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EFFECTS OF THE ANTIESTROGEN LY 117018 ON THE MODULATION BY ETHINYL ESTRADIOL OF THE METABOLISM OF [1-^{14}C]OLEIC ACID BY PERFUSED LIVERS FROM NORMAL AND OVARIECTOMIZED RATS*

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Abstract—Intact female Sprague–Dawley rats (195–249 g) and rats that had been ovariectomized (210–285 g) were injected subcutaneously for 14 days with ethinyl estradiol (15 μg/kg), the antiestrogen LY 117018 (500 μg/kg), both drugs simultaneously, or the vehicle (sesame oil) alone. Livers were removed and perfused *in vitro* in a recycling system. The administration of LY 117018 alone did not affect the secretion of triacylglycerol by livers from normal rats but decreased the secretion of triacylglycerol by livers from ovariectomized rats. When the drugs were administered concurrently to either normal or ovariectomized animals, the increase in the concentration of triacylglycerol and the decrease in the concentration of cholesteryl esters in the plasma produced by ethinyl estradiol were prevented. When administered to either intact or ovariectomized rats, ethinyl estradiol alone stimulated the synthesis and secretion of triacylglycerol and cholesteryl esters by perfused livers isolated from these animals. The simultaneous administration of LY 117018 with ethinyl estradiol prevented this stimulation of the hepatic synthesis and secretion of triacylglycerol and cholesteryl esters. The depression of ketogenesis observed with livers from rats administered ethinyl estradiol alone was reversed by concurrent administration of LY 117018. The concurrent administration of the estrogen and antiestrogen did not result, however, in a complete blockade of the estrogen-induced elevation of hepatic triacylglycerol synthesis and depression of ketogenesis. The incorporation of [1-^{14}C]oleic acid into the triacylglycerol and ketone bodies by livers from ovariectomized rats was less than that of livers from normal rats. It is clear that the antiestrogen antagonizes the actions of ethinyl estradiol on hepatic lipid metabolism. Furthermore, the use of the antiestrogen LY 117018 in these experiments allows the probable conclusion that the modulation of hepatic metabolism of fatty acid by estrogen is mediated by conventional estrogenic receptors.

An inherent characteristic of antiestrogenic drugs is the variable degrees of estrogenicity they possess. LY 117018 [hydroxy-2(*p*-hydroxyphenyl)benzo⟨*b*⟩-thien-3-yl-*p*-(2-pyrollidinyl-ethoxyphenylketone] is a recently developed drug which has minimal estrogenicity as determined by its uterotrophic potential in ovariectomized rats [1]. Drugs with estrogenic potential may be expected to affect hepatic lipid metabolism. It is known that estrogenic drugs elevate serum triacylglycerol concentrations [2], depress serum cholesterol [3, 4], stimulate secretion of very low density lipoprotein (VLDL) triacylglycerol by the liver [5–8] and depress ketogenesis [6]. Furthermore, ovariectomy of the animal results in a decrease in hepatic secretion of triacylglycerol, which is reversible with estrogen therapy [7–9]. The actions of estrogens on the cell are assumed to be mediated by cytosolic receptors [10]. Thompson *et al.* [11] correlated the concentration of hepatic estrogen receptors with the estrogen-mediated elevation of the plasma VLDL. Presumably, the actions of estrogens on hepatic lipid metabolism are mediated by interactions with cytosolic estrogenic receptors. Conversely, antiestrogenic drugs such as LY 117018, which interact with these same receptors, may also affect hepatic lipid metabolism and the concentrations of serum lipids. Since antiestrogenic drugs are administered chronically for therapeutic objectives, it is of value to determine possible effects such drugs may have on hepatic lipid metabolism. These studies were carried out, therefore, in normal and ovariectomized rats, and in perfused livers obtained from these animals.

METHODS

Virgin female Sprague–Dawley rats, or rats that had been ovariectomized, were obtained from Harlan Co., Indianapolis, IN. Treatment of the normal animals with drugs began following a 5-day acclimatization period. During treatment with drugs, the animals were housed individually in metabolic cages under controlled temperature (25°) and lighting (5:00 a.m. to 5:00 p.m.). The animals were divided into four treatment groups, and were injected subcutaneously for 14 days with vehicle, LY 117018 (a gift of E. Diller, Eli Lilly & Co., Indianapolis,

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IN), ethinyl estradiol or both drugs. The LY 117018 (5.0 mg) and the ethinyl estradiol (1.5 mg) were each dissolved in separate aliquots of absolute ethanol (0.5 ml). The drugs were diluted with sesame oil such that 500 μg/kg body weight of LY 117018 and 15 μg/kg body weight of ethinyl estradiol were injected in 1.0 ml sesame oil/kg body weight daily, as indicated. The control group received vehicle alone. The sites of injection were alternated daily.

The dose of LY 117018 used was selected from a preliminary dose/response experiment (the range of LY 117018 was 10–5000 μg/kg body weight/day for 14 days. The selected dose was the highest one that did not affect the secretion of triacylglycerol by the liver (data not shown). This gave a weight ratio of 33/1 (antiestrogen/estrogen) which was necessary to demonstrate an effect of antiestrogen [12]. In our experience [5, 7] and that of others [13], food consumption decreases when estrogenic steroids are administered to rats. Animals, therefore, were match-fed to the group receiving both estrogen and antiestrogen to minimize differences that might have resulted indirectly with the administration of either LY 117018 or ethinyl estradiol. At day 10 of the feeding regimen, all groups of rats were consuming comparable rations. All animals had access to food and water until they were killed. The animals were anesthetized lightly with diethyl ether, and livers were removed surgically for perfusion [14]. Just prior to cannulation of the hepatic portal vein, blood was obtained (2–4 ml) from the abdominal aorta in ethylene diaminetetraacetic acid (0.1%) coated syringes for estimation of plasma lipids. The livers were perfused in a recycling system [15] *in vitro* under conditions described previously [6]. A complex of oleic acid (145 mg) (Nu Chek Prep, Elysian, MN) and [1-^{14}C]oleic acid (10 μCi) (New England Nuclear, Boston, MA) with bovine albumin was prepared [14] and infused to give a steady-state concentration of 0.47 ± 0.03 mM fatty acid in the erythrocyte-free perfusate. Triacylglycerol, cholesterol, cholesteryl esters and fatty acid were determined chemically as described previously [16]. Ketone bodies were determined on an aliquot of cell-free perfusate [17]. The uptake of fatty acid by the liver was calculated as described earlier [17]. The incorporation of [1-^{14}C]oleic acid into lipids [18] or into ketone bodies [19] was determined by liquid scintillation spectroscopy. Statistical evaluation of the differences between means was determined from a two-tailed unpaired Student's *t*-test.

RESULTS

The body and liver weights of normal and ovariectomized rats are presented in Table 1. The amount of fatty acid taken up by the perfused livers from these animals is also given in Table 1. At the initiation of the 14-day treatment period, the ovariectomized rats weighed more than the controls. After the 14-day treatment period, this difference remained. Even though the groups of rats were match fed to the group receiving both drugs, by day 10 of the feeding regimen all groups were consuming similar quantities of ration. Clearly, the body weights of both groups of rats were unaffected by the administration of LY 117018, ethinyl estradiol or a combination of both drugs. However, ovariectomy increased the liver weights in groups receiving LY 117018, ethinyl estradiol or both drugs in the normal animals. In comparison to the control, the liver weights were increased by the administration of either LY 117018 or ethinyl estradiol. Administration of both drugs to normal rats resulted in a decrease in liver weight. The uptake of fatty acid by livers of normal rats was unaffected by any treatment. Ovariectomy reduced fatty acid uptake of livers from rats receiving LY 117018, ethinyl estradiol or both drugs.

In confirmation of previous studies [3, 4], estrogen increased triacylglycerol and decreased cholesteryl esters in the plasma of normal animals (Table 2). The antiestrogen LY 117018 did not affect the concentration of triacylglycerol, cholesterol or cholesteryl esters in the plasma of normal rats. In contrast to previously reported data, for unexplained reasons the concentration of plasma triacylglycerol in the control group was reduced by ovariectomy [8, 10]. The concentration of cholesteryl esters was not affected by ovariectomy, while that of free cholesterol was reduced (Table 2). Treatment with LY 117018

Table 1. Effects of LY 117018 and ethinyl estradiol on body weight, liver weight and uptake of oleic acid by livers from normal and ovariectomized rats

	Group	Body wt (g)		Liver wt (g)		Uptake oleic acid (μmol/g liver/3 hr)	
		N	OVX	N	OVX	N	OVX
(A)	Control	203 ± 5^{c}	227 ± 6^{abc}	5.7 ± 0.3^{gh}	6.1 ± 0.2^{jk}	48.0 ± 3.3	53.9 ± 1.3^{pq}
(B)	LY 117018	216 ± 4^{d}	274 ± 4^{ad}	7.3 ± 0.3^{gm}	8.3 ± 0.2^{gm}	47.2 ± 1.7^{r}	39.7 ± 0.8^{pr}
(C)	Ethinyl Estradiol	203 ± 5^{l}	258 ± 7^{bl}	7.8 ± 0.3^{hin}	10.3 ± 0.2^{kln}	40.5 ± 1.6^{s}	32.2 ± 1.5^{qs}
(D)	LY 117018 plus ethinyl estradiol	213 ± 6^{f}	250 ± 4^{f}	6.8 ± 0.2^{io}	9.0 ± 0.5^{lo}	42.7 ± 1.0^{t}	36.0 ± 1.0^{t}

Normal (N) and ovariectomized (OVX) rats weighed 203 ± 3.3 and 292 ± 3.4 g (means ± SE, N = 16), respectively, at the start of the 14-day treatment period. In this experiment, the rats received no treatment (Group A); LY 117018, 500 μg/kg/14 days (Group B); ethinyl estradiol, 15 μg/kg/14 days (Group C); or both drugs simultaneously (Group D). Values are means ± SE for four observations. Uptake of oleic acid was calculated as reported previously [17]. Numbers in each column with identical superscripts are significantly different from one another with a $P < 0.05$.

Table 2. Effects of LY 117018 and ethinyl estradiol on the concentrations of triacylglycerol and cholesterol and cholesteryl esters in the plasma of normal and ovariectomized rats

	Group	Triacylglycerol (μmol/ml)		Cholesterol (μmol/ml)		Chol. ester (μmol/ml)	
		N	OVX	N	OVX	N	OVX
(A)	Control	0.24 ± 0.04^{ag} (13)	0.06 ± 0.01^{deg} (4)	0.53 ± 0.04^{l}	0.36 ± 0.03^{jkl}	0.92 ± 0.05^{o}	1.19 ± 0.08^{qr}
(B)	LY 117018	0.22 ± 0.02^{bh} (14)	0.41 ± 0.04^{dh} (4)	0.46 ± 0.11	0.56 ± 0.05^{j}	0.85 ± 0.05	0.84 ± 0.04^{q}
(C)	Ethinyl Estradiol	0.64 ± 0.09^{aci} (5)	1.02 ± 0.03^{efi} (4)	0.46 ± 0.03^{m}	0.83 ± 0.07^{km}	0.53 ± 0.02^{opt}	0.24 ± 0.01^{rst}
(D)	LY 117018 plus ethinyl estradiol	0.35 ± 0.06^{bc} (5)	0.48 ± 0.05^{f} (4)	0.46 ± 0.03^{n}	0.73 ± 0.07^{n}	0.90 ± 0.06^{pu}	0.52 ± 0.06^{su}

Plasma was obtained from normal (N) and ovariectomized (OVX) rats that received no treatment (Group A); LY 117018, 500 μg/kg/14 days (Group B); ethinyl estradiol, 15 μg/kg/14 days (Group C); or both drugs simultaneously (Group D). The samples of plasma were obtained from the abdominal aorta just prior to the cannulation of the hepatic portal vein. The number of observations are shown in parentheses. Values given are means ± SE. Numbers in each column with identical superscripts are significantly different from one another with a $P < 0.05$.

Table 3. Effects of LY 117018 and ethinyl estradiol on the secretion of triacylglycerol and incorporation of [1-^{14}C]oleate into perfusate triacylglycerol by livers from normal and ovariectomized rats.

Group		Secretion (μmol/g liver)		Incorporation (dpm/g liver $\times 10^{-3}$)		Specific activity (dpm/μmol $\times 10^{-3}$)	
		N	OVX	N	OVX	N	OVX
(A)	Control	1.86 ± 0.17^{ag}	1.28 ± 0.12^{cdg}	186.0 ± 21.6^{im}	67.8 ± 10.9^{km}	101.5 ± 9.5^{q}	52.3 ± 4.2^{oq}
(B)	LY 117018	1.57 ± 0.15^{h}	0.90 ± 0.04^{efh}	164.2 ± 13.3^{n}	51.1 ± 4.1^{n}	106.9 ± 12.3^{r}	69.7 ± 15.1^{pr}
(C)	Ethinyl estradiol	3.43 ± 0.31^{ab}	3.23 ± 0.35^{de}	361.4 ± 46.2^{ij}	261.7 ± 38.6^{kl}	105.5 ± 12.9^{s}	80.1 ± 3.1^{ops}
(D)	LY 117018 and ethinyl estradiol	1.24 ± 0.17^{b}	1.53 ± 0.12^{ef}	133.3 ± 31.4^{j}	98.5 ± 7.5^{l}	103.3 ± 13.1^{t}	65.8 ± 5.8^{pt}

Livers were removed from normal (N) and ovariectomized (OVX) rats that received no treatment (Group A); LY 117018, 500 μg/kg/14 days (Group B); ethinyl estradiol, 15 μg/kg/14 days (Group C); or both drugs simultaneously (Group D). The livers were removed and perfused in a recycling system. Values for the secretion of triacylglycerol and the incorporation of [1-^{14}C]oleic acid into perfusate triacylglycerol are cumulative amounts at the end of the 3-hr perfusion period. Values given are means ± SE of four observations. Numbers in each column with identical superscripts are significantly different from one another with a $P < 0.05$.

or ethinyl estradiol increased the concentration of triacylglycerol in the plasma of castrated rats, but not when the animals were pretreated with both drugs concurrently. Concurrent administration of both drugs to unoperated rats restored the concentration of triacylglycerol and cholesteryl ester in plasma of normal rats to control levels.

The stimulatory effect of ethinyl estradiol on secretion of triacylglycerol by the perfused liver, and on the incorporation of [1-^{14}C]oleic acid into triacylglycerol, was prevented by LY 117018 with perfused livers from normal and ovariectomized rats (Table 3). LY 117018 alone was without effect on secretion of triacylglycerol. Ovariectomy reduced the output of triacylglycerol in the untreated group and the group of rats that received LY 117018. The incorporation of oleic acid into triacylglycerol was reduced by castration in untreated animals, and in animals that received LY 117018 but not ethinyl estradiol or both drugs. In the castrated group, the specific activity of the secreted triacylglycerol was increased by ethinyl estradiol and reduced by administration of both drugs. Castration reduced the specific activity of triacylglycerol regardless of treatment of the liver donor.

Although the hepatic secretion of total free cholesterol was unaffected by any treatment (data not shown), livers from animals that received ethinyl estradiol secreted more cholesteryl esters than did livers from untreated rats (Table 4). Furthermore, the stimulation of secretion of cholesteryl esters by the estrogen was restored to control levels by simultaneous treatment with LY 117018. Incorporation of [1-^{14}C]oleic acid into cholesteryl esters was stimulated by ethinyl estradiol and reduced by concurrent administration of LY 117018 to the donor rat. LY 117018 also reduced the incorporation of [1-^{14}C]oleic acid into cholesteryl esters with livers from ovariectomized but not with livers from control rats. Specific activity was increased by ethinyl estradiol and was reduced in livers from normal animals that received both drugs.

Table 4. Effects of LY 117018 and ethinyl estradiol on the secretion of cholesteryl esters and incorporation of [1-^{14}C]oleate into perfusate cholesteryl esters by livers from normal and ovariectomized rats

Group		Secretion (μmol/g liver)		Incorporation (dpm/g liver $\times 10^{-3}$)		Specific activity (dpm/μmol $\times 10^{-3}$)	
		N	OVX	N	OVX	N	OVX
(A)	Control	0.41 ± 0.11^{a}	0.19 ± 0.03^{c}	7.1 ± 1.6^{g}	5.7 ± 1.0^{kij}	18.7 ± 2.6^{oq}	35.4 ± 8.5^{q}
(B)	LY 117018	0.29 ± 0.04	0.15 ± 0.02	7.0 ± 0.0^{l}	2.5 ± 0.5^{il}	28.0 ± 7.7	21.9 ± 6.3
(C)	Ethinyl estradiol	0.67 ± 0.05^{abe}	0.49 ± 0.06^{cde}	26.6 ± 1.6^{gnm}	13.1 ± 1.5^{jkm}	39.8 ± 1.9^{opr}	27.6 ± 4.2^{r}
(D)	LY 117018 and ethinyl estradiol	0.25 ± 0.02^{bf}	0.13 ± 0.03^{df}	7.1 ± 0.7^{gmn}	3.7 ± 1.0^{kmn}	28.5 ± 2.6^{p}	22.1 ± 3.6

Livers were removed from normal (N) and ovariectomized (OVX) rats that received no treatment (Group A); LY 117018, 500 μg/kg/14 days (Group B); ethinyl estradiol, 15 μg/kg/14 days (Group C); or both drugs simultaneously (Group D). The livers were removed and perfused in a recycling system. Values for the secretion of cholesteryl esters and the incorporation of [1-^{14}C]oleic acid into perfusate cholesteryl esters are cumulative amounts at the end of the 3-hr perfusion period. Values given are means ± SE of four observations. Numbers in each column with identical superscripts are significantly different from one another with a $P < 0.05$.

Table 5. Effects of LY 117018 and ethinyl estradiol on ketogenesis by livers from normal and ovariectomized rats

Group		Output (μmol/g liver)		Synthesis (dpm/g liver × 10^{-3})		Specific activity (dpm/μmol × 10^{-3})	
		N	OVX	N	OVX	N	OVX
(A)	Control	262.1 ± 49.5^{abg}	150.3 ± 28.7^{deg}	940.2 ± 150.3^{ko}	417.0 ± 123.4^{mo}	3.6 ± 0.1^{s}	3.4 ± 1.5
(B)	LY 117018	134.4 ± 8.4^{ah}	41.5 ± 2.6^{dh}	637.8 ± 77.6^{p}	264.8 ± 75.9^{p}	4.7 ± 0.5	6.5 ± 1.9
(C)	Ethinyl estradiol	46.2 ± 13.7^{bci}	8.3 ± 2.3^{efi}	299.0 ± 42.7^{klq}	48.6 ± 8.4^{mnq}	7.1 ± 1.1^{s}	6.3 ± 0.7
(D)	LY 117018 plus ethinyl estradiol	149.5 ± 17.2^{cj}	57.7 ± 6.7^{fj}	660.5 ± 83.4^{lr}	254.9 ± 51.1^{mnr}	4.7 ± 0.2	4.9 ± 1.5

Livers were removed from normal (N) and ovariectomized (OVX) rats that received no treatment (Group A); LY 117018, 500 μg/kg/14 days (Group B); ethinyl estradiol, 15 μg/kg/14 days (Group C); or both drugs simultaneously (Group D). The livers were removed and perfused in a recycling system. Values for the production of ketone bodies, expressed as μmol acetone, and for the incorporation of [1-^{14}C]oleic acid into ketone bodies in the perfusate are cumulative amounts at the end of the 3-hr perfusion period. Values given are means ± SE of four observations. Numbers in each column with identical superscripts are significantly different from one another with a $P < 0.05$.

As reported previously [6] treatment with ethinyl estradiol reduced ketogenesis by livers from normal rats; similar effects were observed with livers from ovariectomized animals (Table 5). Ketogenesis by livers from animals treated with LY 117018 was reduced by 48.7 and 72.4% in normal and ovariectomized rats respectively (mass output). When both drugs were administered concurrently, the net output of total ketone bodies increased, although the rates did not return to control values. The incorporation of [1-^{14}C]oleic acid into ketone bodies was reduced 69.3% and 88.4% by ethinyl estradiol with livers from normal and ovariectomized rats respectively. Concurrent administration of LY 117018 and ethinyl estradiol increased the rate of incorporation to levels observed with livers from animals treated with the antiestrogen alone.

Effects of LY 117018 and ethinyl estradiol on the concentration of triacylglycerol and on the incorporation of [1-^{14}C]oleic acid into hepatic triacylglycerol are shown in Table 6. The concentration of hepatic triacylglycerol, measured at the termination of the perfusion, was reduced by castration and increased by treatment with LY 117018, ethinyl estradiol, or both drugs. Incorporation of the radioactive oleic acid into triacylglycerol of livers from normal rats was stimulated by ethinyl estradiol, as reported previously [5], and was also increased in livers from castrated animals. The incorporation of oleic acid into triacylglycerol was also stimulated in livers from LY 117018 treated rats. Concurrent administration of both drugs decreased the incorporation of fatty acid into triacylglycerol in livers from normal female rats. In contrast, livers from castrated rats treated with both drugs still incorporated more oleic acid into triacylglycerol than the control group.

The effects of LY 117018 and ethinyl estradiol on the hepatic concentrations of cholesteryl esters and on the incorporation of [1-^{14}C]oleic acid into cholesteryl esters by livers from normal and ovariectomized rats are presented in Table 7. Increased

Table 6. Effects of LY 117018 and ethinyl estradiol on the incorporation of [1-^{14}C]oleate into triacylglycerol in perfused livers from normal and ovariectomized rats

Group		Concentration (μmol/g liver)		Total incorporation (dpm/g liver × 10^{-3})		Specific activity (dpm/μmol × 10^{-3})	
		N	OVX	N	OVX	N	OVX
(A)	Control	2.42 ± 0.28^{abe}	1.62 ± 0.24^{cde}	138.9 ± 23.6^{ghl}	74.7 ± 13.1^{jkl}	57.1 ± 7.3^{no}	46.3 ± 4.6^{q}
(B)	LY 117018	5.96 ± 0.57^{a}	5.35 ± 0.41^{c}	234.4 ± 46.7^{gm}	117.5 ± 14.0^{jm}	39.1 ± 7.0^{nt}	21.9 ± 1.9^{qrt}
(C)	Ethinyl estradiol	4.17 ± 0.33^{b}	4.52 ± 0.43^{d}	320.1 ± 50.4^{hi}	235.1 ± 27.0^{k}	75.7 ± 8.0^{opu}	53.7 ± 3.1^{su}
(D)	LY 117018 plus ethinyl estradiol	3.62 ± 0.32^{f}	5.49 ± 0.27^{ef}	171.8 ± 30.2^{i}	191.2 ± 20.9	46.8 ± 6.5^{p}	35.0 ± 3.9^{rs}

Normal (N) and ovariectomized (OVX) rats received no treatment (Group A); LY 117018, 500 μg/kg/14 days (Group B); ethinyl estradiol, 15 μg/kg/14 days (Group C); or both drugs simultaneously (Group D). After perfusion, the livers were treated as described in Methods. Analyses of the livers were performed on samples of tissue obtained after 3 hr of perfusion. Data are expressed as μmol triacylglycerol/g liver and as incorporation of [1-^{14}C]oleic acid into triacylglycerol (dpm/g liver). Values given are means ± SE of four observations. Numbers in each column with identical superscripts are significantly different from one another with a $P < 0.05$.

Table 7. Effects of LY 117018 and ethinyl estradiol on the incorporation of [1-^{14}C]oleate into cholesteryl esters in perfused livers from normal and ovariectomized rats

	Group	Concentration (μmol/g liver)		Total incorporation (dpm/g liver × 10^{-3})		Specific activity (dpm/μmol × 10^{-3})	
		N	OVX	N	OVX	N	OVX
(A)	Control	0.44 ± 0.06^{ac}	0.61 ± 0.08^{cf}	7.7 ± 1.4^{i}	7.7 ± 2.0^{k}	17.7 ± 2.6^{n}	12.5 ± 2.3
(B)	LY 117018	0.82 ± 0.03^{abh}	1.04 ± 0.05^{eh}	11.0 ± 1.3	7.7 ± 0.7	13.4 ± 1.7^{p}	7.6 ± 0.8^{p}
(C)	Ethinyl estradiol	1.35 ± 0.09^{bcd}	1.19 ± 0.08^{fg}	34.0 ± 6.3^{ijm}	16.7 ± 0.5^{klm}	24.7 ± 1.9^{moq}	14.3 ± 1.1^{q}
(D)	LY 117018 plus ethinyl estradiol	0.77 ± 0.07^{cd}	0.80 ± 0.07^{g}	12.5 ± 4.0^{j}	9.0 ± 1.3^{l}	15.4 ± 3.4^{o}	11.7 ± 2.4

Normal (N) and ovariectomized (OVX) rats received no treatment (Group A); LY 117018, 500 μg/kg/14 days (Group B); ethinyl estradiol, 15 μg/kg/14 days (Group C); or both drugs simultaneously (Group D). After perfusion, the livers were treated as described in Methods. Analyses of the livers were performed on samples of tissue obtained after 3 hr of perfusion. Values given are means ± SE of four observations. Data are expressed as cholesteryl esters, μmol/g liver, and as incorporation of [1-^{14}C]oleic acid into cholesteryl esters (dpm/g liver). Numbers in each column with identical superscripts are significantly different from one another with a $P<0.05$.

hepatic concentrations of cholesteryl esters were observed after perfusion of livers from rats treated with ethinyl estradiol or LY 117018. Ethinyl estradiol stimulated the incorporation into cholesteryl ester by livers from normal and ovariectomized rats. When LY 117018 and ethinyl estradiol were given to either group of rats concurrently, the concentration of cholesteryl esters was reduced, albeit not to control levels.

DISCUSSION

The hypertriglyceridemic effect of ethinyl estradiol was prevented by LY 117018. This, in part, may result from reduction by LY 117018 of the estrogenic effect on stimulation of triacylglycerol output by the liver. Lower doses of ethinyl estradiol (5 μg/kg) apparently do not affect the concentration of triacylglycerol in the serum [5]. At a higher dose (15 μg/kg), hepatic output may exceed clearance and hypertriglyceridemia results. In the ovariectomized rat, estrogen receptors are decreased in number in estrogen responsive tissues such as the liver [10] and, simultaneously, the serum concentration of triacylglycerol is reduced. Perhaps plasma clearance of triacylglycerol is less sensitive to the depletion of estrogen than are the synthesis and secretion of VLDL triacylglycerol by the liver [5]. The livers from ovariectomized rats may also be more sensitive to estrogenic drugs, and this may account for the fact that treatment of the ovariectomized rats with LY 117108, even with its relatively low intrinsic estrogenic activity, resulted in the observed increase in serum triacylglycerol. Nevertheless, when administered concurrently to ovariectomized rats, the antiestrogen lowered, but did not eliminate, the augmentation by ethinyl estradiol of plasma triacylglycerol (Table 2). In contrast to the effects on triacylglycerol, the antiestrogen alone had no effect on the concentration of cholesteryl esters in plasma from castrated animals, but it did reduce the concentration in plasma from normal animals. Furthermore, LY 117018 reversed the depression mediated by ethinyl estradiol on the concentration of cholesteryl esters in the plasma. Presumably, these effects of ethinyl estradiol are mediated by conventional estrogen receptors.

It was reported recently that when cockerels were administered tamoxifen (10–100 mg/kg), an antiestrogen with greater estrogenicity than LY 117018, a reduction in serum triacylglycerol and total cholesterol was observed [20]. The authors concluded that the observed effects on plasma lipids are independent of estrogen receptor action because of the high dose of drug used. In our preliminary experiments, normal female rats that had been administered LY 117018 (5 mg/kg) for 14 days had serum concentrations of 0.05 ± 0.01 (mean ± SE, N = 5) and 0.57 ± 0.08 μmol/ml, for triacylglycerol and total cholesterol respectively. In the present study, the concentration of cholesteryl esters was reduced in the plasma of ovariectomized rats administered LY 117018 (see Table 2).

The effect of LY 117018 on the increased concentration of plasma triacylglycerol induced by ethinyl estradiol was mediated, in part, by the modulation of actions on hepatic fatty acid metabolism. The plasma concentrations of triacylglycerol, to a large extent, reflect the balance between synthesis and secretion by the liver and clearance from the plasma. Ethinyl estradiol affects both of these processes [5]. The hepatic secretion and synthesis of triacylglycerol were stimulated by estrogen and were not affected by the antiestrogen *per se*, under the conditions of our experiments (Table 3). In the group of rats treated with ethinyl estradiol, the secretion of triacylglycerol probably exceeded clearance since the plasma concentration of triacylglycerol was increased. Concurrent administration of both drugs prevented the effects of estrogen from being demonstrated.

Ovariectomy, which reduces the concentration of estrogen receptors in estrogen responsive tissues [10], resulted in decreased rates of hepatic synthesis and secretion of triacylglycerol in the control group and in the groups receiving LY 117018 alone or with ethinyl estradiol. Moreover, with ethinyl estradiol,

the treatment period was sufficient to induce estrogen receptors because no differences were discerned between the normal and castrated groups in triacylglycerol metabolism (Table 3). Administration of LY 117018 decreased the response to estrogen, but since secretion of triacylglycerol and the incorporation of [1-^{14}C]oleic acid into triacylglycerol in the ovariectomized group was greater than that observed with the control, increased sensitivity of the ovariectomized group to estrogen may be responsible. Thus, the agonist action of LY 117018, although low, is more easily manifest when this drug is used to treat the liver donor. The antagonist action is more apparent in castrated animals receiving both drugs because the potent estrogenic action of ethinyl estradiol is blunted.

As reported earlier from our laboratory, ketogenesis is depressed in livers from normal female rats administered ethinyl estradiol [6]. In livers from ovariectomized rats administered LY 117018, ketogenesis was also depressed. However, perhaps reflecting the lower estrogenic potential of the antiestrogen, the ketogenic rate was reduced 51% whereas ethinyl estradiol depressed the rate 83%. Ketogenesis was increased, but did not reach control levels, by livers obtained from normal rats that received both drugs simultaneously. Ovariectomy reduced the ketogenic rates in all groups of rats. Ketogenesis by livers from ovariectomized animals treated with estrogen was decreased 95%. Livers from ovariectomized animals that received both drugs had an increase in ketogenic rates (Table 5), although these rates did not return to control values. The possibility that the effects of ethinyl estradiol and LY 117018 on the metabolism of oleic acid by the liver may result from an effect on the uptake of fatty acid should be considered. Clearly, the effects of ovariectomy were not due to any change in uptake of fatty acid (see Table 1). It is unlikely that the effects of these drugs on the metabolism of oleic acid were the result of a decrease in fatty acid uptake. First, in response to drug treatment, ketogenesis was reduced by either LY 117018 or ethinyl estradiol in livers obtained from either normal or ovariectomized rats and in the presence of both drugs returned to ketogenic rates observed with LY 117018 alone. Second, the uptake of oleic acid by livers from normal animals was not altered by any treatment regimen (Table 1). Third, liver weights in the ovariectomized group were increased as a result of treatment with either LY 117018 or ethinyl estradiol. In fact, uptake per total liver was similar in all ovariectomized groups. Thus, the uptake of oleic acid was 327.5 ± 11.7, 329.1 ± 8.6, 331.9 ± 11.8, and 323.9 ± 11.1 μmol/liver/3 hr for the control, LY 117018, ethinyl estradiol and the group receiving both drugs respectively (see Table 1).

The effect of estrogen on the concentration of free and esterified cholesterol in the plasma is of particular interest. Administration of ethinyl estradiol or mestranol to normal female rats decreases the concentration of total cholesterol in the serum [3], which probably is the result of an effect of the estrogen on the metabolism of cholesteryl esters, as was observed in this work. Simultaneously, the hepatic synthesis and secretion of cholesteryl esters were increased significantly by estrogen. Clearly, the rate of clearance of plasma cholesteryl esters was greater in those animals treated with ethinyl estradiol. It has been reported that administration of high doses (5 mg/kg) of ethinyl estradiol to male rats increases the number of LDL receptors in hepatic cell membranes [21, 22]. The current data suggest that therapeutic doses of ethinyl estradiol (15 μg/kg) like the high dose [21, 22] have a similar action on LDL receptor number even though VLDL output is increased. Concurrent administration of LY 117018 and ethinyl estradiol to female rats returned the serum concentrations and hepatic synthesis and secretory rates of cholesterol to control levels.

Antiestrogens such as LY 117018 are believed to mediate their pharmacological effects by competing for cytoplasmic receptor complex formation, and ultimately preventing the regeneration of the cytoplasmic receptor [23]. Recently, multiple forms of nuclear binding sites were reported to be present in rat liver nuclei [24]. It is also possible that antiestrogens have ancillary actions with specific receptors with which they combine, and which, in turn, regulate the metabolism of fatty acid. Sutherland *et al.* [25] have, in fact, reported that a high affinity antiestrogen binding site distinct from the estrogen receptor exists in rat liver cytosol. This high affinity receptor may be distinct for different antiestrogens. It has been suggested recently that LY 117018 and the antiestrogen tamoxifen act at separate sites or by different molecular mechanisms [26]. The present data suggest that, although intrinsic estrogenicity of LY 117018 is low, it is still present. In this regard, it was observed that LY 117018 administered to ovariectomized rats (500 μg/kg) exhibited weak agonist action in depressing the secretion of leutinizing hormone, and as a partial antagonist to the estrogen-mediated stimulation of the secretion of prolactin.*

It is clear that the antiestrogen LY 117018, alone and in combination with ethinyl estradiol, can modulate the metabolism of fatty acid by the liver. The antiestrogen clearly antagonized the actions of ethinyl estradiol on hepatic liquid metabolism. Furthermore, the use of LY 117018 in these experiments allows the conclusion that the modulation of hepatic metabolism of fatty acid by estrogen was most probably mediated by interaction with conventional estrogenic receptors.

* W. R. Crowley and I. Weinstein, unpublished observations.

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