Atherosclerosis, 67 (1987) 23–31
Elsevier Scientific Publishers Ireland, Ltd.

ATH 03985

Effect of 17α-ethinylestradiol on the catabolism of high-density lipoprotein apolipoprotein A-I in the rat

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(Received 26 August, 1985)
(Revised, received 27 January, 1986; 3 September, 1986; 11 March, 1987)
(Accepted 16 March, 1987)

Summary

The in vivo metabolism and tissue sites of catabolism of high-density lipoproteins (HDL), labelled specifically in the apolipoprotein (apo) A-I moiety, were studied in rats treated with 17α-ethinylestradiol (EE) for 5 days. Apo A-I was labelled either with *O*-(4-diazo-3-[^{125}I]iodobenzoyl)sucrose, a non-degradable labelling compound, or with ^{131}ICl. It was found that EE treatment decreases the serum cholesterol concentration to 10 mg/dl and stimulates the serum decay of apo A-I labelled HDL. The latter effect could be attributed to an increased catabolism of apo A-I labelled HDL in the liver. The increased rates of the serum decay and tissue uptake of apo A-I labelled HDL in EE-treated rats were not affected by a bolus injection of unlabelled human low-density lipoprotein (LDL), administered at the time of the injection of the labelled HDL. When the serum cholesterol concentration was raised to physiological levels by a bolus injection of unlabelled rat HDL, both the serum decay and the tissue uptake of apo A-I labelled HDL were almost completely restored to conditions encountered in control animals. In vitro binding experiments showed that liver membranes obtained from EE-treated rats demonstrated a 6-fold increased specific binding of human ^{125}I-LDL, but virtually unchanged specific binding of rat ^{125}I-HDL, as compared with liver membranes obtained from control rats. It is concluded that rat HDL apo A-I catabolism is hardly mediated by the apo B/E receptor induced by EE treatment.

Key words: HDL; 17α-Ethinylestradiol; Apolipoprotein A-I; Apolipoprotein E

Introduction

Treatment of rats with pharmacological doses of 17 α-ethinylestradiol (EE) increases the number of LDL binding sites in rat liver membranes [1], the catabolism of apo B and/or apo E containing lipoproteins in isolated perfused livers [2], and the in vivo turnover of LDL [1,2]. These studies have

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Abbreviations used: EE, 17α-ethinylestradiol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo, apolipoprotein; DIBS, *O*-(4-diazo-3-iodobenzoyl)sucrose.

led to the notion that the catabolic pathway of LDL, induced by EE treatment, involves an active apo B/E receptor [3]. However, in the rat most of the serum cholesterol is not recovered in LDL but in HDL and the role of the apo B/E receptor in normal rats remains to be explored [1]. While the serum cholesterol concentration is very low in EE-treated rats, it was suggested that HDL catabolism in these animals is enhanced by induction of the apo B/E receptor [1,2]. Indeed, Chao et al. [2] demonstrated that the serum decay of apo E containing rat ^{125}I-HDL is increased in EE-treated rats and also that this rat HDL is catabolized more rapidly in isolated perfused livers obtained from EE-treated rats, as compared with livers from control animals. Since rat HDL contains substantial amounts of apo E, it could be that the apo B/E receptor functions as a receptor for rat HDL, also in the untreated rat. Innerarity et al. [4] showed that HDL_c, an HDL subclass isolated from cholesterol-fed dogs containing predominantly apo E, can be bound by the apo B/E receptor. However, other studies have questioned the role of the apo B/E receptor in HDL catabolism in the rat, e.g. it was found in the perfused liver system that the catabolism of chromatographically isolated rat HDL was not dependent on the apo E content of the HDL particle [5]. Funke et al. [6] reported a normal plasma clearance of HDL_c in cholesterol-fed animals, in which the apo B/E receptor was undetectable. Moreover, it has been shown that apo E-deficient HDL binds to liver cells and isolated liver cell membranes by a saturable and specific process [7–9], indicating that HDL catabolism may be mediated by an HDL receptor which recognises apolipoproteins different from apo E. The latter studies suggest that HDL subfractions, containing predominantly apo A-I, are degraded by the liver via a mechanism which is independent of the apo B/E receptor pathway. In the present study the catabolism of apo A-I labelled HDL in EE-treated rats was studied, in order to determine the relative contribution of the apo B/E receptor pathway to the catabolism of rat HDL apo A-I in vivo.

Experimental procedures

Treatment of rats

Retired breeders of the Wistar strain, weighing 300–400 g, were used. One ml of EE, dissolved in 1,2-dihydroxypropane, was administered subcutaneously in a concentration of 4 mg/kg for 5 consecutive days. Control animals received 1,2-dihydroxypropane only. All animals had free access to food and water. The average body weight of rats treated with EE decreased slightly from 351 ± 23 g to 336 ± 29 g, and of control rats from 352 ± 29 g to 341 ± 25 g (mean ± S.D. for 27 EE-treated and 9 control animals). As shown in Table 1 the wet weights of the adrenal glands were increased by EE-treatment.

Isolation and labelling of lipoproteins

The isolation and labelling of rat HDL, labelled specifically in the apo A-I moiety with ^{131}I, using the ICl method, and with *O*-(4-diazo-3-[^{125}I]iodobenzoyl)sucrose ($D^{125}IBS$) according to [11], was performed as described [12]. Unlabelled human LDL and rat HDL were isolated by sequential centrifugation in a Beckman 50.2Ti rotor (Beckman Instruments Inc., Spinco Division, Palo Alto, CA). Human LDL was isolated from the density range of 1.019–1.063 g/ml of plasma, obtained from healthy female volunteers under the age of 40. Unlabelled rat HDL, for use in the in vivo

TABLE 1

EFFECT OF EE TREATMENT ON THE WET WEIGHT OF 6 TISSUES OF THE RAT

Rats were treated for 5 days with EE, dissolved in 1,2-dihydroxypropane. Control rats were injected with 1,2-dihydroxypropane only. The animals were bled from the abdominal aorta and the tissues were excised and weighed. Results are expressed in g tissue/100 g whole animal and represent the means ± SD for 27 EE-treated rats and 9 controls.

	EE-treated rats	Control rats
Heart	0.27 ± 0.02	0.28 ± 0.03
Liver	4.0 ± 0.4	3.8 ± 0.8
Spleen	0.16 ± 0.03	0.17 ± 0.03
Kidneys	0.70 ± 0.08	0.67 ± 0.09
Adrenal glands	0.012 ± 0.003 *	0.007 ± 0.002
Testes	0.89 ± 0.05	0.90 ± 0.08

* Statistically significantly different from control rats at $p < 0.05$.

experiments, was obtained from the density range of 1.063–1.21 g/ml of serum of animals fasted for 18–24 h. Rat HDL used for in vitro binding studies was isolated from the 1.05–1.21 g/ml density range of serum obtained from animals, fed normal rat chow until being fasted for 18–24 h, and iodinated using ICl [10]. The isolated human LDL and rat HDL were washed once in a Beckman 50H rotor at the densities of 1.063 and 1.21 g/ml, respectively.

Metabolic studies

All lipoprotein preparations were dialyzed overnight against Krebs-Henseleit buffer [13] immediately before use in the in vivo experiments. All animals were fasted for 18–24 h. One minute before the injection of labelled HDL, 2 ml of either unlabelled human LDL, rat HDL, or Krebs-Henseleit buffer alone were injected into a femoral vein of rats anesthetized with diethyl ether. The mixture of ^{131}I-apo A-I HDL and D^{125}IBS-apo A-I HDL was injected in the contralateral vein in a volume of 1 ml. Three minutes after injection of the labelled HDL a small blood sample was obtained from the orbital plexus behind the eye. The animals were bled from the abdominal aorta at various time-points after injection of the labelled HDL. Eleven tissues (heart, lungs, liver, spleen, kidneys, jejunum, adipose tissue, muscle, hide, adrenals and testes) were excised, weighed and counted for radioactivity. The labelled HDL was administered in a dose of 0.05–0.15 mg cholesterol/rat. The unlabelled HDL was administered in a dose of 5–8 mg cholesterol/rat, sufficient to raise the serum cholesterol concentration to physiological levels (Fig. 1). Unlabelled LDL was injected in a dose of 10–15 mg cholesterol/rat. Cholesterol was determined by an enzymatic method [14].

Calculations

The DIBS-dependent accumulation of radioactivity in tissues was calculated as described [12,15], by subtracting the percent of the injected dose of the ^{131}I-apo A-I labelled HDL from the percent of the injected dose of the D^{125}IBS-apo A-I labelled HDL present in each tissue at the indicated times. The blood sample obtained 3 min after injection of the labelled HDL was taken to represent the initial serum radioactivity used for the calculation of the serum decay.

Binding of lipoproteins to isolated liver membranes

Membranes were prepared from liver obtained from rats treated with EE or from control animals. The membranes were isolated by differential centrifugation [1]. The microsomal pellets were frozen and stored at −70 °C. Before use, the pellets were resuspended, by aspiration through a 25 gauge needle (10 ×), to a protein concentration of 5–10 mg/ml of protein in medium consisting of 50 mM NaCl, 20 mM Tris-HCl (pH 7.5) and 1 mM $CaCl_2$. Specific binding of apo E-containing total rat ^{125}I-HDL (the apo E/A-I ratio of the HDL preparations used, determined by electroimmunoassay [16], ranged from 0.25 to 0.30) and human ^{125}I-LDL was measured at 0 °C. The iodinated lipoproteins were incubated, at a concentration of 25 μg protein/ml, with 0.1–0.2 mg of membrane protein in a total volume of 0.08 ml for 1 h, with or without excess unlabelled rat HDL or human LDL (1 mg/ml). The separation of membrane-bound labelled lipoproteins from the bulk of the unbound labelled lipoproteins was performed as described [16].

Materials

Na^{125}I (350–600 Ci/l) and Na^{131}I (40 Ci/l), both carrier free, were obtained from Amersham International PLC, Amersham, U.K. ^{125}I- and ^{131}I-radioactivities were counted in the LKB-Wallac Ultrogamma counter (LKB-Wallac, Turku, Finland). EE was purchased from Sigma Chemical Company, St. Louis, U.S.A.

Results

Effect of EE treatment on the serum decay of rat HDL

As shown in Fig. 1, EE treatment reduced the serum cholesterol concentration from 60 mg/100 ml to approximately 10 mg/100 ml. A bolus injection of either unlabelled rat HDL or human LDL raised the serum cholesterol concentration in EE-treated rats to physiological levels (HDL), or even higher (LDL). The rapid fall in the serum cholesterol concentration after injection of human LDL into EE-treated rats indicates that the apo

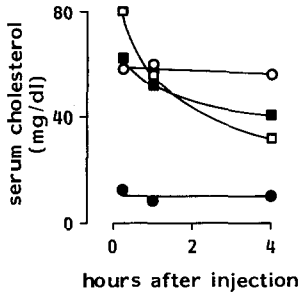

Fig. 1. Serum cholesterol concentration of control rats injected buffer (○), and of EE-treated rats injected buffer (●), unlabelled human LDL (□), or unlabelled rat HDL (■). Results are mean values of two experiments.

B/E receptor is induced by EE treatment (see below).

Fig. 2 shows the serum decay of apo A-I labelled rat HDL in EE-treated and control rats. Only the ^{131}I-apo A-I HDL decays are shown, while no differences were observed between the serum decays of ^{131}I-apo A-I HDL and D^{125}IBS-apo A-I HDL [12]. As shown in Fig. 2, EE treatment enhanced the serum decay of a trace amount of apo A-I labelled HDL. A bolus injection of unlabelled human LDL was without effect on the rapid serum decay of apo A-I labelled HDL in EE-treated animals. In contrast, intravenous injection of unlabelled HDL into EE-treated animals decreased the rate of serum decay of apo A-I labelled HDL again almost to values found in normal rats.

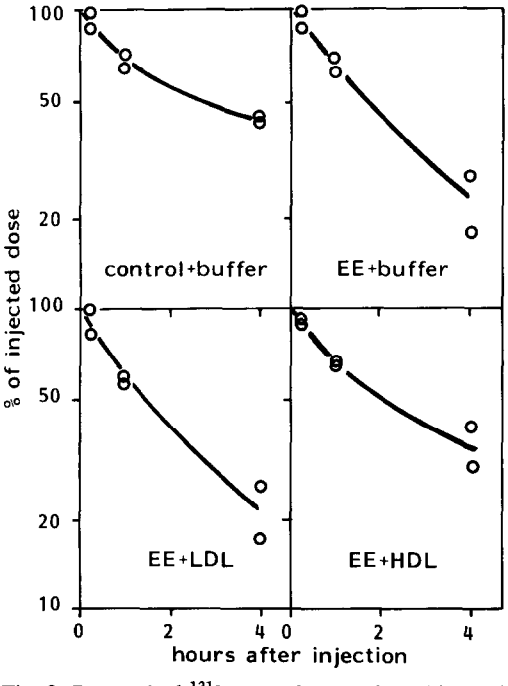

Fig. 2. Removal of ^{131}I-apo A-I HDL from blood plasma of rats. HDL labelled specifically with ^{131}I-apo A-I was injected into a femoral vein. At the indicated time point the animals were bled from the abdominal aorta and serum was obtained by low speed centrifugation. Data obtained from two separate experiments are given. Control+buffer: control rats injected with buffer; EE+buffer: EE-treated rats injected with buffer; EE+HDL: EE-treated rats injected with unlabelled rat HDL; EE+LDL: EE-treated rats injected with unlabelled human LDL. The serum cholesterol concentrations during these experiments are shown in Fig. 1.

Effect of EE treatment on the binding of rat HDL and human LDL to isolated liver membranes

Table 2 shows that the specific binding of total rat ^{125}I-HDL is much higher than the binding of human ^{125}I-LDL. The specific binding was determined at a labelled lipoprotein concentration of 25 μg/ml and taken as the difference between the binding measured with or without excess unlabelled rat HDL or human LDL. Membranes obtained from EE-treated rats bind 6 times more human ^{125}I-LDL than control liver membranes, indicating that the apo B/E receptor is induced by EE treatment. In contrast, EE treatment had

TABLE 2

EFFECT OF EE TREATMENT ON THE SPECIFIC BINDING OF APO E-CONTAINING RAT HDL AND HUMAN LDL TO ISOLATED LIVER MEMBRANES

Microsomal pellets were obtained from livers of rats treated with EE and from livers of control animals. Specific binding was taken as the difference between the values of bound labelled LDL or HDL (25 μg/ml) with and without excess unlabelled lipoprotein (1 mg/ml). Results are expressed as ng LDL or HDL protein bound per mg of membrane protein ± SD for 5 experiments.

	Membranes from	
	EE-treated rats	Control rats
Total rat ^{125}I-HDL (density range 1.05–1.21 g/ml)	678 ± 99	627 ± 87
Human ^{125}I-LDL (density range 1.019–1.063 g/ml)	86 ± 47	15 ± 9

no statistically significant effect on the specific binding of rat ^{125}I-HDL. In one experiment it was found that the ^{131}I-apo A-I labelled HDL preparation, used for the in vivo experiments, had the same binding characteristics (both total binding and specific binding) as the total ^{125}I-HDL, used in the in vitro binding studies.

Accumulation of radioactivity in the liver after intravenous injection of apo A-I labelled HDL

The sites of catabolism of HDL apo A-I were analysed using a method described earlier [12,15]. Fig. 3 shows the accumulation of radioactivity derived from ^{131}I- and D^{125}IBS-labelled apo A-I HDL in livers of EE-treated rats and control animals. The DIBS-dependent accumulation of radioactivity, an index of the rate of catabolism of HDL apo A-I [12,15], is shown by the accentuated lines. An increased DIBS-dependent accumulation was observed in the livers of EE-treated rats 4 h after injection of the labelled HDL. The DIBS-dependent accumulation in the liver of EE-treated animals was not affected when a bolus of unlabelled human LDL was given at the time of injection of labelled HDL. However, the DIBS-dependent accumulation of radioactivity in the liver of EE-treated animals was clearly decreased by injection of unlabelled rat HDL.

Accumulation of radioactivity in extrahepatic tissues after intravenous injection of apo A-I labelled HDL

The accumulation of radioactivity derived from injected ^{131}I- and D^{125}IBS-labelled apo A-I HDL in the kidneys was not influenced by EE treatment or injections of unlabelled lipoproteins. In agreement with [12], approximately 12% of the injected dose was recovered as DIBS-dependent accumulation in this tissue, if measured 4 h after injection of the labelled HDL. Besides the liver and the kidneys, the only tissue showing any DIBS-dependent accumulation was the spleen. The DIBS-dependent accumulation in the spleen (4 h after

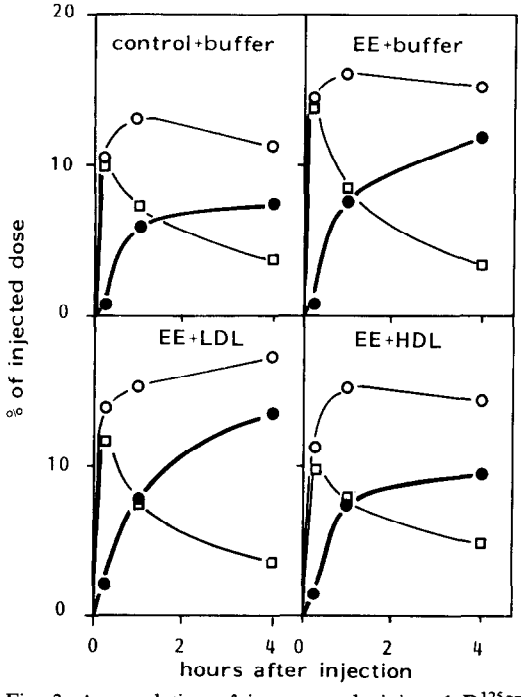

Fig. 3. Accumulation of intravenously injected D^{125}IBS-apo A-I HDL (○) and ^{131}I-apo A-I HDL (□) in the liver. Conditions were identical with those of the experiments shown in Fig. 2. The DIBS-dependent accumulation of radioactivity (●) was calculated. Each point indicates an average value obtained from two rats.

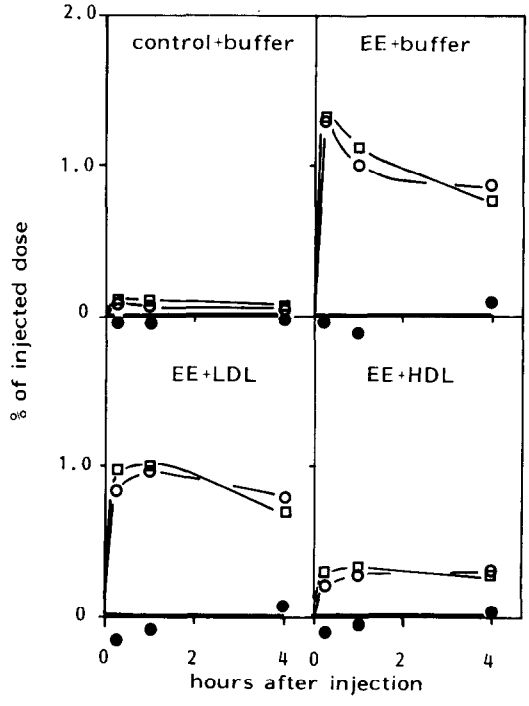

Fig. 4. Accumulation of intravenously injected D^{125}IBS-apo A-I HDL (○) and ^{131}I-apo A-I HDL (□) in the adrenal glands. Conditions were identical with those of the experiments shown in Fig. 2. The DIBS-dependent accumulation of radioactivity (●) was calculated. Average values of two experiments are given.

injection of the labeled HDL) of control animals and of EE-treated animals administered buffer, unlabelled human LDL and rat HDL was 2.1%, 2.0%, 2.3% and 2.1%, respectively.

As illustrated in Fig. 4, the accumulation of radioactivity, derived from apo A-I labelled HDL, in the adrenal glands of rats treated with EE is increased 5–10-fold, as compared with control animals. The increased accumulation in this tissue is not influenced by a bolus injection of unlabelled human LDL, but a bolus injection of rat HDL dramatically reduces the accumulation of radioactivity. Table 1 shows that EE treatment results in an increase in the wet weight of adrenal glands. When the accumulation of radioactivity in the adrenal glands is expressed per gram tissue, no differences are present between the values for control animals and rats treated with EE and injected with a bolus of unlabelled HDL. No DIBS-dependent accumulation of radioactivity derived from apo A-I labelled HDL was found in the adrenal glands under any condition.

Discussion

As documented in this study, EE treatment increases the rate of removal of intravenously injected apo A-I labelled HDL from rat serum. This increase can be attributed to an increased fractional rate of catabolism of apo A-I labelled HDL in the liver. These results agree with the conclusions drawn by Chao et al. [2], who showed that the catabolism of apo E containing rat HDL, isolated from the density interval 1.09–1.21 g/ml, is increased in isolated perfused livers obtained from EE-treated rats. While there is considerable evidence that EE treatment induces an apo B/E receptor in rat liver [1,2], these observations suggest that this apo B/E receptor is involved in the catabolism of HDL apo A-I. However, in the present study it was found that the rapid serum decay and the high fractional rate of catabolism of apo A-I labelled HDL in EE-treated rats are not decreased when the serum cholesterol concentration in the EE-treated rats is increased by a bolus injection of unlabelled human LDL. This indicates that LDL is not competing with the apo A-I labelled HDL for binding and degradation via the apo B/E receptor.

In agreement with Kovanen et al. [1], it was found that EE treatment results in an increased specific binding of human LDL to rat liver membranes (see Table 2). We also observed, in agreement with earlier data [1,2], that intravenously injected human LDL was removed much faster from the blood of EE-treated rats than from the blood of control rats (not shown). These results indicate that the apo B/E receptor is induced in the EE-treated rats. Indeed, as shown in Fig. 1, the serum cholesterol concentration of EE-treated rats, injected with unlabelled human LDL, decreases rapidly during the experiment. Still, at the end of the 4 h experiment, the serum cholesterol concentration is approximately 3-fold higher than the serum cholesterol concentration of EE-treated rats injected with buffer only. This suggests that at the end of the 4-h experiment, a considerable amount of unlabelled LDL is still present in serum, which could potentially compete with apo A-I labelled HDL for the same receptor.

The lack of competition in vivo between LDL and apo A-I labelled HDL shows that the turnover of HDL apo A-I is mediated by a mechanism different from the apo B/E receptor system. This conclusion is supported by lipoprotein binding studies to liver membranes of untreated and EE-treated rats. It was shown by Kovanen et al. [1] that binding of human ^{125}I-HDL was not increased by EE treatment. Windler et al. [30] have reported that rat HDL shows poor competition with the binding of lipoproteins to the apo B/E receptor of liver membranes from EE-treated rats. In the present study we observed that binding of total rat ^{125}I-HDL to rat liver membranes is not significantly influenced by EE treatment. Thus, both in vivo competition studies and in vitro binding studies support the concept that HDL apo A-I catabolism in the liver of EE-treated rats is not mediated by the apo B/E receptor to any appreciable extent.

It was shown by Sigurdsson et al. [17] that the fractional catabolic rate of rat ^{125}I-HDL in the isolated perfused liver is increased at low HDL concentrations. In agreement with this observation we have recently found that in 4APP-treated rats (4APP treatment reduces the serum cholesterol concentration to values similar to those found in EE-treated animals) the increase in the fractional

rate of catabolism of apo A-I labelled HDL can be completely abolished when the serum HDL concentration is restored to physiological levels [18]. This suggests that the increased rate of catabolism of apo A-I labelled HDL in hypolipemic rats is due to a decreased serum HDL concentration and is not related to an increased capacity of the liver to catabolize HDL apo A-I. In the present study it was found that the injection of unlabelled rat HDL in EE-treated rats reduces the fractional rate of catabolism of apo A-I labelled HDL. This experiment shows that the increase in fractional rate of catabolism of apo A-I labelled HDL in EE-treated rats is mostly due to a decreased pool size of endogenous HDL, resulting in less competition with the labelled HDL. However, as compared with control animals, the fractional rate of catabolism of apo A-I labelled HDL in EE-treated rats is not completely normalized by administration of a bolus of unlabelled HDL. This indicates that EE treatment may induce a slight increase in the capacity to catabolize HDL apo A-I. As discussed above, this effect of EE treatment is probably not mediated by an induced apo B/E receptor. However, treatment of rats with estrogen may be associated with proliferation of endothelial cells and activation of Kupffer cells [19], suggesting a marked effect of this drug on various hepatic functions. A slightly increased capacity of the liver to catabolize HDL apo A-I may be related to these changes.

We observed a dramatically increased association of radioiodine derived from apo A-I labelled HDL with the adrenal glands of EE-treated rats. A bolus injection of unlabelled rat HDL decreased the recovery of radioiodine in the adrenal glands of EE-treated rats. Intravenous injection of unlabelled human LDL had no such effect. As discussed above for the liver these results show that the LDL receptor, induced in rat adrenals by EE treatment [20], is not involved in HDL apo A-I metabolism by this tissue. No significant DIBS-dependent accumulation of apo A-I radioactivity was found in the adrenals under any condition tested, indicating that the apo A-I labelled HDL is not catabolized by the adrenal glands. This conclusion is in agreement with recent studies which have shown that both apo A-I HDL as well as apo E HDL are not, or only poorly, catabolized in the adrenal glands of normal rats in vivo [12,21–23]. In contrast, several in vivo studies have shown that a substantial fraction of the cholesteryl ester moiety of HDL is catabolized by the adrenal glands [22,24]. In vitro studies of adrenal cells in culture have shown that the cholesteryl ester moiety of HDL is readily internalized by these cells, while the protein moiety of HDL is only poorly degraded [9,25]. These in vivo and in vitro studies indicate that the binding sites for HDL on the adrenal glands [26–28] are not involved in a process which results in the internalization and degradation of the whole HDL particle, but could be important in the uptake of HDL cholesteryl esters by the cells via a still unknown mechanism. As discussed previously for the liver, the increased association of radioactivity in the adrenal glands of EE-treated rats after injection of apo A-I labelled HDL may be due to the decrease in the serum HDL concentration or to an increased number of binding sites for HDL. The large reduction of HDL apo A-I radioactivity, recovered in the adrenal glands of EE-treated rats after injection of a bolus of unlabelled HDL, provides strong evidence in favor of a competition between labelled HDL and unlabelled HDL for a limited number of binding sites. The observation that a bolus of unlabelled LDL does not influence the recovery of radioactivity in the adrenal glands of EE-treated rats is evidence for a specific HDL binding. As illustrated in Table 1 the wet weight of the adrenal glands in the EE-treated rats is increased. When the accumulation of HDL apo A-I radioactivity in the adrenal glands is expressed per gram tissue, no significant difference is observed any more between control animals and EE-treated rats administered a bolus of unlabelled HDL. It is therefore concluded that the increased recovery of radioactivity in the adrenal glands of EE-treated rats, injected with apo A-I labelled HDL, is not due to an increase in the number of specific binding sites for HDL in this tissue, but is related to the low serum HDL concentration and to the increase in adrenal weight.

Recent studies have provided evidence for a role of the kidneys in the catabolism of HDL apo A-I [12,21–23]. The cholesteryl ester moiety of HDL is however poorly catabolized by this tissue [22,24]. It was suggested by Glass et al. [22] that in

the circulation some of the HDL apolipoproteins dissociate from the HDL particles, are filtered by the glomeruli and subsequently catabolized in the tubular cells of the kidney. Although this hypothesis awaits further experimental proof, the data presented in this paper are not in conflict with this model. We found no increase in the association of radioactivity derived from intravenously injected apo A-I labelled HDL in the kidneys, nor was the rate of catabolism of apo A-I labelled HDL increased in this tissue by EE treatment. These results again show that the mechanism of HDL catabolism in the kidneys is different from that in the liver and adrenal glands.

The present study provides evidence that the reduced HDL concentration in EE-treated rats is not due to an increased catabolism of HDL apo A-I via the apo B/E receptor. To account for the low HDL levels in EE-treated rats, several alternative hypotheses may be put forward: (1) the low level of HDL could be explained by a reduction in HDL synthesis in EE-treated animals. Although both Chao et al. [2] and Weinstein et al. [31] did not find a decreased rate of synthesis of apo A-I by perfused liver of EE-treated male rats, apo E synthesis was decreased, as compared with control animals [2]. Krause et al. [29] have shown that the lymphatic output of apo A-I is markedly decreased in EE-treated rats, indicating a possible reduction in intestinal apo A-I synthesis. However, it is not clear whether these reduced rates of synthesis of HDL apolipoproteins can fully explain the decreased levels in EE-treated rats. (2) It may be that EE-treated rats secrete HDL particles of different composition as compared with HDL secreted by normal rats. While the HDL levels in EE-treated rats were extremely low, all of the HDL preparations used in the present study were obtained from untreated animals. It can therefore not be excluded that a changed composition of endogenous HDL present in EE-treated rats leads to a more rapid catabolism of these particles. (3) As discussed above, it cannot be excluded that the catabolism of HDL A-I via a still unknown pathway, distinct from the apo B/E receptor, is increased in the EE-treated rats.

Acknowledgements

We thank Mr. John F.C. Molenaar for his excellent technical assistance, Ir. Gees Dallinga-Thie for helping with the purification of rat apolipoproteins, and Miss A. Cecile Hanson and Miss Martha I. Wieriks for typing the manuscript. Dr. Anton S.H. de Jong is thanked for generously providing *O*-(4-aminobenzoyl)sucrose. These studies were supported by grant 30.002 from the Dutch Heart Foundation.

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