may, in part, depend on the chemical configuration of the complex. It may well be that the unsaturated esters,

because of their angular configuration, may possess a different cellular membrane gradient than the saturated esters.

Our studies suggest that hypocholesterolemic agents stimulate the formation of cholesterol esters *in vitro*. As noted above, hypocholesterolemic agents may modify cholesterol metabolism at different levels; that is, absorption, synthesis, or degradation. It is possible that the stimulation of cholesterol ester formation may be not only a pharmacological resultant of the drug but modification of compartmentalization as the free or ester form in itself may be of physiological significance.

Summary. The hypocholesterolemic agents Atromid-S and Choloxin stimulate the *in vitro* synthesis of cholesterol esters from free cholesterol by a rat liver microsomal preparation. Atromid-S enhances, in particular, the formation of cholesteryl oleate and linoleate. Choloxin has its primary effect on cholesteryl oleate. SU-13437 stimulates the formation of all cholesterol esters. SaH-2348 primarily affects the rate of formation of cholesteryl oleate and linoleate.

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