Regulation of low-density-lipoprotein metabolism in the rat

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The mechanism of regulation of plasma low-density-lipoprotein (LDL) metabolism in the rat was studied under a number of experimental conditions. LDL clearance and uptake in the liver was measured after intravenous pulse injection of [^{14}C]sucrose-labelled LDL alone or in combination with reductively methylated [^3H]sucrose-labelled LDL. Hyperthyroid rats showed a significant increase in fractional catabolic rate (FCR) and the proportion of LDL degraded in the liver, whereas the synthetic rate of LDL increased by 50%. Receptor-mediated clearance increased 2-fold. Hypothyroid rats showed a significant increase in LDL concentration. The FCR and proportion of LDL degraded in the liver were decreased significantly. Receptor-mediated clearance was also reduced. Cholesterol feeding increased chylomicron, very-low-density- and intermediate-density-lipoprotein cholesterol concentrations, but there was no change in the LDL concentration, FCR or the synthetic rate of LDL. Cholestyramine feeding did not induce changes in the kinetic parameters. These results indicate that, in the rat, the hepatic LDL-receptor pathway is under hormonal control, whereas cholesterol and cholestyramine feeding have no demonstrated effect on LDL metabolism.

INTRODUCTION

The major plasma cholesterol-transporting lipoprotein in man and some animal species is low-density lipoprotein (LDL). In the absence of a change in the rate of synthesis of LDL, accelerated catabolism or decreased removal can bring about changes in plasma LDL concentrations. The factor that determines removal of LDL from plasma is the rate at which it is taken up and degraded in various tissues. It has now been established that the liver is quantitatively the most important organ for LDL removal, with more than a third of the plasma LDL being catabolized in this tissue (Stein *et al.*, 1981; Pittman *et al.*, 1982). LDL uptake in the liver is mainly a receptor-mediated process which is known to be regulated by certain drugs (Windler *et al.*, 1980; Carew *et al.*, 1982).

Despite similarities between the rat and other species in the mode of uptake of LDL by the liver, the plasma concentration of LDL is relatively low in the rat. Unlike other species, the rat is unique in not developing atherosclerosis under certain experimental conditions. This raises the possibility that regulation of LDL metabolism in the rat is different from that in other species. To explore this, we studied the mechanism of hepatic regulation of LDL metabolism under a number of appropriate experimental conditions. Our results indicate a mode of regulation of LDL metabolism that is somewhat different from that in other species.

EXPERIMENTAL

Animals, diet and treatment

Male Wistar rats were fed a standard commercial diet consisting of (w/w) carbohydrate (70%), protein (20%),

fat (5%), fibre, minerals and vitamins (5%) (Fidelity Feed, Harden, N.S.W., Australia). They had free access to food and water and were maintained under conditions of alternate 12 h periods of light and dark. The rats were subjected to various manipulations listed below.

One group of rats (200–250 g) were rendered hyperthyroid by subcutaneous injections of L-thyroxine sodium salt (50 $\mu\text{g/day}$ per 100 g). Injections were carried out between 09:00 and 10:00 h daily for 7 days. The drug was dissolved in 0.9% NaCl/1 M-NaOH, pH 8.5. Control rats were injected with the same vehicle. A second group of rats (150–200 g) were rendered hypothyroid by addition of propylthiouracil (0.1%) to their drinking water for a period of 21 days. Controls were pair-fed to match the weights of treated animals. A third group of rats were fed for 1 week on a diet containing cholestyramine. The drug was added (4%, w/w) to the powdered chow, water was added to give a thick slurry, and pellets were prepared and dried for 2 days at room temperature. A fourth group of rats were fed for 2 weeks on a diet containing cholesterol (2%, w/w) and olive oil (10%, w/w) prepared in the same way. Control rats were fed a diet containing olive oil (10%, w/w) for the same period.

Preparation, labelling and reductive methylation of LDL, turnover studies and other assay procedures

Chylomicrons and VLDL ($\rho < 1.006 \text{ g/ml}$), IDL ($\rho 1.006\text{--}1.020 \text{ g/ml}$) and LDL ($\rho 1.020\text{--}1.050 \text{ g/ml}$) were isolated from rat plasma by ultracentrifugation (Havel *et al.*, 1955). LDL apo B was labelled with [^{14}C]sucrose as described by Pittman *et al.* (1979). The procedure has been described in detail previously (Bhattacharya *et al.*, 1984). Modification of the lysine residues of LDL by reductive methylation was performed by the method of Weisgraber *et al.* (1978). This technique enabled the

Abbreviations used: LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; [^3H]sucrose-MeLDL, reductively methylated [^3H]sucrose-labelled LDL; FCR, fractional catabolic rate; ACR, absolute catabolic rate; IVP, intravascular pool; apo B, apolipoprotein B.

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Table 1. Metabolic parameters: controls versus hyper- and hypo-thyroid rats

Rats were injected with thyroxine (10 µg/day per 100 g) (hyperthyroid) or were fed 0.05% propylthiouracil for 21 days (hypothyroid). Intravenous pulse injections of [¹⁴C]sucrose-LDL were given and blood samples were taken periodically over 12 h. All values represent means ± s.d. (*n* = 6). Statistical analyses were carried out by Student's *t* test. Abbreviation: NS, not significant.

Condition	LDL cholesterol (mg/dl)	IVP of LDL apo B (µg)	FCR (h ⁻¹)	ACR (mg/day per kg)	Dose in liver at 12 h (% of that injected)
Control	7.83 ± 1.87	521 ± 58	0.135 ± 0.015	5.62 ± 1.68	27.28 ± 3.31
Hypothyroid	19.66 ± 7.02	792 ± 87	0.115 ± 0.005	7.54 ± 1.44	18.50 ± 2.87
Significance...	<i>P</i> < 0.01	<i>P</i> < 0.001	<i>P</i> < 0.05	NS	<i>P</i> < 0.001
Control	7.77 ± 1.37	504 ± 31	0.142 ± 0.012	5.75 ± 1.05	31.32 ± 4.63
Hyperthyroid	7.60 ± 1.46	518 ± 47	0.176 ± 0.018	8.63 ± 1.17	39.00 ± 4.58
Significance...	NS	NS	<i>P</i> < 0.01	<i>P</i> < 0.001	<i>P</i> < 0.05

assessment of the percentage of LDL that was removed via receptor-mediated pathways. LDL kinetic parameters after intravenous pulse injections of labelled LDL were measured as previously described (Bhattacharya *et al.*, 1984). In addition to the animals that were injected and underwent periodic tail-vein bleeding, there were groups of rats (control and treated) that were exsanguinated through the abdominal aorta at the beginning of the experiment. LDL was isolated and the IVP of LDL apo B was measured in each case. Results indicated that the IVP of LDL apo B remained unchanged before and after the completion of tail-vein sampling. This confirmed that plasma LDL levels remained in a steady state throughout the course of the experiments. Fractional catabolic rates were calculated as described by Matthews (1957), using a two-compartment model for the disappearance of LDL from plasma. The liver accumulation of [¹⁴C]sucrose-LDL was measured after perfusing and homogenizing the liver.

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Cholesterol concentrations in various lipoprotein fractions were determined by g.l.c. with stigmasterol as internal standard, after extraction and alkaline hydrolysis. Thyroxine levels in plasma were measured by using standard radioimmunoassay techniques.

RESULTS

Effect of thyroxine treatment

Rats treated with thyroxine were hyperthyroid as judged by plasma thyroxine levels (156 ± 9 as against 37 ± 4 nmol/litre, *n* = 5, *P* < 0.001) and showed normal growth rates. There was no significant change in the LDL cholesterol concentration or the intravascular pool of LDL apo B (Table 1). The FCR of LDL apo B was significantly increased in hyperthyroid rats, accompanied by a significant increase in the proportion of ¹⁴C radioactivity appearing in the liver. The ACR showed a 50% increase. The percentage of LDL removal via receptor-mediated pathways was measured in two rats of each group. The treated rats showed a 2-fold increase compared with controls (range 75–84% as against 41–45%).

Effect of propylthiouracil treatment

Propylthiouracil treatment significantly decreased plasma thyroxine concentrations (7 ± 1 as against 59 ± 2 nmol/litre, *n* = 5, *P* < 0.001), indicating hypo-

thyroidism. The intravascular pool of LDL increased significantly (Table 1). There was a significant decrease in both the FCR of LDL and the proportion of radioactivity accumulated in the liver in the treated rats. There was a 34% increase in the ACR of LDL apo B, but this was not statistically significant. Receptor-mediated clearance of LDL apo B was significantly decreased in the treated rats (range 20–26% as against 40–46%).

Effect of cholesterol and cholestyramine feeding

Table 2 summarizes data from cholesterol- and cholestyramine-fed rats. Cholesterol feeding significantly increased chylomicron, VLDL and IDL cholesterol concentrations, but without causing significant changes in the LDL cholesterol or LDL apo B concentration. The FCR, ACR and the amount of radioactivity accumulated in the liver of the cholesterol-fed rats did not significantly differ from that observed in controls. Cholestyramine feeding was not associated with significant changes in any of the kinetic parameters.

DISCUSSION

The results presented here indicate that, in the rat, as in other species, thyroid hormones influence LDL metabolism. However, unlike in other species, increases in dietary cholesterol intake or manipulations that accelerate the conversion of cholesterol into bile acids do not have an apparent effect on LDL metabolism.

Thyroxine treatment in man has been shown to increase the FCR of LDL by increasing its catabolism via receptor-mediated pathways (Thompson *et al.*, 1981). Our results for the rat show that the increase in the FCR of LDL with thyroxine treatment is a result of increased hepatic uptake facilitated by an increased activity of LDL receptors. In other species this increased LDL clearance is accompanied by a decrease in plasma LDL concentration. In the rat, increased LDL catabolism does not lead to a decrease in plasma LDL concentration because of a concomitant increase in LDL apo B synthesis. The rats rendered hypothyroid showed some effects opposite to those of thyroxine treatment. Here we have found evidence of a decreased FCR of LDL, associated with a decrease in receptor-mediated LDL catabolism in the liver. This presumably gives rise to a decrease in the ACR of LDL which results in an increase in the intravascular pool of LDL. A new steady rate is eventually reached in

Table 2. Metabolic parameters: controls versus cholesterol-fed and cholestyramine-treated rats

'Control I' rats were fed chow containing 10% olive oil and cholesterol-fed rats were given chow containing 2% cholesterol and 10% olive oil for a period of 2 weeks. 'Control II' rats were fed standard laboratory chow. Cholestyramine-treated rats were given chow containing 4% cholestyramine for 1 week. All animals were injected with [¹⁴C]sucrose-LDL and blood samples were taken periodically over 12 h. Abbreviations: chylo, chylomicrons; NS, not significant. All values represent means ± s.d. (n = 6). Statistical analyses were carried out by Student's *t* test.

Conditions	Cholesterol (mg/dl) in:				IVP of LDL apo B (μg)	FCR (h ⁻¹)	ACR (mg/day per kg)	Dose in liver at 12 h (% of that injected)
	Chylo + VLDL	IDL	LDL	LDL				
Control I	13.12 ± 5.02	3.08 ± 0.52	7.89 ± 1.63	539 ± 63	0.131 ± 0.008	5.64 ± 1.23	27.00 ± 4.23	
Cholesterol-fed	40.53 ± 10.47	11.78 ± 3.59	8.01 ± 2.58	534 ± 51	0.130 ± 0.010	5.47 ± 1.00	24.20 ± 2.77	
Significance...	<i>P</i> < 0.001	<i>P</i> < 0.001	NS	NS	NS	NS	NS	
Control II	—	—	7.98 ± 2.02	524 ± 49	0.136 ± 0.005	5.67 ± 1.99	25.67 ± 3.39	
Cholestyramine-fed	—	—	7.86 ± 1.99	498 ± 6	0.139 ± 0.006	5.53 ± 1.37	26.00 ± 2.01	
Significance...	—	—	NS	NS	NS	NS	NS	

which the original ACR is attained, but this can be achieved only through an increase in the proportion of LDL catabolized via a non-receptor pathway.

We have previously shown (Bhattacharya *et al.*, 1984) that, when rats are treated with oestradiol, plasma LDL concentrations decrease owing to increased clearance of LDL via the hepatic receptor-mediated pathway. In conjunction with the present results, we conclude that hormones regulate plasma LDL concentration in the rat mainly through changes in hepatic LDL receptor activity and to a lesser extent through changes in LDL synthesis.

Cholesterol-feeding experiments in rabbits (Kovanen *et al.*, 1981) and dogs (Hui *et al.*, 1981) have been shown to trigger a series of events. The liver cholesterol concentration increases, LDL receptors are suppressed, LDL clearance rate is decreased and the plasma LDL concentration rises. In the rat, cholesterol feeding also leads to accumulation of cholesterol in the liver, suppression of 3-hydroxy-3-methylglutaryl-CoA reductase (Balasubramaniam *et al.*, 1978) and an increase in the cholesterol concentrations in chylomicron, VLDL and IDL fractions (Table 2). However, neither the hepatic clearance of LDL nor the plasma LDL concentration is altered. Therefore the classical regulation of LDL receptors by cholesterol feeding does not seem to operate in the rat. It is possible that dietary cholesterol influences cholesterol metabolism without affecting LDL-receptor pathways. The ingested cholesterol is carried as chylomicron remnants which are rapidly cleared by the liver (Wade *et al.*, 1984), thus increasing liver cholesterol concentration and decreasing cholesterol synthesis. The accumulated cholesterol is then either transported from the liver as VLDL and IDL or is converted into biliary steroids and excreted in the bile. The observation that the plasma LDL concentration does not increase, despite an increase in plasma VLDL or IDL, supports the previous findings of Fidge & Poulis (1978) that, in the rat, very little is derived from the VLDL-IDL pathway and the major portion of it is derived independently of this pathway.

Cholestyramine treatment has been shown to increase the receptor-mediated clearance of LDL, thereby decreasing the plasma LDL concentration in man and the rabbit (Shepherd *et al.*, 1979; Slater *et al.*, 1980). Since cholestyramine leads to increased excretion of bile acids, it can be inferred that increased hepatic clearance of LDL is the mechanism by which cholestyramine lowers plasma LDL concentration in these species. In the rat, as in other species, cholestyramine increases cholesterol synthesis and bile-acid excretion (Boyd *et al.*, 1969). However, neither the plasma LDL clearance rate nor hepatic LDL uptake is altered. Cholestyramine treatment does not stimulate LDL receptor activity in the rat, but causes a local effect in the liver whereby increased bile-acid excretion is balanced by increased synthesis of cholesterol.

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