Effect of hypocholesterolemic doses of 17α-ethinyl estradiol on cholesterol balance in liver and extrahepatic tissues

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Abstract This study was performed to investigate the effects of 17α-ethinyl estradiol, a potent hypocholesterolemic agent at pharmacological doses, on cholesterol balance in the liver and extrahepatic tissues of the rat in vivo. Female Sprague-Dawley rats were treated with 17α-ethinyl estradiol (5 mg/kg per day s.c. for 5 days) or with 4-aminopyrazolo(3,4-d) pyrimidine (20 mg/kg per day i.p. for 3 days). Both drug regimens suppressed plasma total and low density lipoprotein-cholesterol by more than 80%. Analysis of the kinetic parameters of low density lipoprotein transport did not show increased receptor activity in extrahepatic tissues during either treatment. 17α-Ethinyl estradiol significantly increased low density lipoprotein tissue spaces and clearance rates in the liver, with a 5-fold increase in low density lipoprotein-receptor activity, whereas 4-aminopyrazolo(3,4-d)pyrimidine suppressed hepatic transport of low density lipoprotein probably due to a nonspecific toxic effect. Treatment with 17α-ethinyl estradiol markedly enhanced the hepatic expression of low density lipoprotein-receptor protein and mRNA despite a 7-fold increase in hepatic cholesteryl ester levels. Finally, treatment with both drugs increased cholesterol synthesis in several extrahepatic tissues, such as adrenals, ovaries, small bowel, and spleen. These findings confirm that 17α-ethinyl estradiol at pharmacological doses markedly increases synthesis and expression of low density lipoprotein-receptor in the liver. Hypocholesterolemia, whether induced by activation of low density lipoprotein-receptors or by other mechanisms, fails to up-regulate low density lipoprotein transport in extrahepatic tissues, which rather respond by increasing local sterol synthesis. This suggests the occurrence of separate regulatory mechanisms for low density lipoprotein transport and cholesterol synthesis.—**Bertolotti, M., and D. K. Spady.** Effect of hypercholesterolemic doses of 17α-ethinyl estradiol on cholesterol balance in liver and extrahepatic tissues. *J. Lipid Res.* 1996. **37:** 1812–1822.

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All cells can acquire cholesterol from local de novo synthesis and/or from uptake of cholesterol-containing plasma lipoproteins (1). The uptake of low density lipoproteins (LDL), the prevalent lipoprotein fraction in humans, takes place largely via a highly specific receptor-mediated pathway which has been recently characterized (2). The liver plays a key role in the maintenance of whole body cholesterol homeostasis in many animal species including humans (3). In quantitative terms the liver is the most important organ contributing to cholesterol synthesis and LDL transport in the organism; furthermore, elimination of cholesterol from the body takes place largely via biliary excretion, as intact cholesterol or in the form of bile acids. Alterations in hepatic cholesterol balance and most importantly in hepatic LDL receptor expression are well known to affect circulating levels of total and LDL-cholesterol, with obvious pathophysiological and clinical relevance (2).

Both cholesterol synthesis and LDL receptor activity appear to be subjected to metabolic regulation, possibly involving transcriptional and post-transcriptional levels of control (4). Whether the two pathways are controlled independently of each other or share common regulatory mechanisms, however, is still incompletely understood. In some experimental models excess cholesterol was shown to suppress both LDL receptor expression and the activity of the rate-limiting enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) (4–6). In contrast, several observations suggest that these processes can be independently regulated in the liver and in the small bowel of the experimental animal in vivo (7–9). As a

Abbreviations: ACAT, acyl-coenzyme A:cholesterol acyltransferase; APP, 4-aminopyrazolo(3,4-d) pyrimidine; DPS, digitonin-precipitable sterols; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; J^m, maximal rate of receptor-dependent LDL transport; LDL, low density lipoprotein.

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general rule, many dietary and pharmacological interventions can profoundly affect cholesterol synthesis whereas changes in LDL transport rates are much more difficult to achieve, particularly when utilizing the rat as an experimental model (9).

In the present studies we utilized two drugs presenting potent hypocholesterolemic activity at pharmacological doses, in order to investigate their effects on cholesterol homeostasis in the rat in vivo. The synthetic estrogen compound 17α-ethinyl estradiol has long been shown to stimulate LDL receptor activity in rat liver (10–13) whereas the adenine analogue 4-aminopyrazolo(3,4-d) pyrimidine (APP) is likely to induce hypocholesterolemia by inhibiting hepatic lipoprotein secretion (14, 15). We looked at the perturbations induced by these compounds on cholesterol synthesis and LDL transport in liver and extrahepatic organs. Our findings confirm a marked stimulation of the biosynthesis of LDL receptor protein in the liver by 17α-ethinyl estradiol; whereas neither drug was able to enhance LDL transport in extrahepatic tissues, both agents significantly induced cholesterol synthesis in a number of peripheral organs.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats were obtained from Sasco Laboratories, Inc. (Omaha, NE). Rats were subjected to light cycling for at least 2 weeks before use, with free access to water and pelleted rat chow (Teklad, Winfield, IA). Experimental protocols were started with animals of 200–250 g. All subsequent experiments were performed during the mid-dark phase of the light cycle.

Groups of animals were injected subcutaneously with 5 mg/kg body weight of 17α-ethinyl estradiol (Sigma Chemical Co., St. Louis, MO) per day, for 5 days; the drug was dissolved in ethanol, and then in propylene glycol, to a final concentration of 6 mg/ml. Control animals received an equal volume of diluent alone and were pair-fed, so that at the moment of the study the groups weighed the same.

Separate groups of animals were treated with APP (Sigma Chemical Co., St. Louis, MO), injected intraperitoneally at the dose of 20 mg/kg body weight per day, for 3 days. The drug was dissolved in a buffer solution containing Na phosphate (25 mM) and NaCl (154 mM) at a pH of 4.0, to a final concentration of 2 mg/ml. Controls received buffer alone. During the treatment period, both APP-treated and control animals were fasted but allowed free access to water, as previously described (16). After 3 days of treatment, both groups had lost 30 to 40 g of body weight. Approximately 12–15 h before the experiments, each animal was fitted with a femoral vein catheter and placed in an individual restraining cage. Subsequently, animals were infused i.v. at a rate of 1.2 ml/h with a solution containing NaCl (77 mM), K_2HPO_4 (8.3 mM), KCl (16.7 mM), and glucose (370 mM) until studies were conducted.

Determination of sterol synthesis rates in vivo

Sterol synthesis rates in different tissues were measured under in vivo conditions in the separate groups of animals. As previously described in detail (16, 17), animals were administered a bolus of [^{3}H]water (≅ 50 mCi) intravenously and thereafter were kept under fume hoods until they were killed by exsanguination 1 h later. Aliquots of plasma were taken for the measurement of plasma water specific activity and aliquots of blood and of the following tissues were taken for isolation of digitonin-precipitable sterols (DPS), after alkaline extraction: liver, adrenal glands, ovary, spleen, lung, kidney, adipose tissue, muscle, and brain. The small bowel and carcass were saponified as a whole. Rates of tissue sterol synthesis (newly synthesized sterol content) were expressed as the nmol of [^{3}H]water incorporated into DPS per h per g of tissue (nmol/h per g) (16, 17).

Lipoprotein preparation

Rat LDL was isolated from the plasma of donor rats receiving a low cholesterol diet in the density range of 1.020–1.055 g/ml, and purified as described (8, 13); on polyacrylamide gel electrophoresis this preparation contained essentially only apolipoprotein B. LDL was labeled with [^{14}C]sucrose (ICN Pharmaceuticals Inc., Irvine, CA) as described (18, 19). Labeled lipoproteins were dialyzed against phosphate buffer and saline and used within 24 h of preparation. LDL was filtered through a 0.45-μm Millipore filter prior to utilization. A cumulated lipoprotein fraction ($d < 1.210$ g/ml) was also harvested by rat plasma ultracentrifugation, followed by repeated dialysis. This fraction was used in separate groups of treated animals to restore normal plasma cholesterol levels.

Determination of the rates of tissue clearance and uptake of LDL in vivo

Rats received intravenously a priming dose of a trace amount of [^{14}C]sucrose-LDL followed by a constant infusion of the same molecule for 6 h (19). The radioactivity present in the priming dose relative to the radioactivity administered in the constant infusion was adjusted so as to maintain a constant specific activity of the lipoprotein in the plasma throughout the experimental period (9, 19). In general, the same amount of labeled LDL that was administered as a bolus was thereafter administered as a 6-h infusion. In the case of estrogen-

treated animals only, the amount of tracer administered as an infusion was doubled compared to the priming dose; this was done because of the more rapid rate of disappearance of LDL from plasma. At the end of the infusion, the specific activity of plasma LDL was comparable to that measured at the end of the bolus injection; in estrogen-treated animals only, plasma LDL radioactivity was on average 15% lower, despite the higher rate of infusion. This small fall in specific activity over 6 h did not significantly affect calculation of clearance rates. Some rats from each experimental group received only the bolus and were killed after 10 min. Separate groups of rats treated with 17α-ethinyl estradiol or with APP were also administered a mass amount of unlabeled lipoprotein (density < 1.210 g/ml), either as a bolus or as a primed-continuous infusion, along with the [^{14}C]sucrose-LDL. This was done in order to restore plasma total and LDL-cholesterol levels that were markedly reduced by both agents (see later) to normal. In estrogen-treated animals, LDL-cholesterol levels after continuous lipoprotein infusion were on average 18% lower than after the bolus; again, this difference did not significantly affect calculation of clearance rates.

At the end of the experiment rats were killed by exsanguination and aliquots of different organs were removed: liver, small bowel, adrenal glands, ovary, spleen, lung, kidney, adipose tissue, muscle, and brain. Tissue spaces of LDL at 10 min and at 6 h were calculated as described (9, 13, 19). The space of distribution at 10 min expresses LDL binding to the different tissues; the hourly increase in tissue space represents the rate of LDL clearance, expressed as the volume of plasma cleared of LDL per h per g of tissue. The rate of LDL-cholesterol uptake was calculated by multiplying clearance rates by plasma LDL-cholesterol concentration.

Assay of plasma and liver cholesterol concentration

Cholesterol concentration in the different lipoprotein density fractions (< 1.020; 1.020–1.055; > 1.055 g/ml) was measured after ultracentrifugation of pooled plasma samples. The cholesterol content of each fraction was measured colorimetrically (20). Hepatic concentration of free and esterified cholesterol was assayed as described (9, 21).

Determination of LDL receptor mRNA

Tissue levels of LDL receptor mRNA were determined in separate groups of control animals and animals treated with 17α-ethinyl estradiol. The contents of mRNA for LDL receptor and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined by nuclease protection (22–24). Single stranded ^{32}P-labeled cDNA probes were synthesized as previously described (22, 23). After tissue homogenization in guanidinium thiocyanate and isolation of RNA, total RNA (20–40 μg) was hybridized with the cDNA probes at 48°C overnight. Unhybridized probe was then digested with mung bean nuclease and the mRNA-protected ^{32}P-labeled probes were separated on 7 M urea, 6% polyacrylamide gels together with ^{32}P-labeled *Msp*I-digested pBR 322 size standards and identified by autoradiography (22–24). The radioactivity in each band, as well as background radioactivity, was quantitated using an isotopic imaging system (Ambis, Inc., San Diego, CA). The level of GAPDH, which did not change with estrogen treatment, was used to correct for any procedural losses.

Determination of LDL receptor protein levels

Tissue membrane proteins were solubilized according to Schneider et al. (25) and LDL receptor protein was determined by immunoblotting as described (23, 24). Solubilized proteins were separated by polyacrylamide gel electrophoresis and transferred to polyvinyldifluoride membrane. Membranes were blocked with 5% nonfat dried milk and incubated with a 1:200 dilution of polyclonal monospecific LDL receptor antiserum prepared in New Zealand White rabbits against the COOH-terminal peptide of the rat LDL receptor. After incubation with ^{125}I-labeled donkey anti-rabbit antibody, radiolabeled bands were identified by autoradiography, excised, and assayed for radioactivity in a gamma counter.

Calculations

Mean values ± SEM for groups of data are presented and when appropriate differences in mean values were tested for significance by Student's *t* test for unpaired data. All *P* values < 0.05 were considered statistically significant.

The evaluation of LDL transport in the different experimental conditions was performed by superimposing the experimental values of LDL-cholesterol uptake to the kinetic curves defining LDL uptake rates in the different tissues of the rat, as a function of circulating LDL-cholesterol. Such curves have been previously obtained in control rats infused with various amounts of lipoprotein-cholesterol, by non-linear regression analysis (26, 27). Transport parameters as previously described in the different tissues (27) were used.

RESULTS

Table 1 shows the effects of 17α-ethinyl estradiol and APP on plasma cholesterol levels. As shown, both drugs induced marked changes in circulating levels of total and LDL-cholesterol. Plasma total cholesterol fell from

TABLE 1. Effect of treatment with 17α-ethinyl estradiol and with APP on cholesterol concentration in plasma and in the different lipoprotein density fractions

Group	Plasma Cholesterol Concentration			
	Total	<1.020	1.020-1.055	>1.055
	mg/dl			
Controls	58 ± 3	3	11	44
EE-treated	10 ± 1[a]	4	2	4
Fasted controls	53 ± 5	3	9	41
APP-treated	5 ± 1[a]	1	2	2

The first two groups of animals received a daily injection of either diluent or 17α-ethinyl estradiol (EE) at the dose of 5 mg/kg s.c. for 5 days. The latter two groups were fasted throughout the treatment period and were injected daily with either diluent or APP (20 mg/kg) i.p. for 3 days; they received a continuous i.v. infusion of glucose and electrolytes throughout the 12–15 h preceding the experiment. Total plasma cholesterol was determined in individual animals and mean values ± SEM are shown for 11 animals in each group. The distribution of plasma cholesterol among the lipoprotein density fractions was determined in two pooled plasma samples from each group; mean values are shown.

[a] $P < 0.05$ versus the corresponding control value, Student's t test for independent data.

58 ± 3 to 10 ± 1 mg/dl after treatment with 17α-ethinyl estradiol and from 53 ± 5 to 5 ± 1 mg/dl after treatment with APP. Cholesterol concentration in the density fraction 1.020–1.055 g/ml, largely composed of LDL, dropped from 11 to 2 mg/dl with 17α-ethinyl estradiol and from 9 to 2 mg/dl with APP. Cholesterol content was also markedly reduced in the fraction greater than 1.055 g/dl with both treatments whereas changes in the lower density fraction were not detectable due to the very low basal values.

LDL transport is a curvilinear function of circulating LDL-cholesterol levels, and drastic reduction of LDL-cholesterol concentration per se, in the absence of changes in LDL receptor activity, may induce an increase in LDL clearance rates (27). Evaluation of LDL transport must therefore take into account the changes in LDL-cholesterol levels. This was done restoring plasma LDL-cholesterol levels to near-normal levels by concomitant infusion of mass amounts of lipoprotein to treated animals. Plasma total cholesterol concentration rose to 149 ± 13 mg/dl in estrogen-treated rats and to 110 ± 3 mg/dl in APP-treated rats (n = 11 in each group) after lipoprotein administration; LDL-cholesterol concentration was brought respectively to 9 mg/dl and 14 mg/dl (mean value from two pooled plasma samples in each group).

Figure 1 illustrates the effects of treatment with 17α-ethinyl estradiol (panel A) and with APP (panel B), with and without lipoprotein infusion, on specific rat LDL tissue spaces in liver, adrenals, ovary, and small bowel. As is shown, tissue spaces in the liver increased significantly after estrogen treatment and remained significantly higher even after restoring normal LDL-cholesterol levels. In the other organs tissue spaces also increased in treated animals, but decreased back to normal after lipoprotein infusion. These findings underline the importance of taking into account absolute LDL-cholesterol levels when evaluating changes in LDL transport. In comparison, after treatment with APP, a non-significant increase was observed in the organs studied whereas after lipoprotein infusion tissue spaces were significantly lower in the liver.

Figure 2 shows the effects of 17α-ethinyl estradiol (panel A) and APP (panel B), with or without lipoprotein infusion, on the rates of LDL clearance in all tissues examined. Whereas a significant increase in clearance rates was detected in several tissues after estrogen treatment, in the liver an important (nearly 5-fold) increase

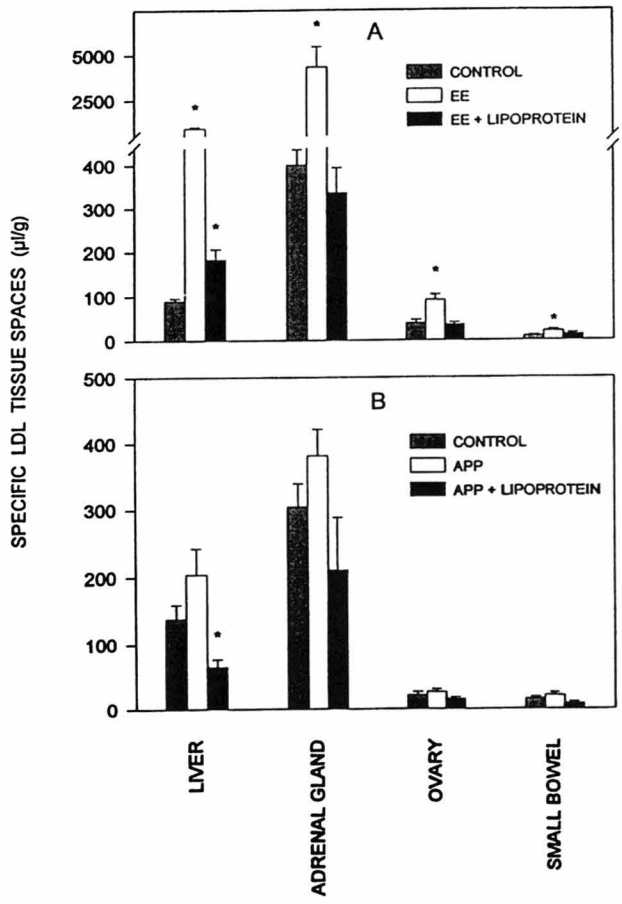

Fig. 1. Effect of 17α-ethinyl estradiol (EE) (panel A) and APP (panel B) on rat LDL tissue spaces in liver, adrenal gland, ovary, and small bowel. Animals were treated as described in Table 1. Tissue spaces of [^{14}C]sucrose-LDL were determined after i.v. administration, as described in the Materials and Methods section (9, 13, 19). Separate groups of treated rats also received mass amounts of homologous unlabeled lipoprotein along with the labeled LDL, to restore plasma LDL-cholesterol levels to normal. The values represent the mean ± SEM from 5 rats in each experimental group. *$P < 0.05$ versus controls, Student's t test for independent data.

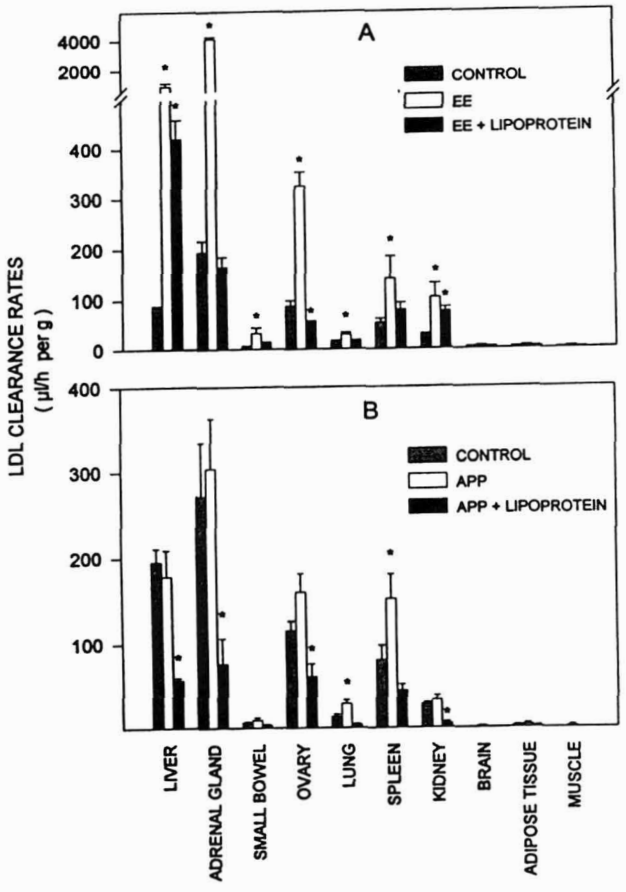

Fig. 2. Effect of treatment with 17α-ethinyl estradiol (EE) (panel A) and with APP (panel B) on the clearance rates of rat LDL. Animals were treated as described in Table 1. The figure shows the data from controls, animals treated with either drug, and treated animals that received a continuous infusion of homologous unlabeled lipoprotein, thus presenting nearly normal levels of circulating LDL-cholesterol. Data represent mean values ± SEM from 6 animals in each group. $*P < 0.05$ versus controls, Student's t test for independent data.

was still present after lipoprotein infusion. The kidney was the only other organ showing a statistically significant increase, though of lower magnitude. On the other hand, APP treatment significantly increased LDL clearance in lung and spleen, whereas after lipoprotein administration clearance rates were reduced in several tissues, including the liver. This finding is likely to reflect a nonspecific toxic effect, and supports the view that the hypolipidemic effect may be due to reduced lipoprotein production by the liver (14, 15).

The effect of treatment with 17α-ethinyl estradiol on LDL transport is better illustrated in **Fig. 3,** showing LDL-cholesterol uptake rates in liver and in the most important extrahepatic tissues. Uptake rates in control animals and treated plus lipoprotein-infused animals are plotted against the curves of LDL uptake as a function of LDL-cholesterol concentration, previously obtained in untreated rats (27). The changes observed in the liver are consistent with a 5-fold increase in LDL receptor number (as reflected by a corresponding change in J^m).

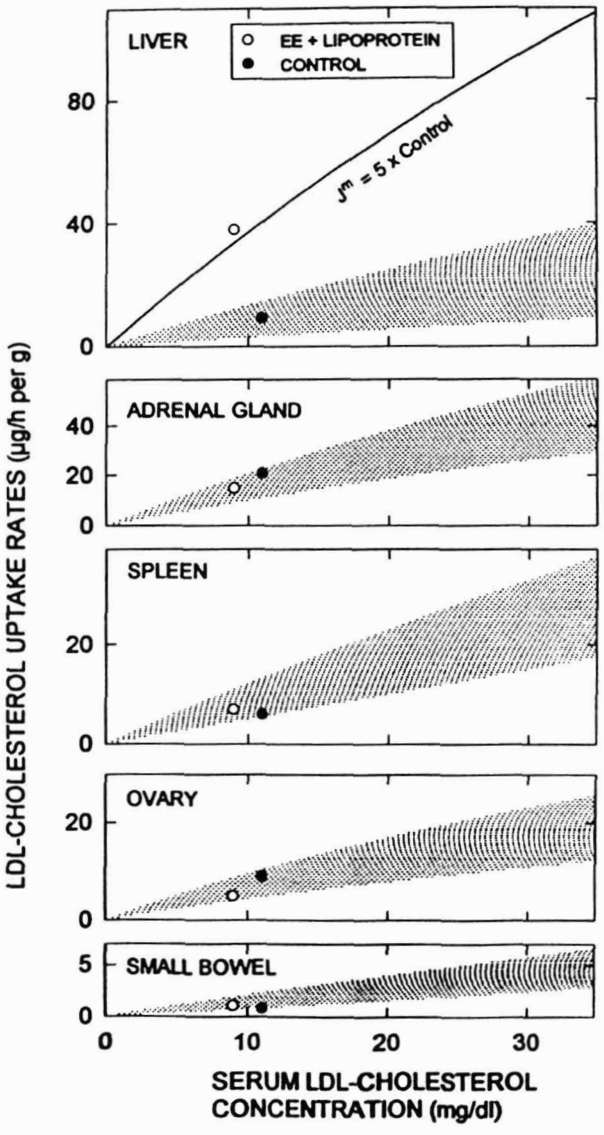

Fig. 3. Plots of the uptake rates of rat LDL versus plasma LDL-cholesterol concentration in five tissues in control animals (closed circles) and in animals treated with 17α-ethinyl estradiol (EE) (open circles) as indicated in Table 1. Treated animals also received a continuous infusion of unlabeled lipoprotein to maintain near normal values of LDL-cholesterol. The experimental results are superimposed on computer-generated nonlinear regression plots, whose kinetic parameters were derived from data obtained in normal rats infused with varying amounts of LDL-cholesterol (27). The shaded areas represent the kinetic curves relating LDL transport to LDL-cholesterol concentration in normal conditions. The continuous line shows the transport curve in the liver calculated with a maximal transport capacity (J^m) for LDL 5-fold greater than normal while the other constants were left unchanged. Data represent the mean values obtained from 6 animals in each group, with LDL-cholesterol values obtained from pooled plasma samples.

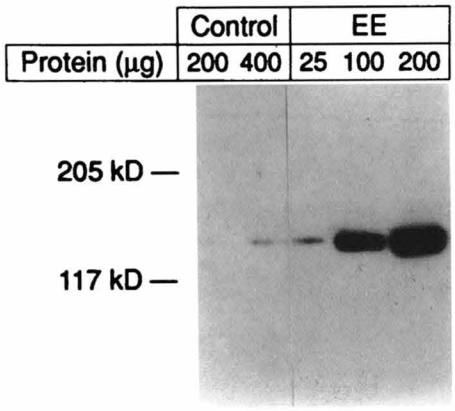

Fig. 4. Effect of treatment with 17α-ethinyl estradiol (EE) on hepatic content of LDL receptor protein. Animals were treated as detailed in Table 1. Immunoblotting of LDL receptor was performed from solubilized liver membrane proteins separated on polyacrylamide gels and then transferred to polyvinyldifluoride membranes. Membranes containing the indicated amount of protein were incubated with a 1:200 dilution of a monospecific rabbit antiserum raised against the COOH-terminal peptide of the rat LDL-receptor and then with ^{125}I-labeled donkey anti-rabbit antibody, and the dried membranes were subjected to autoradiography (23–25).

No changes in LDL receptor activity were detected in adrenal gland, spleen, ovary, and small bowel, as well as in the remaining tissues investigated (data not shown).

Figure 4 shows the effect of treatment with 17α-ethinyl estradiol on hepatic expression of LDL receptor protein. The changes observed with estrogen treatment are consistent with a more than 10-fold increase in receptor protein mass. In extrahepatic tissues LDL receptor protein bands could barely be shown by immunoblotting, with no detectable differences between treated and untreated animals (data not shown).

Figure 5 illustrates the changes induced by 17α-ethinyl estradiol on LDL receptor mRNA levels in liver (panel A) and in small bowel, spleen, kidney, and adrenal gland (panel B). The findings are summarized in panel C. As shown, hepatic mRNA content was increased 3.4-fold by estrogen treatment; in extrahepatic organs much smaller changes were observed and only in the kidney, which showed a 67% increase, was the difference statistically significant. In the rest of the tissues mRNA expression was barely detectable, with no apparent differences (data not shown).

Hepatic concentrations of esterified cholesterol were also profoundly affected by estrogen treatment: whereas the content of intrahepatic free cholesterol was essentially unchanged (controls: 2.9 ± 0.1 mg/g of liver; estrogen-treated: 3.1 ± 0.2 mg/g of liver) cholesteryl ester levels were significantly increased after 17α-ethinyl estradiol (controls: 0.4 ± 0.1 mg/g of liver; estrogen-treated: 3.0 ± 0.4 mg/g of liver, $P < 0.05$ vs. controls, Student's t test for unpaired samples).

Finally, **Fig. 6** shows the effects of 17α-ethinyl estradiol and APP on cholesterol synthesis in vivo in the different tissues. Treatment with 17α-ethinyl estradiol decreased cholesterol synthesis in the liver by 36% and significantly increased synthetic rates in a number of extrahepatic tissues, including small intestine, ovary, and spleen. An increased synthetic rate was also observed in the adrenal gland, although the difference was not statistically significant due to large data scatter. Treatment with APP almost completely suppressed cholesterol synthesis in the liver, consistent with a hepatotoxic effect, and significantly increased synthesis in several peripheral tissues (adrenals, small bowel, lung, spleen, and kidney).

DISCUSSION

The liver plays a central role in the regulation of cholesterol balance in the whole organism (3); changes in hepatic cholesterol synthesis and in hepatic lipoprotein synthesis or catabolism can significantly affect circulating cholesterol levels. In the experimental conditions of the present study, the marked hypocholesterolemic effects observed with 17α-ethinyl estradiol and APP is most likely to be due to a pharmacological effect at the hepatic level.

In the case of APP this hypolipidemic effect is not accompanied by increased expression of hepatic LDL receptors; LDL transport, on the other hand, is significantly depressed. This finding suggests a nonspecific toxic effect on the liver, leading to reduced production of lipoprotein (15, 16). This view is supported by the fact that hepatic cholesterol synthesis is also markedly suppressed.

On the other hand, hypocholesterolemia induced by treatment with 17α-ethinyl estradiol associates with, and is most likely due to, enhanced hepatic LDL receptor activity. Estrogen treatment was previously shown to stimulate hepatic LDL receptor expression (10–13) and LDL transport in the animal in vivo (28); data in humans also appear to support the occurrence of increased LDL turnover (29). Our experimental model, utilizing a primed-continuous infusion of labeled homologous LDL, clearly document a significant increase of in vivo LDL transport in rat liver. The present results are consistent with a nearly 5-fold increase in LDL receptor number (as reflected by the change in J^m), which is associated with a parallel increase of hepatic LDL receptor mRNA and protein content. The latter finding is consistent with previous evidence obtained in both rats and rabbits (30–32) and strongly suggests a direct effect at the transcriptional level.

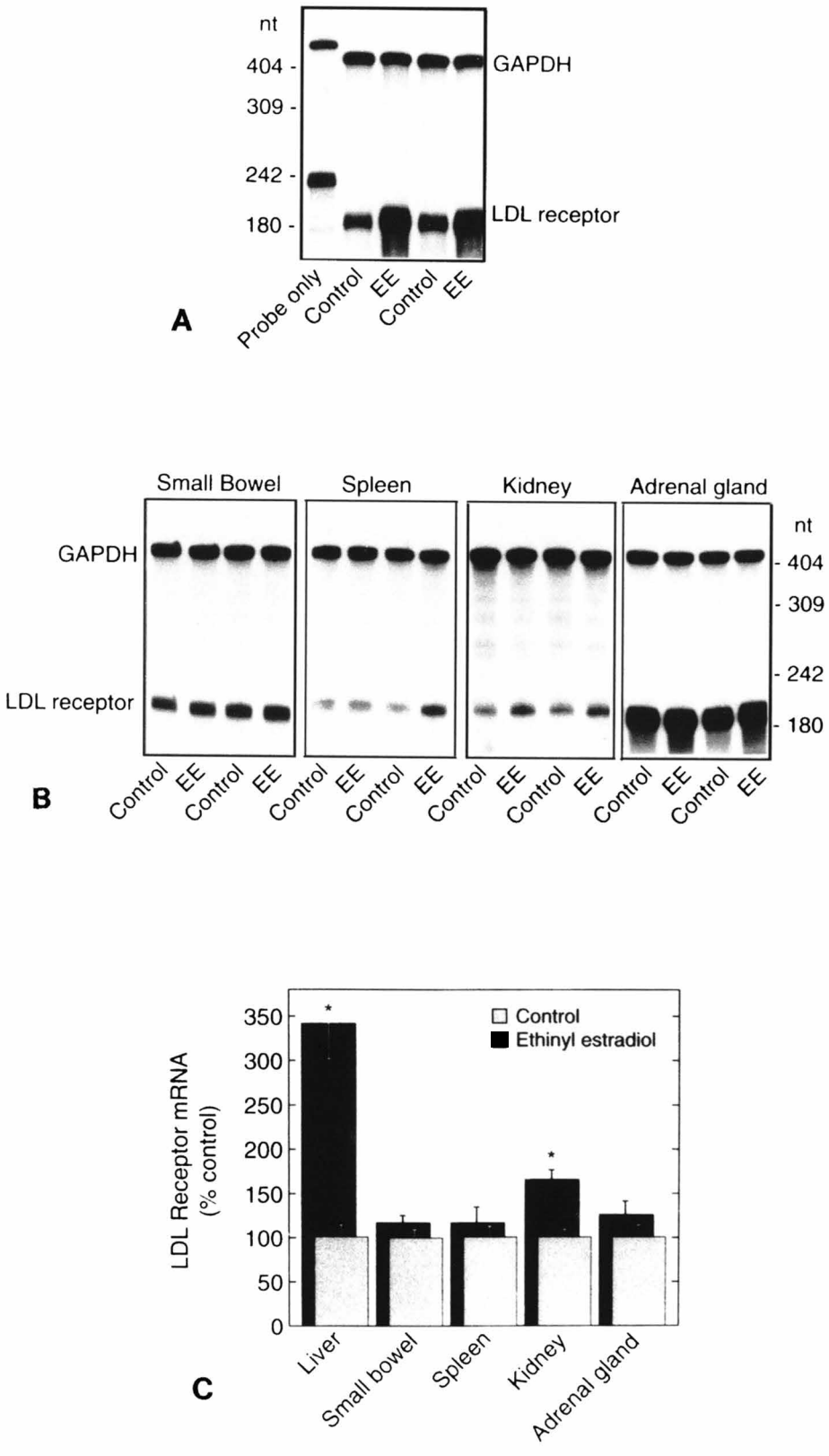

Fig. 5. Effect of treatment with 17α-ethinyl estradiol (EE) on the expression of LDL receptor mRNA in liver (panel A) and in four extrahepatic tissues (panel B). Animals were treated as detailed in Table 1. Hepatic RNA was hybridized with ^{32}P-labeled single-stranded cDNA probes and the protected bands resistent to mung bean nuclease digestion were analyzed by polyacrylamide gel electrophoresis followed by autoradiography (22–24). Panel C summarizes the findings from 5 animals in each group (mean percent value of control ± SEM). *$P <$ 0.05 versus controls, Student's t test for independent data.

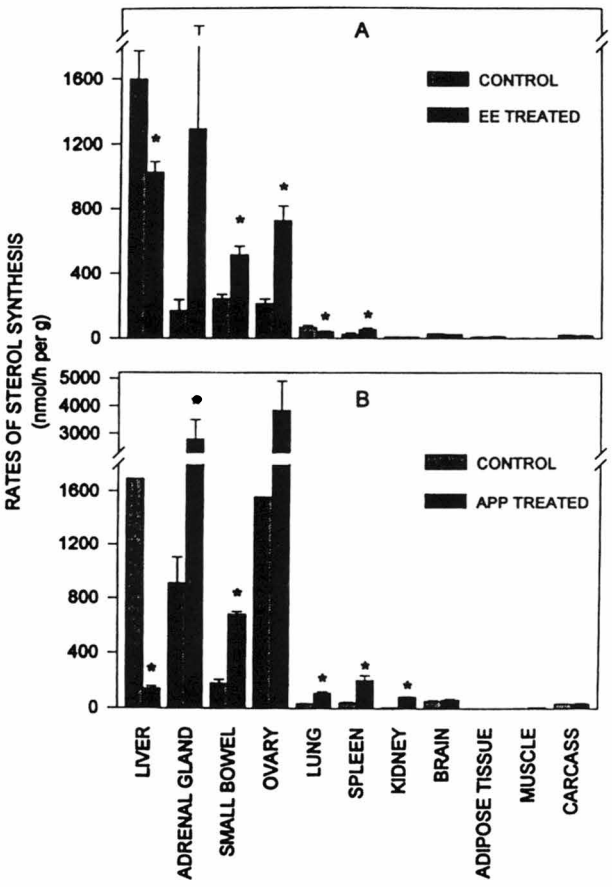

Fig. 6. Effect of 17α-ethinyl estradiol (EE) (panel A) and APP (panel B) on cholesterol synthesis in vivo in the different tissues. Animals were treated as described in Table 1. The rates of sterol synthesis were calculated from the amount of ^3H present in DPS after i.v. injection of a bolus of [^3H]water and were expressed as the nmol of [^3H]water incorporated per h per g of tissue (16, 17). Data represent the mean ± SEM obtained from 6 animals in each group. $*P < 0.05$ versus controls, Student's t test for independent data.

Interestingly, in other rodent species (hamsters and mice) estrogen treatment failed to induce any alteration in LDL receptor expression (30, 33) suggesting the presence of species-specific control sites; the discrepancy, at least with mice, seems to involve different regulation mainly at the post-transcriptional level (30). Alternatively, this might involve different regulatory elements in the LDL receptor gene. A different interference of regulatory hormones such as growth hormone (34) must also be considered.

On the other hand, hamsters are rather prone to modulate the activity of LDL receptors in conditions of altered cholesterol balance, whereas rats appear to be extraordinarily refractory to show changes in LDL transport with most experimental manipulations (9); this is probably due to the extremely high capacity to compensate for changes in cholesterol availability by adjusting the rates of cholesterol synthesis in this species. Indeed, estrogen treatment seems to be the most effective method capable of inducing a significant increase in hepatic LDL receptor activity in the rat.

We also observed a marked decrease in the circulating levels of cholesterol bound to the density fraction higher than 1.055 g/dl, mainly consisting of high density lipoprotein. With estrogen treatment, increased internalization of apolipoprotein E-containing lipoproteins by the liver might account for this finding (10, 13). The effect of APP treatment is more likely related to reduction in hepatic lipoprotein production; additionally, with both drugs, increased extrahepatic uptake of high density lipoprotein or high density lipoprotein-cholesterol (35, 36) might take place.

We cannot exclude the occurrence of changes in the qualitative composition of plasma lipoproteins, in particular of the apolipoprotein moiety. Previous evidence showed that treatment with either 17α-ethinyl estradiol or APP can shift the peak density for high density lipoprotein to higher density values (36). This finding, though, was not found to interfere with high density lipoprotein catabolism (36). We believe that under the present experimental conditions the effects of the dramatic reduction in circulating levels of lipoprotein prevail over those induced by possible qualitative alterations of apolipoprotein composition.

Treatment with 17α-ethinyl estradiol also induced a marked increase in hepatic concentration of cholesteryl esters with no changes in free cholesterol levels. This suggests activation of the microsomal enzyme acyl-CoA:cholesterol acyltransferase (ACAT). This relationship has long been suggested to be a consequence of increased internalization of cholesterol via the receptor-mediated pathway (2). Interestingly, recent evidence in the hamster also showed a strict correlation between the activity of the esterification pathway and LDL receptor expression in particular experimental conditions when the net sterol balance across the liver was not affected (37).

Cholesterol synthesis tended to be depressed in the liver of animals treated with 17α-ethinyl estradiol; this once again might be interpreted as a metabolic consequence of increased internalization of LDL-cholesterol (2). The finding, obtained in an experimental model where LDL receptor expression is markedly enhanced, most likely at the transcriptional level, brings support to the view of an independent regulation of the two pathways (see later).

In conditions of reduced systemic availability of cholesterol due to marked hypocholesterolemia, as observed with both 17α-ethinyl estradiol and APP, extrahepatic tissues did not respond by increasing LDL receptor activity, as reflected by the data on the parame-

ters of LDL transport. In some organs of estrogen-treated animals an increase in LDL tissue spaces and clearance rates could be observed due to desaturation of the LDL transport system in conditions of low LDL-cholesterol levels, but almost no tissue showed a significant increase of LDL clearance when LDL-cholesterol levels were brought back to normal by lipoprotein infusion; only in the kidney did LDL clearance rates remain significantly higher, even when the increase was much lower than in the liver, and not suggestive of a change in LDL transport parameters.

The findings with LDL receptor mRNA expression strongly support this view. Treatment with 17α-ethinyl estradiol induced a more than 3-fold increase in LDL receptor mRNA content in the liver whereas changes in the extrahepatic tissues were minor; again, among these only the kidney showed a statistically significant increase. The data are in close agreement with recent experimental evidence in the rat (32). Considering the complex physiology of the kidney, comparable to that of the liver, and its limited involvement in cholesterol homeostasis, we speculate a direct effect of 17α-ethinyl estradiol at the transcriptional level, as in hepatic tissue, rather than a metabolic effect mediated by hypocholesterolemia.

On the other hand, a number of tissues showed a significant decrease in LDL clearance and LDL transport parameters after treatment with APP, possibly due to a toxic effect.

Several organs instead showed a significant increase of in vivo cholesterol synthesis; these included mainly the organs with relevant receptor-dependent LDL uptake (27) and rapidly exchanging cholesterol with the blood and tissues. The brain and the tissues of the carcass, whose cholesterol mainly belongs to the nonexchangeable body pool (1), were not able to derepress cholesterol synthesis even in conditions of extreme hypocholesterolemia.

The dissociation between LDL transport and cholesterol synthesis, observed in the majority of extrahepatic organs, is in disagreement with previous findings obtained in different experimental models, where the two steps were shown to vary in parallel (5) and with the evidence of a coordinate regulation of HMG-CoA reductase and LDL receptor protein at the transcriptional level (4, 6, 38).

On the other hand, the data are strictly in line with a body of evidence obtained in the experimental animal in vivo, in the liver (8, 9, 39) and in the small bowel (7). In conditions of altered functional need, most organs appear to respond primarily by modulating the rates of de novo cholesterol synthesis; only when this mechanism proves insufficient, do alterations in LDL receptor expression appear to take place (9). The present findings for the first time bring clear additional information regarding the other extrahepatic tissues, and strongly support the hypothesis of an independent regulation of cholesterol synthesis and LDL transport under in vivo conditions.

This might involve the presence of separate functional pools of intracellular free cholesterol, or a different level of regulation of the two pathways by a single cholesterol pool. It is worth recalling on this regard that coordinate transcription of the HMG-CoA reductase and LDL receptor genes, as recently shown, does not necessarily imply coordinate changes in the activity of the two proteins (22) and that the activity of HMG-CoA reductase, unlike the expression of LDL receptors and of other enzymes involved in the control of cholesterol homeostasis, has been shown to be regulated mainly at the post-transcriptional level (40).

In conclusion, the present findings, complementing the analysis of lipoprotein transport in vivo and the assay of the hepatic content of receptor mRNA and protein mass, clearly confirm the marked stimulatory effect of pharmacological doses of 17α-ethinyl estradiol on the expression of hepatic LDL receptors in the rat. Pharmacologically induced hypocholesterolemia is able to derepress cholesterol synthesis, but not LDL receptor activity, in extrahepatic tissues. Diversity in the levels of regulation of these two pathways may certainly account for distinct metabolic behaviors regarding cholesterol homeostasis and its perturbations, observed in different individuals and in different species.

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