Endotoxin suppresses rat hepatic low-density lipoprotein receptor expression

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Endotoxin induces hyperlipidaemia in experimental animals. In the current study, we investigated whether endotoxin alters hepatic low-density lipoprotein (LDL) receptor expression in rats. Endotoxin treatment suppressed hepatic LDL receptor expression in a dose- and time-dependent manner. Eighteen hours after intraperitoneal injection of increasing amounts of endotoxin, LDL receptor and its mRNA levels were determined by ligand blot and solution hybridization respectively. LDL receptor expression was inhibited by about 70% at a dose of 500 µg/100 g body weight. However, LDL receptor mRNA levels were markedly increased in all endotoxin-treated groups at this time point (by 83–136%; $P < 0.001$). Time-course experiments showed that LDL receptor expression was already reduced by 48% 4 h after endotoxin injection and was maximally reduced

(by 63–65%) between 8 and 18 h. Changes in hepatic LDL receptor mRNA showed a different pattern. By 4 h after endotoxin injection, LDL receptor mRNA had decreased by 78% ($P < 0.001$). However, by 8 h after endotoxin injection, LDL receptor mRNA had returned to levels similar to controls, and 18 and 24 h after endotoxin injection, they were increased by about 60% ($P < 0.05$). Separation of plasma lipoproteins by FPLC demonstrated that endotoxin-induced changes in plasma triacylglycerols and cholesterol were due to accumulation of plasma apolipoprotein B-containing lipoproteins among very-low-density lipoprotein, intermediate-density lipoprotein and LDL. It is concluded that endotoxin suppresses hepatic LDL receptor expression *in vivo* in rats.

INTRODUCTION

Administration of endotoxin results in profound effects on the metabolism of plasma lipoproteins. Thus, hyperlipidaemia occurs in endotoxin-treated animals ([1–17], reviewed in [18,19]) and during infections with Gram-negative bacteria, parasites, or viruses [20–28]. Plasma triacylglycerols are increased in endotoxin-treated animals. Plasma cholesterol is increased within 24 h of administration of endotoxin into rabbits [1,2], mice [11], rats [7,12] and hamsters [17]. However, plasma cholesterol is decreased 48 h after endotoxin injection in non-human primates [13–15]. After endotoxin administration, low-density lipoprotein (LDL)-cholesterol is increased [5,17], whereas high-density lipoprotein (HDL)-cholesterol is reduced [5,12–15,17]. Thus, endotoxin-induced hyperlipidaemia results from accumulation of lipids within very-low-density lipoprotein (VLDL) and LDL [18]. Inhibition of lipoprotein lipase (LPL) by endotoxin is one important mechanism for endotoxin-induced accumulation of VLDL and hypertriacylglycerolaemia.

How endotoxin causes LDL accumulation is largely unknown. LDL receptors in the liver play an important role in the clearance of plasma lipoproteins such as LDL and intermediate-density lipoprotein (IDL). In the current study, we addressed the question of whether endotoxin alters hepatic LDL receptor expression in rats. It was demonstrated that administration of endotoxin reduces hepatic LDL receptor expression and induces accumulation of plasma apolipoprotein B (apo-B)-containing lipoproteins (VLDL, IDL and LDL).

MATERIALS AND METHODS

Materials

Endotoxin from *Escherichia coli* O55B5 was purchased from

Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents and chemicals were from sources described previously [29,30].

Animals and experimental procedure

Male Sprague–Dawley rats (about 250 g) were maintained under standardized conditions with free access to chow and water. The light cycle hours were between 06:00 h to 18:00 h. Animals were allowed to adapt to the environment for 1 week before starting the experiments. Experiments were started at 16:00 h by withdrawing food supplies from the animals. Rats were then injected with endotoxin intraperitoneally at the indicated dose and time using saline (0.9% NaCl) as vehicle; control rats received saline. Each group consisted of six animals. At 16:00 h on the following day, animals were anaesthetized with ether, and blood was taken into EDTA-containing tubes (Vacutainer; Becton Dickinson, France) by puncture of the abdominal aorta. Animals were killed by cervical dislocation and the livers were removed and immediately frozen in liquid nitrogen and later stored at -70°C . All protocols were approved by the institutional Animal Care and Use Committee.

Size-fractionation of lipoproteins by FPLC

Size-fractionation of lipoproteins was performed by FPLC [29,31]. Equal volumes of plasma from every rat in each group were pooled (5 ml), and the density was adjusted to 1.21 g/ml with solid KBr. After ultracentrifugation at 100 000 *g* for 48 h, the removed supernatant (lipoprotein fraction) was adjusted to 2 ml by adding FPLC elution solution (0.15 M NaCl, 0.01% EDTA, 0.02% sodium azide, pH 7.3). After filtration through a 0.45 µm-pore-size filter, 1 ml (corresponding to 2.5 ml of plasma)

Abbreviations used: apo-B, apolipoprotein B; apo-E, apolipoprotein E; b.w., body weight; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase; TNA, total nucleic acid; VLDL, very-low-density lipoprotein.

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was injected on to a 54 cm $\times$ 1.8 cm Superose 6B column; 2 ml fractions were collected.

SDS/PAGE separation of apolipoproteins

For separation of apolipoproteins in FPLC fractions, 1.1 ml of the elution fraction was precipitated with trichloroacetic acid (15%), washed twice with acetone, and solubilized in 120 μ l of loading buffer as described elsewhere [32]. Samples were boiled for 5 min in the presence of 5% (v/v) 2-mercaptoethanol, and 80 μ l was loaded on 4–20% gradient SDS/polyacrylamide gels for apolipoprotein separation (4 h, 45 mA/gel) as described [32]. Gels were stained with Coomassie Blue. For reference, human LDL (apo-B100), and high- (Bio-Rad Laboratories, Richmond, CA, U.S.A.) and low- (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) molecular-mass standards were used.

Preparation of hepatic membranes and ligand blot assay of LDL receptors

Hepatic membranes were prepared from pooled liver samples (0.5 g) of each group as described [30]. The liver samples were homogenized with a Polytron (Kinematica, type PT 10/35; Kriens, Lucerne, Switzerland) at 4 °C in 1 ml of buffer (2 mM CaCl_2 , 0.5% Triton X-100, 1 mM leupeptin, 1 mM phenanthroline, 1 mM PMSF, 50 mM Tris/HCl, pH 7.5). After quick sonication, homogenates were centrifuged for 10 min (4 °C, 15800 g) in a microcentrifuge followed by 7 min ultracentrifugation at 206.7 kPa (30 p.s.i.) in a Beckman Airfuge at room temperature, using a prechilled ice-cold rotor. The supernatant was collected and assayed for protein [33], using reagents from Bio-Rad. The membrane preparation was mixed with loading buffer (10% glycerol, 0.5% SDS, 2 mM CaCl_2 , 0.5% Triton X-100, 0.05% Bromophenol Blue and 50 mM Tris/HCl, pH 6.8), separated without reduction on SDS/6%-polyacrylamide gels, and then electrotransferred on to 0.45 μ m nitrocellulose filters (type BA 85, Schleicher & Schuell) [30]. After 1 h preincubation in 5% (w/v) BSA, 2 mM CaCl_2 , 1 mM KI, 50 mM Tris/HCl, pH 8.0, the filters were incubated for 1 h with ^{125}I -labelled rabbit β -migrating VLDL (5 μ g/ml). Filters were washed with 0.5% BSA, 2 mM CaCl_2 , 50 mM Tris/HCl, pH 8.0, and thereafter with the washing buffer without albumin, as described in [30]. Filters were exposed to Kodak XAR-film for about 8 h for autoradiography. LDL receptor activity in blots was quantified by using a Bio-Imaging Analyser (Fujix, BAS 2000, Japan). Background levels measured in irrelevant filter areas were subtracted.

Total nucleic acid (TNA) preparation and analysis of LDL receptor mRNA

TNA was prepared according to Durnam and Palmiter [34]. The liver samples were homogenized with a Polytron in 4 ml of buffer (1% SDS, 10 mM EDTA, 20 mM Tris/HCl, pH 7.5), and digested for 45 min at 45 °C with proteinase K (200 μ g/ml). TNA was precipitated by adding 2 vol. of pure ethanol after phenol-chloroform extraction, and the pellet was suspended in an appropriate volume of the buffer. Quantification of LDL receptor mRNA was done by a solution-hybridization titration assay using a mouse [^{35}S]UTP-labelled cRNA probe [29]. The slopes of the linear hybridization signals were calculated by the method of least squares and compared with the slope generated from a synthetic mouse LDL receptor mRNA standard. Data are expressed as attomoles (amol) per μ g of TNA.

Cholesterol and triacylglycerol assays

Cholesterol and triacylglycerols in plasma and FPLC fractions were assayed with commercial kits (Boehringer Mannheim, Germany).

Statistics

Data are presented as means $\pm$ S.E.M. Significances of differences between groups were evaluated by Student's *t*-test.

RESULTS

In order to determine the effect of the dose of endotoxin, we first injected groups of rats with increasing amounts of endotoxin. The rats were killed 18 h after injection since significant effects on plasma cholesterol have been reported at this time point [5,12,17]. Endotoxin clearly reduced hepatic LDL receptor expression in a

Figure 1 Effect of endotoxin dose on hepatic LDL receptor expression and LDL receptor mRNA

Groups of rats (each group consisted of six animals) were injected with the indicated amounts of endotoxin at 22:00 h. Eighteen hours after injection, the animals were killed and livers obtained for determination of LDL receptor expression and LDL receptor mRNA levels as described in the Materials and methods section. For ligand blot assay of LDL receptor, hepatic membranes from each group were prepared from pools of liver samples ($n = 6$). For each group, 50, 100 and 200 μ g of membrane protein per lane were loaded on the gel as indicated. LDL receptor activity in blots was quantified using a Bio-Imaging Analyser. LDL receptor mRNA was quantified individually. (A) Ligand blots for LDL receptor. The molecular mass is indicated in kDa on the left-hand side of the Figure. (B) Quantification of LDL receptor expression (data are expressed as percentages of the control value) and LDL receptor mRNA (data are expressed as means plus S.E.M. of six animals). * $P < 0.001$, compared with control.

Table 1 Effect of endotoxin dose on plasma triacylglycerol and total cholesterol

Groups of rats (each group consisted of six animals) were injected with the indicated amounts of endotoxin at 22:00 h. Eighteen hours after injection, blood was taken for measuring triacylglycerol and total cholesterol, as described in the Materials and methods section. Data are means $\pm$ S.E.M. of six animals. The values in the parentheses are percentages of the control values. * $P < 0.001$. ** $P < 0.02$.

Endotoxin injected ($\mu\text{g}/100 \text{ g b.w.}$)	Triacylglycerol (mM)	Cholesterol (mM)
0 (control)	0.69 ± 0.11 (100)	2.18 ± 0.07 (100)
100	$1.12 \pm 0.07^{**}$ (162)	$2.47 \pm 0.006^{**}$ (113)
300	$1.53 \pm 0.04^{*}$ (220)	2.36 ± 0.06 (108)
500	$1.47 \pm 0.11^{*}$ (213)	$2.52 \pm 0.09^{**}$ (116)
700	$1.40 \pm 0.10^{*}$ (201)	2.39 ± 0.09 (110)

dose-dependent manner (Figure 1). At a dose of $100 \mu\text{g}/100 \text{ g}$ body weight (b.w.), endotoxin inhibited LDL receptor expression by 36 %. Maximal inhibitory action (by about 70 %) was obtained at a dose of $500 \mu\text{g}/100 \text{ g b.w.}$ However, determination of LDL receptor mRNA demonstrated increased levels in all endotoxin-treated groups (by 83–136 %; $P < 0.001$) (Figure 1B).

The plasma triacylglycerol levels were markedly increased, whereas the plasma total cholesterol levels were increased only slightly (Table 1). To characterize the plasma lipoprotein changes, lipoproteins were separated by FPLC for subsequent analysis of triacylglycerols and cholesterol. Changes in lipoprotein pattern after challenge with increasing doses of endotoxin are shown in Figure 2. Administration of endotoxin markedly increased tri-

acylglycerols in VLDL, IDL and LDL. The cholesterol increase occurred mainly in LDL and IDL, whereas in the smaller HDL fractions cholesterol was, if anything, reduced. SDS/PAGE separation of proteins in lipoprotein fractions showed that apo-B100 and apo-B48 increased in endotoxin-treated animals (results not shown).

The marked discrepancy between LDL receptor protein expression and LDL receptor mRNA levels prompted us to study the changes of these two parameters with time. Rats were injected with $500 \mu\text{g}/100 \text{ g b.w.}$ of endotoxin at different hours and all killed at 16:00 h. By 4 h after endotoxin injection, LDL receptor expression was decreased by 48 % (Figure 3B). After another 4 h, there was a further reduction of LDL receptor expression which remained essentially unchanged 18 h after endotoxin injection. However, 6 h thereafter, the LDL receptor expression was clearly increased, although still lower (by 38 %) than that observed in the control group. Changes in LDL receptor mRNA showed a different pattern (Figure 3B). By 4 h after endotoxin injection, LDL receptor mRNA was decreased by 78 % ($P < 0.001$). By 8 h after endotoxin injection, LDL receptor mRNA had returned to levels similar to controls, and 18 and 24 h after endotoxin injection, they were further increased by 62 % and 59 %, respectively ($P < 0.05$).

The plasma triacylglycerol levels were increased by almost 2-fold throughout 4–24 h after endotoxin injection ($P < 0.001$; Table 2). The plasma total cholesterol level was unchanged 4 h after injection. Increases in the plasma total cholesterol levels could be seen between 8 and 24 h after injection. As seen by FPLC analysis, the triacylglycerol concentration was increased in VLDL as well as in LDL and IDL fractions (Figure 4). Within 8 h of endotoxin injection, triacylglycerols had increased substantially in LDL and IDL fractions. After endotoxin injection, cholesterol increased in VLDL, IDL and LDL fractions (except

Figure 2 Effect of endotoxin dose on plasma lipoprotein pattern

Groups of rats (each group consisted of six animals) were injected with the indicated amounts of endotoxin at 22:00 h. Eighteen hours after injection, blood was obtained and lipoproteins were separated by FPLC, as described in the Materials and methods section.

Figure 3 Time course for effect of endotoxin on hepatic LDL receptor expression and LDL receptor mRNA

Groups of rats (each group consisted of six animals) were injected with endotoxin (500 μg/100 g b.w.) at 4, 8, 18, or 24 h before killing. Control rats received an equal volume of the vehicle 24 h before killing. The livers were obtained for determination of LDL receptor expression and LDL receptor mRNA levels, as described in the Materials and methods section. For ligand blot assay of LDL receptor, hepatic membranes from each group were prepared from pools of liver samples ($n = 6$). For each group, 50, 100 and 200 μg of membrane protein per lane were loaded on the gel as indicated. LDL receptor activity in blots was quantified using a Bio-Imaging Analyser. LDL receptor mRNA was quantified individually. (A) Ligand blots for LDL receptor. The molecular mass (kDa) is indicated on the left-hand side of the Figure. (B) Quantification of LDL receptor expression (data are expressed as percentages of the control value) and LDL receptor mRNA (data are expressed as means plus S.E.M. of six animals). * $P < 0.001$, compared with control; ** $P < 0.05$, compared with control.

Table 2 Time course for effect of endotoxin on plasma triacylglycerol and total cholesterol

Groups of rats (each group consisted of six animals) were injected with endotoxin (500 μg/100 g b.w.) at 4, 8, 18, or 24 h before blood sampling. Control rats received equal volume of the vehicle 24 h before blood sampling. Blood was obtained for measuring triacylglycerol and total cholesterol, as described in the Materials and methods section. Data are means $\pm$ S.E.M. of six animals. The values in the parentheses are percentages of the control values. * $P < 0.001$. ** $P < 0.01$. *** $P < 0.02$.

Time after endotoxin injection (h)	Triacylglycerol (mM)	Cholesterol (mM)
0 (control)	0.53 $\pm$ 0.04 (100)	1.80 $\pm$ 0.14 (100)
4	1.46 $\pm$ 0.13* (275)	1.86 $\pm$ 0.11 (103)
8	1.56 $\pm$ 0.06* (293)	2.13 $\pm$ 0.13 (118)
18	1.43 $\pm$ 0.13* (270)	2.47 $\pm$ 0.13** (136)
24	1.52 $\pm$ 0.12* (287)	2.33 $\pm$ 0.09*** (129)

that cholesterol slightly decreased in the LDL fractions 4 h after endotoxin injection) (Figure 4). However, a slight decrease or no change could be seen for cholesterol in the smaller HDL fractions.

Separation of apolipoproteins in FPLC fractions showed that apo-B100, apo-B48 and apolipoprotein E (apo-E) increased in VLDL and IDL fractions 4–24 h after endotoxin injection, and that apo-B100 and apo-B48 increased in LDL fractions 8–24 h after endotoxin injection (Figure 5). It should be noted that 4 h after endotoxin injection, apo-B100 and apo-B48 increased in VLDL and IDL fractions, but decreased in LDL fractions, which is consistent with the cholesterol changes in the lipoprotein fractions.

DISCUSSION

A dose- and time-dependent inhibition of rat hepatic LDL receptors was obtained following endotoxin administration. LDL receptor expression was already reduced by 48 % within 4 h of endotoxin injection, and maximal suppression of the LDL receptor (63–65 %) occurred between 8 and 18 h after endotoxin injection. Changes in LDL receptor mRNA showed a different pattern. A marked reduction of hepatic LDL receptor mRNA (by 78 %) occurred 4 h after endotoxin injection. However, 18 and 24 h after endotoxin injection, LDL receptor mRNA increased markedly. The difference between LDL receptor activity and its mRNA level may reflect, in part, the relative delay in turnover of the LDL receptor protein.

Rat was chosen as the model since a number of published data have been obtained with this species and rats respond to endotoxin with hyperlipidaemia and hypercholesterolaemia [7–10,12,16]. The doses of endotoxin used in the present study were sublethal and relatively low compared with most previous studies in the rat (e.g. [7–9,16]). The inhibitory effects of endotoxin on hepatic LDL receptor expression in the current study were obtained using similar doses which affect LPL activity and triacylglycerol clearance [10]. Recently, Feingold et al. [17] reported no change, or a slight increase, in hepatic LDL receptor expression 16 h after endotoxin injection in the hamster (0.1 μg or 100 μg/100 g). The reason for this difference is unclear. It could be due to different responses to endotoxin challenge in rats and hamsters.

We also found that triacylglycerols, total cholesterol, apo-Bs and apo-E increased in VLDL, LDL and IDL in the plasma of endotoxin-treated rats. Endotoxin induces hypertriacylglycerolaemia and inhibits LPL (reviewed in [18]). Thus, the accumulation of VLDL in endotoxin-treated animals is perhaps largely due to the inhibition of LPL activity. Stimulation by endotoxin of hepatic synthesis and secretion of lipids may also contribute to this hyperlipidaemia in certain experimental conditions [10]. The present study showed that at an early stage (4 h after endotoxin injection), VLDL accumulation was primarily responsible for the increase in plasma triacylglycerols. At later time points, LDL and IDL also made substantial contributions to the increase in plasma triacylglycerols. Since the endotoxin-induced increase in triacylglycerols of LDL and IDL fractions was proportionately much larger than that in cholesterol, these lipoproteins became enriched in triacylglycerols. A similar triacylglycerol enrichment in lipoproteins also occurs in hamsters treated with endotoxin [17]. The reason for the triacylglycerol enrichment in LDL and IDL is not clear, but it may be caused by an inhibition of LPL and hepatic lipase that also occurs in endotoxin-treated rats [7].

The increase in plasma cholesterol following endotoxin treatment occurred mainly within the LDL and IDL fractions. The suppression of hepatic LDL receptors was already seen 4 h after endotoxin injection, a time point where LDL-apo-Bs and LDL-cholesterol were not increased. Inhibition of LPL activity by endotoxin should lead to a diminished formation of LDL from

Figure 4 Time course for changes of plasma lipoprotein patterns after endotoxin challenge

Groups of rats (each group consisted of six animals) were injected with endotoxin (500 μ g/100 g b.w.) at 4, 8, 18, or 24 h before blood sampling. Control rats received an equal volume of the vehicle 24 h before blood sampling. Blood was obtained and lipoproteins were separated by FPLC, as described in the Materials and methods section.

VLDL. In the present study, apo-Bs and cholesterol increased in IDL and VLDL fractions but decreased in LDL fractions 4 h after endotoxin injection, suggesting a decreased conversion of VLDL into LDL. The continued suppression of hepatic LDL receptor expression may in part contribute to the subsequent accumulation of LDL and IDL in the endotoxin-treated animals. In the circulation, endotoxin-lipoprotein complexes are formed (reviewed in [35]), which are known to decrease cellular endocytotic catabolism of LDL *in vitro* [36,37]. Sakaguchi has shown a concomitant increase in serum lipid peroxide and LDL cholesterol levels in endotoxin-treated animals [5]. Formation of endotoxin-lipoprotein complexes, lipoprotein oxidation and other unknown mechanisms may also contribute to the LDL accumulation.

How endotoxin suppresses hepatic LDL receptor expression is unclear. It is believed that cytokines, especially tumour necrosis factor, interleukins 1 and 6, and interferon γ , mediate the biological activities of endotoxin *in vivo*. These cytokines induce hyperlipidaemia (reviewed in [18,19]). However, tumour necrosis factor, interleukins 1 and 6, and oncostatin M have been shown to stimulate LDL receptor expression in cultured hepatocytes [38–41] and other cells [42,43]. Endotoxin also induces release of hormones such as catecholamines and glucocorticoids. Catecholamines suppress LDL receptors in human mononuclear leucocytes [44], but stimulate LDL receptor activity in rat hepatocytes [45]. *In vivo*, dexamethasone stimulates hepatic LDL receptor expression in rats [46]. Recently, it was reported that endotoxin injection results in a dramatic (by 80–90% after 1–4 h), but transient (normalized after 24 h), reduction of plasma growth hormone levels [47]. Insulin-like growth factor-I levels are also reduced in response to endotoxin. These findings are of particular interest, since it has previously been shown that the presence of growth hormone is of importance in the regulation of hepatic

LDL receptors [30,48]. Clearly, it will be of interest to study further the role of hormonal mediators in the endotoxin-induced effects on hepatic LDL receptor expression.

It has been reported that endotoxin treatment increases cholesterol synthesis through induction of 3-hydroxy-3-methylglutaryl CoA reductase in mice and hamsters [11,17]. Thus it is possible that the initial inhibitory effects of endotoxin on LDL receptor and its mRNA are mediated through an enhanced hepatic cholesterol synthesis induced by endotoxin. We also observed an increased mRNA level of 3-hydroxy-3-methylglutaryl CoA reductase, but hepatic total cholesterol was not increased after endotoxin treatment (W. Liao, M. Rudling and B. Angelin, unpublished work). It will be of interest to study further whether changes in hepatic sterol metabolism are involved in endotoxin-induced inhibition of hepatic LDL receptor expression.

Regulation of LDL receptors is usually linked to parallel changes in LDL receptor mRNA. However, glucagon administration to rats increases hepatic LDL receptor without an increase of receptor mRNA [32], suggesting that post-transcriptional regulation of hepatic LDL receptors may occur. The present study showed that the endotoxin-induced inhibition of hepatic LDL receptor expression could not be simply explained by changes in LDL receptor mRNA, also suggesting the existence of post-transcriptional regulation of LDL receptors in this situation. Thus, the model of endotoxin treatment may provide a system to better understand the regulation of hepatic LDL receptors *in vivo*.

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Figure 5 Separation of apolipoproteins of FPLC fractions from time-course experiment

Groups of rats (each group consisted of six animals) were injected with endotoxin (500 μ g/100 g b.w.) at 4, 8, 18, or 24 h before blood sampling. Control rats received an equal volume of the vehicle 24 h before blood sampling. Blood was obtained and lipoproteins were separated by FPLC. Apolipoproteins in FPLC fractions were further separated by SDS/PAGE, as described in the Materials and methods section. The molecular mass (kDa) is indicated on the left-hand side of the Figure. Abbreviation: A1, apolipoprotein A1.

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