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Studies with etofibrate in the rat. Part II: a comparison of the effects of prolonged and acute administration on plasma lipids, liver enzymes and adipose tissue lipolysis

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To contribute to the understanding of the hypolipidemic action of etofibrate, which is the 1,2-ethandiol ester of clofibric acid and nicotinic acid, 300 mg of this drug/kg body weight or of the medium were administered daily by a stomach tube to normolipidemic rats. Some animals were decapitated at the 10th day of daily treatment (prolonged treatment), whereas others were studied at different times after one single administration (acute treatment). In animals on prolonged treatment etofibrate decreased plasma levels of cholesterol, triacylglycerols, free fatty acids (FFA) and glycerol, as well as the total and unesterified cholesterol concentrations, in liver microsomes. In these rats, etofibrate increased the activity of liver cytosolic glycerol-3-*P* dehydrogenase, whereas it decreased the activity of both microsomal HMG-CoA reductase and cholesterol 7α-hydroxylase and did not affect acyl-CoA : cholesterol acyltransferase (ACAT). At 3, 5 and 7 h after acute treatment, etofibrate decreased plasma levels of triacylglycerols, glycerol and FFA, and this effect disappeared at 24 h, whereas plasma cholesterol did not change 3 h after etofibrate but decreased at 5 and 7 h and remained low after 24 h, and a similar change was found in the liver microsomes free cholesterol concentration. However, with the exception of a significant reduction in cytosolic glycerol-3-*P* dehydrogenase at 7 h and in ACAT at 5 h, acute etofibrate treatment did not affect the activity of the liver enzymes studied. At low concentrations (10^{-5} M) in the incubation medium, etofibrate decreased the release of both FFA and glycerol by epididymal fat pad pieces incubated in vitro. These findings together with those previously reported by us in rats using a similar etofibrate treatment protocol [6] indicate that etofibrate decreases the availability of lipolytic products in the liver by acting on their release from adipose tissue and on their intrinsic hepatic metabolism. Consequently, this drug would decrease liver VLDL triacylglycerol synthesis and secretion, which together with facilitating the clearance of circulating triacylglycerols causes its hypotriglyceridemic effect. The hypocholesterolemic effect of etofibrate after acute treatment may be a secondary consequence of the reduced liver VLDL production caused by decreased adipose tissue lipolysis, but after prolonged treatment, this effect also seems to be influenced by the inhibition of HMG-CoA reductase activity which would reduce cholesterol synthesis.

Introduction

The combination of clofibrate and nicotinic acid in the rat was shown to produce greater decreases in serum lipids than equimolar doses of either drug alone [1] and the same combined treatment was also found to be efficient in reducing both plasma lipids and myocardial reinfarction in men [2,3]. This motivated the development of etofibrate (Lipo-Merz, Merz & Co., Frankfurt/Main, Germany), the ethandiol-1,2 diester of the nicotinic and clofibric acids, which has been shown to be a potent hypolipidemic agent in animals [4–7] and humans [8–10]. Although the hypolipidemic effect of etofibrate is not completely understood, in rats it decreases the conversion of intravenously administered labelled palmitate or glycerol into plasma VLDL-glycerides and enhances reesterification of fatty acids and glycerol, lipoprotein lipase activity in adipose tissue and liver cytosolic glycerol-3-phosphate dehydrogenase activity [6]. In hamsters, etofibrate treatment decreases liver HMG-CoA reductase and acyl-CoA: cholesterol acyltransferase activities (ACAT) without modifying the activity of cholesterol 7α-hydroxylase [7]. However, it is not known whether these changes in liver enzyme activities are directly related to the hy-

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potriglyceridemic or the hypocholesterolemic action of this drug. To test this, the present work has determined both the plasma lipid levels and the liver activity of these enzymes in normolipidemic rats chronically treated with a very high dose of etofibrate or at different times after receiving an acute load of the drug. In addition, although we were unable to detect a decreased in vitro lipolytic activity in adipose tissue from etofibrate treated rats [6], both nicotinic acid and clofibrate have been shown to decrease adipose tissue lipolytic activity when added in vitro [11,12]. We have therefore extended the present study to determine whether the presence of etofibrate in the incubation medium would also decrease in vitro adipose tissue lipolytic activity.

Methods

Materials. [3-^{14}C]HMG-CoA (spec. act. 52 mCi/mmol), DL-[2-^{3}H]mevalonic acid lactone (sp. act. 1 Ci/mmol) and [1-^{14}C]oleyl-CoA were obtained from Amersham International, Amersham, UK. [1,2,6,7-^{3}H]cholesteryl oleate (sp act. 82 Ci/mmol) was obtained from New England Nuclear, USA. Unlabeled mevalonic acid lactone, cholesteryl oleate, $NADP^+$, EDTA, dithiothreitol, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and fatty acid-free bovine albumin were purchased from Sigma Chemical Co., USA. Standard of 7α-hydroxycholesterol was obtained from Steraloids, USA. All other reagents were of analytical quality and obtained from Igoda-Merck, Spain, organic solvents for HPLC from Ferosa, Spain, and silica-gel chromatographic plates from Whatman, USA. The HPLC chromatograph was composed of a pump (model 510), injector (U6K), data module integrator (730) and precolumn module (P/N 080040) with a resolve Si cartridge from Millipore-Waters (USA), a detector (model 165) from Beckman Instruments (USA) and a Spherisorb S5W column (5 μm and 250 mm $\times$ 4.9 mm i.d.) from Kontron, USA.

Animals, drug administration and collection of the samples. Male Sprague-Dawley rats weighing 200–250 g, fed ad libitum purina chow diet (Panlab, Barcelona, Spain) and subjected to a 12 h on-off light cycle and 22–24°C were used. In one set of animals, on days 1–9 of the experiment, food was removed from the cages at the onset of the light cycle (7.00 a.m.), and 3 h later, either 300 mg etofibrate freshly suspended in 2% Tween-80/kg body weight or the medium (controls) was administered to the rats by stomach tube without anesthesia. After treatment, rats were again allowed free access to food. On the 10th day, the animals were treated as above, but they were kept fasted up to killing, which was done by decapitation 3 h after drug administration. In another set of animals, fed rats were given either 300 mg of etofibrate/kg body weight suspended in 2% Tween-80 or the medium (controls) by stomach tube at 10.00 a.m. and were then decapitated 3, 5, 7 or 24 h later.

Blood was collected from the neck into ice chilled heparinized tubes and centrifuged at 3000 $\times g$ for 10 min. Plasma was kept at −24°C until processed for enzymatic determination of cholesterol [13], triacylglycerols [14] (commercial kits from Menarini, Italy), free fatty acids (FFA) [15] and glycerol [16]. Liver was immediately dissected and placed in ice-cold 0.9% NaCl to be processed as indicated below.

Preparation of liver cytosolic and microsomal fractions. About 1 g of fresh liver was minced and homogenized with a loose-fitting Teflon pestle in 3 vols. of 50 mM potassium phosphate buffer (pH 7.4) containing 30 mM Na_2-EDTA, 250 mM NaCl and 1 mM dithiothreitol. After 15 min centrifugation at 6000 $\times g$ the supernatant was centrifuged for 15 min at 18000 $\times g$. The resulting supernatant was centrifuged for 60 min at 105000 $\times g$ to separate the cytosolic and the microsomal fractions which were kept at −70°C until processed.

The contents of protein in the cytosolic and microsomal fractions were determined by a modification [17] of the method of Lowry et al. [18]. Total and free cholesterol [19,20] and choline phospholipids [21] were measured in microsomal fractions.

Assay of glycerol-3-phosphate dehydrogenase. After thawing, the cytosolic fraction (10–16 μg protein) was used to directly measure glycerol-3-phosphate dehydrogenase activity following a colorimetric method [22].

Assay of HMG-CoA reductase activity. The microsomal fractions (100–130 μg protein) were resuspended in 1 ml of homogenization buffer and HMG-CoA reductase activity was measured following the method described by Shapiro et al. [23] using [3-^{14}C]HMG-CoA as radioactive substrate and DL-[2-^{3}H]mevalonic acid lactone as internal standard.

Assay of ACAT activity. Microsomal ACAT activity was measured following a method based on others previously described [24–26]. The assay system contained 0.175 ml of the resuspended microsomal preparation (about 150 μg of protein) in 2.857 mM potassium buffer (pH 7.4), containing 1.714 mM Na_2-EDTA, 14.286 mM NaCl, 114.29 mM Tris-HCl, 0.143 mM dithiothreitol and 68.57 μM fatty acid-free bovine serum albumin. This mixture was preincubated at 37°C for 15 min and the reaction was initiated by the addition of 25 μL of a solution containing 12 nmol (0.1 μCi) of [1-^{14}C]oleyl-CoA. The entire process was done in the dark to prevent oxidation due to light sensitivity. After 10 min incubation the reaction was stopped by adding 0.2 ml ethanol containing the internal standard, 0.2 ml methanol and 1 ml chloroform/hexane (72:28, v/v). After thorough mixing and centrifugation at 600 $\times g$ for 5 min, the organic phase was collected and

evaporated to dryness under N_2. The residue was redissolved in 0.2 ml chloroform/methanol (2:1, v/v) and subjected to thin layer chromatography in 0.25 mm thick silica-gel plates. The chromatogram was developed in hexane/ethyl acetate (9:1, v/v). The cholesteryl oleate zone was visualized with iodine vapor and scraped off into a counting vial containing 10 ml of PPO/POPOP toluene/methanol-based scintillation cocktail.

Assay of cholesterol 7α-hydroxylase activity. The method was based on those previously described [27,28]. The assay system contained 0.225 ml of microsomal preparation (about 300 μg protein) in 0.1 M potassium phosphate buffer (pH 7.4), containing 6.7 mM Na_2-EDTA, 0.222 mM dithiothreitol, 55.6 mM NaCl and 5.6 mM 2-mercaptoethylamine. After 5 min preincubation at 37°C, the reaction was initiated by adding 25 μl of a solution containing 96.1 mM potassium phosphate buffer (pH 7.4), 170 mM glucose-6-phosphate, 16.8 mM $NADP^+$ and 0.289 units of glucose-6-phosphate dehydrogenase. After 15 min, the reaction was stopped by the addition of 50 μl of a solution containing 4 mM potassium phosphate buffer (pH 7.4), 70 mM sodium cholate, 869 mM glycerol and 25 μl of cholesterol oxidase (0.156 units), and the mixture was incubated for another 15 min. All this process was carried out in the dark to protect against oxidation. The last incubation was stopped by adding 0.3 ml methanol, and after being thoroughly stirred, the mixture was extracted twice with 1 ml of n-hexane followed by 5 min centrifugations at $600 \times g$. The pooled hexane extracts were evaporated to dryness under N_2. The residue was redissolved in n-hexane/isopropyl alcohol/acetic acid (95:5:2, v/v) and subjected to high-performance liquid chromatography using n-hexane/isopropyl alcohol/acetic acid (95:5:2) as eluent at a flow of 1.4 ml/min during minutes 0 to 5, and 1.9 ml/min during minutes 5 to 8. Standards containing 100, 200 and 300 pmol of 7α-hydroxycholesterol were also run under the same conditions and quantification was made by using peak areas of 7α-hydroxy-4-cholesten-3-one (retention time of about 7 min) on the chromatogram.

Validation of enzyme determinations. The measured enzyme activities always changed linearly in the presence of increasing amounts of cytosolic or microsomal proteins in the range of the assayed samples, with correlation coefficient values above 0.99 in all cases. The activity of the studied enzymes was stable even after the cytosolic or microsomal fractions had been stored for 49 days at −70°C.

In vitro adipose tissue lipolysis. Lipolytic activity in vitro in epididymal fat pad pieces from untreated fed rats in the presence or absence of etofibrate in the medium was determined following the method already described by us [29,30], with few modifications. After being weighed, fresh tissue was cut into small pieces and 18–20 mg were placed in vials containing 2.0 ml of Krebs Ringer bicarbonate buffer (pH 7.4) supplemented with fatty acid-free bovine albumin (20 mg/ml). At zero time, 20 μl of dimethyl sulfoxide with or without etofibrate for a final concentration of 10^{-5} M in the incubation medium was added to each vial. Incubations were carried out under an O_2/CO_2 (95:5) atmosphere for 120 min and stopped by placing the vials in an ice-bath. An aliquot of each medium was treated with 10% $HClO_4$ for protein precipitation and the neutralized supernatants were used for glycerol determination [16], whereas another aliquot of the medium was used directly for FFA determination [15].

Statistical analysis. Results are expressed as means ± SE, and statistical comparison between the groups was evaluated by the Student's *t*-test.

Results

Long-term treatment

As shown in Table I, 10 days of etofibrate treatment in rats did not affect body weight compared to controls, although their liver weight was about 25% heavier than in the latter. The concentrations of cholesterol, triacylglycerols, FFA and glycerol in plasma were significantly lower in the etofibrate treated rats than in controls (Table I). As also shown in Table I, a decreased concentration of total cholesterol which corresponded to its free fraction was also found in liver microsomes from etofibrate treated rats (values of total minus free-cholesterol did not differ between the two

TABLE I

Body and liver weights and plasma and liver microsomal lipids in etofibrate-treated and control rats

Values are expressed as means ± S.E., corresponding to 6–10 rats/group. Rats received the treatment for 10 days and were killed after 6 h of fasting, corresponding to 3 h after the last drug administration. n.s., not significant.

	Control	*P*	Etofibrate-treated
Body weight (g)			
at day 1	251.3 ± 5.9	n.s.	255.2 ± 6.3
at day 10	292.5 ± 8.6	n.s.	285.0 ± 6.8
Liver weight (g)	10.3 ± 0.4	< 0.01	13.2 ± 0.7
Plasma			
Cholesterol (mg/dl)	57.9 ± 3.4	< 0.01	39.8 ± 1.4
Triacylglycerols (mg/dl)	77.1 ± 6.3	< 0.001	19.0 ± 1.6
FFA (μM)	575.0 ± 104.5	< 0.01	136.5 ± 41.5
Glycerol (μM)	69.9 ± 7.9	< 0.05	47.2 ± 4.0
Liver microsomes			
Total cholesterol (μg/mg protein)	24.6 ± 0.7	< 0.05	21.4 ± 1.0
Unesterified chol. (μg/mg protein)	19.2 ± 0.5	< 0.05	17.2 ± 0.7
Phospholipids (nmol/mg protein)	300.3 ± 7.1	n.s.	284.3 ± 8.0

groups, data not shown). However, the microsomal phospholipid concentration did not differ between etofibrate treated rats and controls (Table I).

Enzyme activities found in liver cytosolic or microsomal fractions in rats after 10 days of treatment are summarized in Table II. Cytosolic glycerol-3-phosphate dehydrogenase activity was higher in etofibrate treated rats than in controls, whereas both microsomal HMG-CoA reductase and cholesterol 7α-hydroxylase activities were significantly lower in the former, with a difference between the two groups that was of the order of 50% for both enzymes. However, the microsomal activity of ACAT did not differ between the two groups.

Acute treatment

As shown in Fig. 1a, plasma triacylglycerols were greatly reduced 3 h after the acute treatment with etofibrate as compared to controls, and this difference was maintained to the 7th h studied but had completely disappeared at 24 h. The changes in both plasma glycerol (Fig. 1b) and FFA levels (Fig. 1c), were similar to those in triacylglycerols, with significant reductions from 3 until 7 h after etofibrate treatment and restoration of control levels at 24 h. As shown in Fig. 2a, differently from the plasma triacylglycerols values, cholesterol level do not change at 3 h after etofibrate treatment but decrease significantly 5 h after treatment and remain decreased even at 24 h. The change in free cholesterol concentration in microsomes was similar to that of plasma cholesterol since, as shown in Fig. 2b, the concentration does not differ between etofibrate and control rats at 3 h and decreases significantly in the former at 5 h. Values for the microsomal free cholesterol concentration remained lower in etofibrate

TABLE II

Effect of etofibrate treatment for 10 days on liver enzyme activities in the rat

Values are expressed as means ± S.E. of six rats/group. Other experimental details as indicated in legend for Table I and in the text.

	Controls	*P*	Etofibrate
Cytosolic glycerol-3-*P* dehydrogenase (μmol/mg protein per min)	0.89 ± 0.02	< 0.001	1.18 ± 0.06
HMG-CoA reductase (pmol/mg protein per min)	155.2 ± 13.8	< 0.01	71.5 ± 16.3
Cholesterol 7α-hydroxylase (pmol/mg protein per min)	19.2 ± 1.3	< 0.001	8.6 ± 1.0
Acyl-CoA : cholesterol acyltransferase (pmol/mg protein per min)	287.8 ± 18.7	n.s.	294.2 ± 25.1

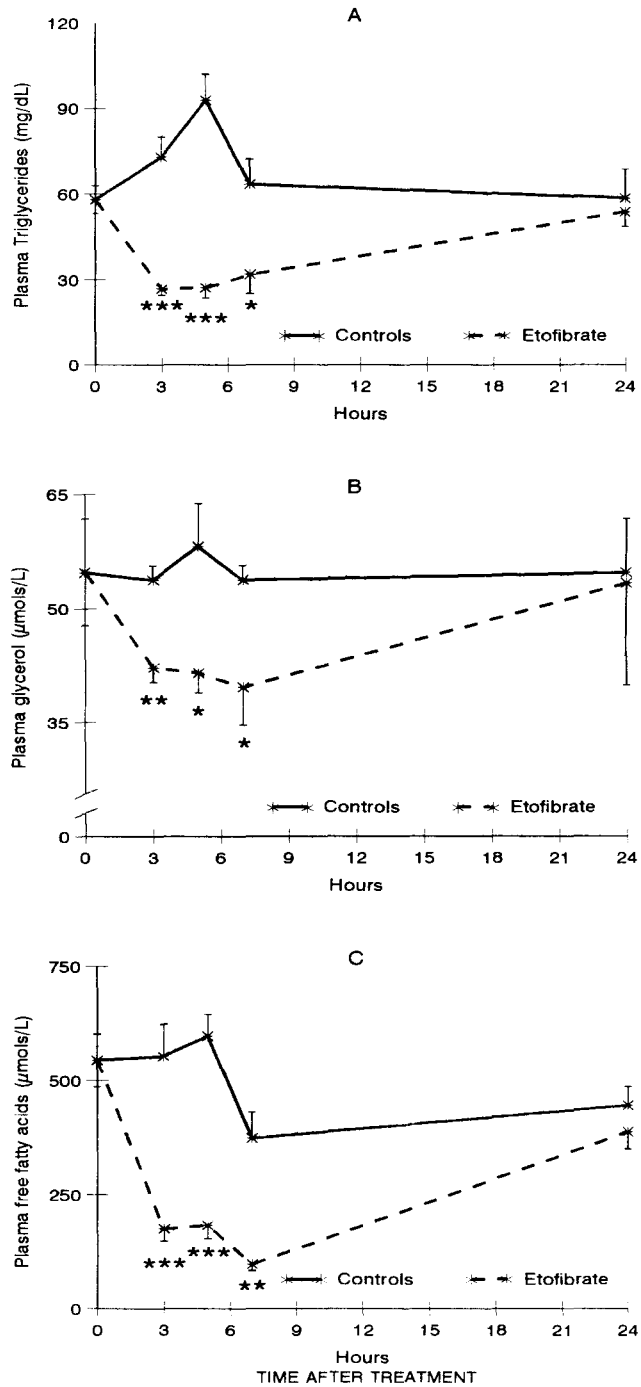

Fig. 1. Concentrations of plasma triacylglycerols (A), glycerol (B) and free fatty acids (C) at different times after a single oral administration of etofibrate in the rat. Means ± SE of 6–10 rats/group. Asterisks correspond to the statistical comparison between values from etofibrate and control animals (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

than in control rats at 7 and 24 h, although the difference between the two groups was not significant. The change for microsomal total cholesterol was similar to that found for microsomal free cholesterol contents, whereas microsomal phospholipid content did not differ between the two groups at any of the times studied

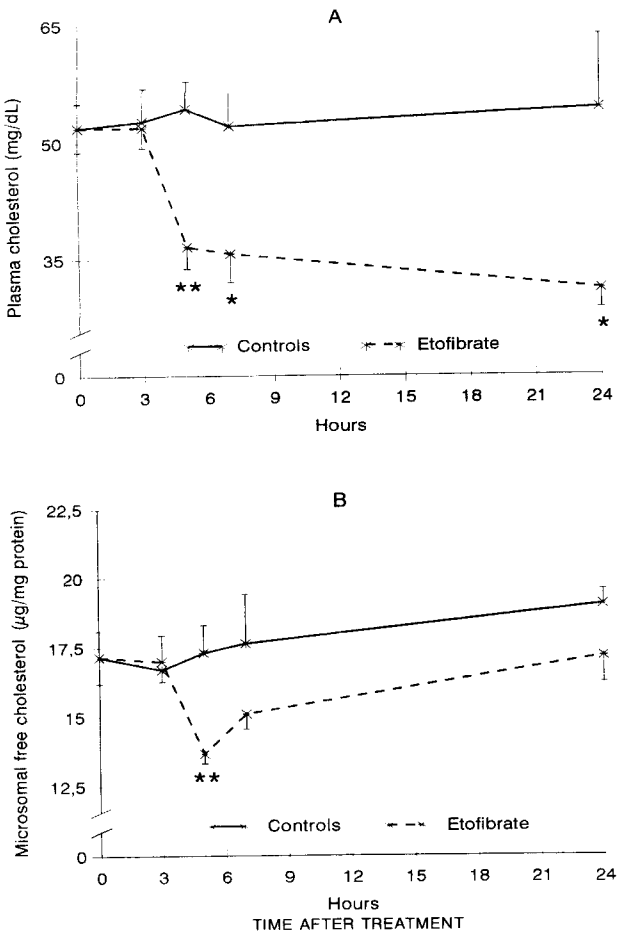

Fig. 2. Plasma total cholesterol (A) and microsomal free cholesterol (B) concentrations at different times after a single oral administration of etofibrate in the rat. Means ± S.E. of 6–10 rats/group. Asterisks correspond to the statistical comparison between values from etofibrate and control animals (* $P < 0.05$, ** $P < 0.01$).

(data not shown).

Table III summarizes the activity of liver cytosolic glycerol-3-phosphate dehydrogenase and microsomal HMG-CoA reductase, cholesterol 7α-hydroxylase and ACAT in etofibrate treated rats and controls at different times after acute treatment. Cytosolic glycerol-3-phosphate dehydrogenase activity in the control rats decreased from the 3rd to the 5th h after receiving the medium to recover initial values from the 7th h. In the etofibrate treated rats cytosolic glycerol-3-phosphate dehydrogenase activity was very similar to that in controls until 5 h, and then it decreased at 7 h when values became significantly lower than in controls ($P < 0.001$), before recovering again at 24 h (Table III). Microsomal HMG-CoA reductase and cholesterol 7α-hydroxylase activities changed during the day in the controls as would be expected in light of their known circadian rhythms [31,32] increasing at 7 h after receiving the medium, which corresponded to 17.00 p.m., and returning to basal values at 24 h after the medium (10.00 a.m. on the next day) (Table III). No statistically significant difference was found, however, for either of these enzyme activities between the etofibrate treated rats and their controls at any of the time points studied, although values of HMG-CoA reductase activity in the etofibrate group were always lower than those in controls (Table III). Microsomal ACAT activity progressively increased in the control rats after receiving the medium. Mean values of ACAT activity were also lower in the etofibrate group than in controls at all the times studied and the difference between both groups was only statistically significant ($P < 0.01$) at 5 h (Table III), an effect that coincided with the lowest microsomal free cholesterol concentration present in the acutely treated rats (Fig. 2b).

Adipose tissue lipolytic activity

As shown in Table IV, the presence of 10^{-5} M etofibrate in the incubation medium significantly decreased the in vitro release of both free fatty acids and glycerol by epididymal fat pad pieces. The difference between both groups appeared greater when values in the tissues incubated in the presence of etofibrate were expressed as a percentage of the values in the controls for the corresponding experiment (Table IV). These

TABLE III

Liver enzyme activities at different times after acute etofibrate administration in the rat

Values are expressed as means ± S.E. of 5 rats/group. The superscript letters correspond to the statistical comparison between the groups at each of the time points (hours after treatment) studied: the same letter within one enzyme means that there is no statistical difference between the corresponding groups ($P < 0.05$). Statistical comparisons between etofibrate and control groups are shown by the P values.

	Controls	P	Etofibrate
Cytosolic glycerol-3-*P* dehydrogenase (μmol/mg protein per min)			
3 h	0.94 ± 0.03 ^{ac}	n.s.	0.85 ± 0.04 ^{a}
5 h	0.83 ± 0.03 ^{b}	n.s.	0.75 ± 0.05 ^{ab}
7 h	0.98 ± 0.03 ^{a}	< 0.001	0.69 ± 0.03 ^{b}
24 h	0.87 ± 0.02 ^{bc}	n.s.	0.87 ± 0.06 ^{a}
HMG-CoA reductase (pmol/mg protein per min)			
3 h	339.5 ± 42.8 ^{ab}	n.s.	199.5 ± 45.5 ^{a}
5 h	315.4 ± 33.4 ^{a}	n.s.	254.2 ± 84.6 ^{a}
7 h	735.2 ± 171.4 ^{b}	n.s.	658.1 ± 151.2 ^{b}
24 h	482.9 ± 112.4 ^{ab}	n.s.	379.4 ± 147.4 ^{ab}
Cholesterol 7α-hydroxylase (pmol/mg protein per min)			
3 h	32.8 ± 2.1 ^{a}	n.s.	46.2 ± 6.9 ^{a}
5 h	32.4 ± 3.8 ^{a}	n.s.	29.4 ± 5.3 ^{a}
7 h	46.0 ± 4.2 ^{b}	n.s.	40.0 ± 5.8 ^{a}
24 h	35.0 ± 5.0 ^{ab}	n.s.	28.7 ± 4.7 ^{a}
Acyl-CoA : cholesterol acyltransferase (pmol/mg protein per min)			
3 h	205.0 ± 12.7 ^{a}	n.s.	188.2 ± 5.8 ^{a}
5 h	248.6 ± 9.0 ^{b}	< 0.01	189.9 ± 10.6 ^{a}
7 h	306.6 ± 38.4 ^{b}	n.s.	291.1 ± 19.1 ^{b}
24 h	451.5 ± 48.5 ^{c}	n.s.	291.3 ± 61.9 ^{ab}

TABLE IV

Glycerol and free fatty acid release by epididymal fat pieces from untreated rats incubated in vitro for 120 min in the presence or absence of etofibrate

Values are means ± S.E. Values in parentheses indicate the total number of vials from four separate experiments. Values in square brackets indicate the percentage change from controls (incubations without the drug).

Metabolites released in the medium	Controls	*P*	Etofibrate (10^{-5} M)
Free fatty acids (nmol/100 mg tissue)	1302.7 ± 79.6 (14) [100]	< 0.05 [< 0.001]	925.3 ± 47.6 (8) [75.6]
Glycerol (nmol/100 mg tissue)	487.6 ± 40.5 (14) [100]	< 0.05 [< 0.05]	347.9 ± 22.5 (7) [76.7]

findings therefore show that etofibrate decreased adipose tissue lipolytic activity in vitro.

Discussion

In the present study, etofibrate treatment for either 10 days or given acutely to normal rats decreased plasma FFA, glycerol, triacylglycerol and cholesterol levels; however, prolonged treatment enhanced liver cytosolic glycerol-3-phosphate dehydrogenase activity and decreased both microsomal HMG-CoA reductase and cholesterol 7α-hydroxylase activities, although these changes did not appear after acute treatment. The drug decreased the lipolytic activity of adipose tissue in vitro. It may then be concluded that, whereas the hypolipidemic effect of etofibrate after prolonged treatment may be influenced by the changes that were found in the studied enzyme activities, its effect after acute treatment seems to be independent of these changes and may be a consequence of its antilipolytic effect.

Hypotriglyceridemic effect

The decrease in plasma FFA, glycerol and triacylglycerol levels produced in normolipemic rats after 10 days' etofibrate treatment confirms previous findings [4,6], and we have seen here that a similar effect also occurs shortly after acute treatment. The increased activity of liver cytosolic glycerol-3-phosphate dehydrogenase in the etofibrate rats would facilitate the oxidation of glycerol-3-phosphate (a necessary precursor of liver triacylglycerols) to dihydroxyacetone phosphate and result in decreased triacylglycerol synthesis, which has been previously shown by us in rats subjected to similar treatment [6]. Other mechanisms also seem to contribute to the hypotriglyceridemic action of etofibrate. Since we know that adipose tissue from etofibrate treated rats shows enhanced in vitro utilization of glycerol and heparin-released lipoprotein lipase activity [6], the drug may improve plasma triacylglycerol clearance. Although a decreased lipolytic activity was not previously found by us when adipose tissue from etofibrate treated rats was studied in vitro [6], an inhibitory action of the drug on this pathway is seen here when it was offered in vitro to tissue from untreated rats. This different lipolytic response to the drug under in vitro versus ex vivo conditions may be a consequence of the need for the drug to be present to manifest its effect. However, the intense reduction of both plasma FFA and glycerol levels found here in rats on etofibrate treatment indicates the efficient antilipolytic action of the drug in vivo. Circulating FFA and glycerol are mainly taken up by the liver where they are partially converted into triacylglycerols for later export to the circulation in the form of VLDL triacylglycerols [33,34]. It is therefore proposed that in the rats receiving a prolonged treatment with etofibrate, the reduced availability of lipolytic products in the liver and enhanced oxidation of glycerol-3-phosphate are responsible for the inhibition of liver VLDL triacylglycerol synthesis and secretion previously found in etofibrate treated rats [6].

The intense reduction in plasma triacylglycerols already seen at 3 h after a single administration with the drug is coincident with the known peak in plasma etofibrate concentration after a single dose in the rat [35] and appears before any change in liver cytosolic glycerol-3-phosphate dehydrogenase can be detected. On the basis of the unique antilipolytic effect of etofibrate seen in vitro and the rapid decrease in the plasma level of the two lipolytic products, FFA and glycerol, it may be proposed that a reduced flux of these products to the liver for triacylglycerol synthesis and release is the main factor responsible for the hypotriglyceridemic effect of acute etofibrate administration.

Hypocholesterolemic effect

Several simultaneous effects also seem to contribute to the hypocholesterolemic action of etofibrate. The effect of 10 days treatment with etofibrate in inhibiting hepatic HMG-CoA reductase in rats conforms with a similar effect found in the hamster [7] and the decreased cholesterol synthesis found in the rat after receiving etofibrate treatment [4]. This effect of etofibrate on hepatic HMG-CoA reductase activity is greater than that reported for clofibrate and other fibric acid derivatives [36,37]. Treatment with nicotinic acid has been shown to not affect liver cholesterol synthesis in rats [38]. Therefore, the present findings indicate a synergistic inhibitory effect of the two molecular moieties in the structure of etofibrate on the key regulating enzyme of liver cholesterol synthesis.

Present results show that, under certain conditions, etofibrate also decreased liver cholesterol-7α hydroxylase activity without modifying ACAT activity, although an inhibitory effect was found for the latter enzyme at 5 h after acute treatment. Albeit similar changes were previously seen in the hamster receiving this drug [7] and in the rat being treated with other fibrates [36,37], no relationship can be found between these changes and the hypocholesterolemic effect of etofibrate.

As shown in this study, a single administration of etofibrate to rats rapidly decreases plasma triacylglycerols, FFA and glycerol levels. Based on the unique antilipolytic effect of etofibrate and the time sequence of these effects it may be proposed that the reduced VLDL production caused by the decreased flux of FFA and glycerol to the liver after acute etofibrate treatment limits the conversion of VLDL to LDL, and thus reduces plasma cholesterol levels. This hypothesis is supported by the fact that the acute hypocholesterolemic effect of etofibrate appeared later than its hypotriglyceridemic effect. A similar explanation has been given previously to justify the hypocholesterolemic effect of short-term nicotinic acid or its analogues [39] such as acipimox, which seem to be effective antilipolytic agents [40–42], although their effect on cholesterol metabolism is minor or absent [38,42,43]. An additional (or alternative) mechanism based on the increased clearance of LDL as the result of activation of the LDL receptor pathway could also be claimed to explain the hypocholesterolemic effect of etofibrate after acute treatment, as has been proposed in hypercholesterolemic individuals [10], but, quantitatively, this mechanism seems to be of small importance in the present study, since normolipidemic rats were used, and LDL-cholesterol levels [44] and hepatic uptake of LDL [45, 46] are much lower in this species than in humans.

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