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# Effects of ciprofibrate and 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate (POCA) on the distribution of carnitine and CoA and their acyl-esters and on enzyme activities in rats

## Relation between hepatic carnitine concentration and carnitine acetyltransferase activity

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The effects of feeding the peroxisome proliferators ciprofibrate (a hypolipidaemic analogue of clofibrate) or POCA {2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate} (an inhibitor of CPT I) to rats for 5 days on the distribution of carnitine and acylcarnitine esters between liver, plasma and muscle and on hepatic CoA concentrations (free and acylated) and activities of carnitine acetyltransferase and acyl-CoA hydrolases were determined. Ciprofibrate and POCA increased hepatic [total CoA] by 2 and 2.5 times respectively, and [total carnitine] by 4.4 and 1.9 times respectively, but decreased plasma [carnitine] by 36–46 %. POCA had no effect on either urinary excretion of acylcarnitine esters or [acylcarnitine] in skeletal muscle. By contrast, ciprofibrate decreased [acylcarnitine] and [total carnitine] in muscle. In liver, ciprofibrate increased the [carnitine]/[CoA] ratio and caused a larger increase in [acylcarnitine] (7-fold) than in [carnitine] (4-fold), thereby increasing the [short-chain acylcarnitine]/[carnitine] ratio. POCA did not affect the [carnitine]/[CoA] and the [short-chain acylcarnitine]/[carnitine] ratios, but it decreased the [long-chain acylcarnitine]/[carnitine] ratio. Ciprofibrate and POCA increased the activities of acyl-CoA hydrolases, and carnitine acetyltransferase activity was increased 28-fold and 6-fold by ciprofibrate and POCA respectively. In cultures of hepatocytes, ciprofibrate caused similar changes in enzyme activity to those observed *in vivo*, although [carnitine] decreased with time. The results suggest that: (1) the reactions catalysed by the short-chain carnitine acyltransferases, but not by the carnitine palmitoyltransferases, are near equilibrium in liver both before and after modification of metabolism by administration of ciprofibrate or POCA; (2) the increase in hepatic [carnitine] after ciprofibrate or POCA feeding can be explained by redistribution of carnitine between tissues; (3) the activity of carnitine acetyltransferase and [total carnitine] in liver are closely related.

## INTRODUCTION

Mitochondria oxidize long-chain fatty acids rapidly and completely to acetyl-CoA, although they only oxidize very-long-chain fatty acids slowly [1-3]. Liver peroxisomes oxidize long-chain and very-long-chain fatty acids to medium-chain acyl-CoA esters by successive removal of acetyl groups as acetyl-CoA. Medium-chain acyl-CoA esters formed by partial peroxisomal $\beta$-oxidation transfer their acyl groups to carnitine catalysed by COT and CAT in peroxisomes [2,3]. These acylcarnitine esters then diffuse to the mitochondria, where the acyl groups are transferred to CoA in the matrix by the action of CPT I, CPT II, COT and CAT, where their oxidation to acetyl-CoA is completed. Acetyl groups formed by peroxisomal $\beta$-oxidation are also transferred to the mitochondrial matrix as acetylcarnitine. The capacity of the liver to oxidize long-chain and very-long-chain fatty acids is therefore partly determined by the activities of the carnitine acyltransferases. In addition to the activities of mitochondrial and peroxisomal enzymes, and the mitochondrial redox state, the capacity for $\beta$-oxidation depends on [carnitine] and [CoA]. Carnitine is essential for the transfer of long-chain fatty-acyl groups into mitochondria, and buffers free [CoA]. Administration of hypolipidaemic drugs of the fibrate group including clofibrate, ciprofibrate and bezafibrate [4], and the hypoglycaemic compound POCA, the CoA ester of which powerfully inhibits CPT I [5], induces both proliferation of peroxisomes and peroxisomal $\beta$-oxidation, and the capacity for mitochondrial $\beta$-oxidation of palmitoylcarnitine [6,7]. Fibrates also increase [carnitine] [6,8] and [CoA] [9–11] in rat liver, and clofibrate increases both mitochondrial and cytosolic [CoA] [12].

Although the metabolism of carnitine and CoA is intimately related through the action of the carnitine acyltransferases, [carnitine], [CoA], [short-chain acylcarnitine], [short-chain acyl-CoA], [long-chain acylcarnitine] and [long-chain acyl-CoA] do not appear to have been determined in the same internally consistent experiment. In this study we used ciprofibrate {2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropionic acid} [13] and

Abbreviations used: POCA, 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate; POCA-CoA, 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carbonyl-CoA; CAT, carnitine acetyltransferase; COT, carnitine octanoyltransferase; CPT, CPT I, CPT II, carnitine palmitoyltransferase (EC 2.3.1.21), its overt and latent forms.

POCA to change fatty acid metabolism so as to investigate the relation between [carnitine], [CoA], [acylcarnitine] and [acyl-CoA] and the activities of CAT and acyl-CoA hydrolases in liver and skeletal muscle, and [carnitine] and [acylcarnitine] in plasma.

## MATERIALS AND METHODS

### Materials

Ciprofibrate was a gift from Sterling–Winthrop Research, POCA was from Byk Gulden Chemische Fabrik (Konstanz, Germany), and L-carnitine was from Sigma-Tau (Rome, Italy). [*methyl*-$^3$H]Carnitine was from Amersham International (Amersham, Bucks., U.K.). Enzymes and confactors were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or from Boehringer Corp. (Mannheim, Germany). Sources of culture media were as in ref. [14].

### Animals

Male albino Wistar rats (180–220 g body wt.) were bred locally, and guinea pigs (250–300 g, body wt.) were obtained from Bantin and Kingman, Grimston, Aldborough, Hull, U.K. They were fed on standard rat or guinea-pig chow (Scientific Diets Services, Stepfield, Witham, Essex, U.K.) *ad libitum*. Ciprofibrate and the ethyl ester of POCA were dissolved in acetone as 10% (v/v) solutions and sprayed on the chow, to give concentrations of 0.1% and 0.2% (w/w) respectively, which were checked by h.p.l.c. analysis [15]. The rats were fed on control diet or diet containing drugs for 5 days. In Expt. 1 the rats were caged in groups of five for 4 days, and on day 5 they were put singly in metabolism cages for collection of urine. In Expt. 2 the rats were caged in groups of five for 5 days until used. The animals were killed by intraperitoneal injection of pentobarbital (60 mg/kg body wt.) between 09:00 and 10:00 h. Blood was withdrawn from the inferior vena cava, and the liver was rapidly removed and weighed. Part of the liver was immediately freeze-clamped, and part was homogenized in a medium containing 50 mM-Tris/HCl, pH 7.0, 100 mM-KCl, 5 mM-EDTA, 20 mM-KF and 0.05% (w/v) Lubrol PX, for enzyme assays. Enzyme activities were determined the same day. Skeletal muscle (hind limb, gastrocnemius, quadriceps) was rapidly removed and freeze-clamped.

### Studies *in vitro* using cultured hepatocytes

Parenchymal hepatocytes were isolated from normal rats (180–200 g) by collagenase perfusion of the liver [16] and cultured in monolayer in 25 cm$^2$ flasks as described previously [14]. Minimum essential medium supplemented with 5% (v/v) fetal bovine serum was used for the initial attachment stage (4 h). Subsequently cells were maintained in serum-free medium supplemented with 10 nM-dexamethasone, 10 nM-insulin and the concentrations of ciprofibrate indicated. Medium was changed daily and enzyme activities were determined after 4 days. The hepatocyte monolayer was washed twice with 0.14 M-NaCl and then extracted in the medium used for fresh tissue (above).

### Concentrations of carnitine and acylcarnitine esters in tissues, plasma and urine

Carnitine was determined radioenzymically [17] with some modifications. Freeze-clamped liver or muscle (approx. 100 mg) was homogenized with a Polytron homogenizer in 600 $\mu$l of 1 M-$HClO_4$, to which was added palmitoyl-L-[*methyl*-$^3$H]carnitine (73 pmol; 8000 d.p.m.; $1.85 \times 10^6$ Bq/$\mu$mol) as internal standard. The homogenate was centrifuged (10000 ***g***), and free and short-chain acylcarnitine esters were determined in the neutralized supernatant. For determination of long-chain acylcarnitine esters, the pellet was dissolved in 200 $\mu$l of 1 M-KOH and incubated at 50 °C for 2 h. Protein was precipitated by 300 $\mu$l of 1 M-$HClO_4$ and removed by centrifugation (10000 ***g***). The supernatant (400 $\mu$l) was neutralized with 95 $\mu$l of 1 M-KOH and 200 $\mu$l of 1 M-Hepes, pH 7.2 and precipitated $KClO_4$ was removed by centrifugation (10000 ***g***). Free carnitine formed by hydrolysis was determined and corrected for incomplete recovery by reference to the internal standard. With plasma and urine samples the homogenization step was omitted.

### Determination of free CoA and acyl-CoA esters

Frozen liver (about 200 mg) was homogenized in 1.0 ml of 1 M-$HClO_4$ containing 2 mM-dithiothreitol, and centrifuged (10000 ***g***). The supernatant was neutralized to pH 5–6 with 3 M-KOH (150 $\mu$l), and half was used directly for determination of free CoA. The rest was used for the determination of acid-soluble acyl-CoA, after hydrolysis of the esters by adding 30 $\mu$l of 3 M-KOH and incubating at 45 °C for 30 min and then neutralizing with 5 M-HCl, to give total CoA (acid-soluble CoA plus free CoA). The protein precipitate (containing acid-insoluble long-chain acyl-CoA) was hydrolysed by adding 200 $\mu$l of 1 M-KOH and 500 $\mu$l of 10 mM-dithiothreitol and incubation at 45 °C for 20 min. Protein was precipitated with 300 $\mu$l of 1 M-$HClO_4$ and the supernatant neutralized.

CoA in the extracts was determined enzymically by using 2-oxoglutarate dehydrogenase, by an automated fluorimetric assay using a centrifugal analyser (Cobas Bio 8326; Hofmann–La Roche). The assay medium contained 100 mM-Tris/HCl (pH 7.9), 5 mM-$MgCl_2$, 2 mM-$NAD^+$, 5 mM-2-oxoglutarate, 2 mM-dithiothreitol, 0.4 mM-thiamin pyrophosphate and 2-oxoglutarate dehydrogenase (10 munits) in a final volume of 200 $\mu$l, and 10 $\mu$l of either unhydrolysed or hydrolysed extract or CoA standards (25, 50 or 100 $\mu$M). The reaction was started by addition of 2-oxoglutarate dehydrogenase after a preincubation spin of 50 s at 100 ***g***. The increase in fluorescence (excitation 340 nm, emission 450–460 nm) after addition of enzyme was monitored at 30 s intervals for 8 min at 25 °C. The CoA concentration was determined from the increase in fluorescence by end-point analysis. End-points were reached at 100–150 s, and the increase in fluorescence was linear with [CoA] up to 100 $\mu$M.

### Measurement of enzyme activities

The following enzymes were assayed in homogenates of fresh liver or cultured hepatocytes spectrophotometrically at 30 °C using a centrifugal analyser (Cobas Bio 8362) as described previously: glutamate dehydrogenase (EC 1.4.1.2) and citrate synthase (EC 4.1.3.7) [18]; hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) [19]; CAT (EC 2.3.1.7), palmitoyl-CoA hydrolase (EC 3.1.2.2) and acetyl-CoA hydrolase (EC 3.1.2.1) [20]. The last enzyme was assayed in the presence of 2 mM-ATP or 1 mM-ADP, which respectively activate or inhibit the cytoplasmic enzyme [21]. All assays were run against

**Table 1. Concentrations of CoA, carnitine and their acyl esters in livers from control, ciprofibrate-fed and POCA-fed rats**

Concentrations are given as nmol/g wet wt., as means ± S.E.M.; significances of differences from controls: $*P < 0.05$; $**P < 0.01$; $***P < 0.005$. The apparent mass-action ratios for the reactions catalysed by the carnitine short-chain acyl- and palmitoyltransferases are defined as [carnitine][short-chain acyl-CoA]/[CoA][short-chain acylcarnitine] and [carnitine][long-chain acyl-CoA]/[CoA][long-chain acylcarnitine] respectively. The apparent mass-action ratios as measured depend on whole-tissue concentrations of CoA, carnitine and their acyl esters. However, the carnitine/acylcarnitine carrier in the mitochondrial inner membrane enables equilibration of the acylation state of the mitochondrial and cytosolic pools of CoA and carnitine, so that these concentrations define approximately the mass-action ratios (see the text).

| | Control ($n = 7$) | Ciprofibrate-fed ($n = 9$) | POCA-fed ($n = 5$) | Guinea pig ($n = 2$ or 3) |
|---|---|---|---|---|
| Concentrations | | | | |
| CoA | 43.0±2.6 | 71.5±7.4** | 84.6±14.0** | 33.8, 51.6 |
| Short-chain acyl-CoA | 12.2±2.5 | 35.2±6.1** | 38.7±3.1*** | 21.2, 13.5 |
| Long-chain acyl-CoA | 68.7±4.1 | 147.0±11.7*** | 189.0±16.8*** | 62.8, 45.6 |
| Total CoA | 124.0±6.2 | 253.0±13.7*** | 313.0±13.3*** | 118.0, 111.0 |
| Carnitine | 167.0±7.41 | 645.0±14.8*** | 328.0±11.6*** | 316.0±36.0*** |
| Short-chain acyl-carnitine | 40.0±4.3 | 291.0±22.0*** | 71.0±6.8*** | 86.0±17.0*** |
| Long-chain acyl-carnitine | 22.0±2.2 | 67.0±3.5*** | 29.0±1.6* | 61.0±7.0*** |
| Total carnitine | 229.0±6.6 | 1003.0±22.2*** | 428.0±13.5*** | 463.0±47.4*** |
| Concentration ratios | | | | |
| Carnitine/CoA | 3.70±0.69 | 9.09±2.48* | 4.00±1.44 | 7.14 |
| Short-chain acyl-CoA / Short-chain acyl-carnitine | 0.39±0.13 | 0.10±0.05 | 0.53±0.15 | 0.20 |
| Long-chain acyl-CoA / Long-chain acyl-carnitine | 3.03±0.83 | 2.08±0.56 | 6.25±1.95 | 1.12 |
| Apparent mass-action ratios | | | | |
| For short-chain esters | 1.32±0.16 | 0.99±0.38† | 2.17±0.24** | 1.12 |
| For long-chain esters | 11.6±0.16 | 20.0±12 | 13.2±9.7 | 66.7 |

† $n = 7$.

blanks with water in place of enzyme extract. Controls for CAT were run with enzyme extract and omission of L-carnitine. Reaction rates were monitored for 210 s and determined by a Linear Search and Regression program. The rates were corrected by subtraction of control values. Acyl-CoA oxidase activity with 25 μM-palmitoyl-CoA as substrate was recorded colorimetrically [22] with a dual-wavelength spectrophotometer.

## RESULTS

### Body weight and liver weight of drug-treated rats

Administration of ciprofibrate or POCA in the diet for 5 days had no significant effect on the body weights of the rats: in Expt. 1, controls, 204±9 g, ciprofibrate, 192±3 g, POCA, 198±16 g; in Expt. 2, controls, 203±6 g, ciprofibrate, 212±5 g (means ± S.E.M.). Ciprofibrate, but not POCA, increased the liver weights by 60%: in Expt. 1, controls 9.4±0.2 g, ciprofibrate, 15.1±0.3 g, POCA, 8.7±0.3 g; in Expt. 2, controls, 10.0±0.3 g, ciprofibrate, 16.3±0.9 g (means ± S.E.M.). All data for Expts. 1 and 2 were very similar and were pooled.

### Concentrations of CoA and acyl-CoA esters in liver

Ciprofibrate caused a 2-fold increase in [total CoA] (free plus acylated) in liver, and POCA a 2.5-fold increase (Table 1). Approx. 10% of the CoA was esterified with short-chain acyl groups in the controls, and 12% in both treated groups, and 55% with long-chain acyl groups in the controls and 60% in the treated groups (Table 1).

### Concentrations of carnitine and acylcarnitine esters in liver

Ciprofibrate caused a 4-fold increase in hepatic [carnitine] and a 7-fold increase in [short-chain acylcarnitine] (Table 1), with doubling of the [short-chain acylcarnitine]/[carnitine] ratio). POCA, by contrast, increased [carnitine] and [short-chain acylcarnitine] by 2-fold without affecting the [short-chain acylcarnitine]/[carnitine] ratio. However, it caused a small but significant increase in the [long-chain acyl-CoA]/[long-chain acylcarnitine] ratio (Table 1).

### Concentrations of carnitine and acylcarnitine esters in plasma, skeletal muscle and urine

The plasma [total carnitine] decreased in both ciprofibrate- and POCA-treated rats (Table 2), largely owing to decreases in [carnitine] (51 and 36% respectively). POCA also decreased [short-chain acylcarnitine] and [long-chain acylcarnitine].

Muscle [total carnitine] was 10% lower after administration of ciprofibrate, owing to lower [acylcarnitine] but not [carnitine] (Table 2). POCA did not decrease muscle [carnitine], in agreement with previous results [15]. In control and POCA-treated rats the percentage of the carnitine acylated was higher in skeletal muscle and in liver than in plasma (Tables 1 and 2).

Ciprofibrate increased the urinary excretion of acyl-

**Table 2. Concentrations of carnitine and acylcarnitine esters in rat skeletal muscle and plasma**

Experimental details are given in the Materials and methods section. Concentrations are expressed as nmol/g wet wt. or nmol/ml of plasma, and are means ± S.E.M. with the numbers of observations in parentheses. Significances of differences from controls: $*P < 0.05$; $**P < 0.01$; $***P < 0.005$.

| Tissue | Concn. | | | | Percentage of carnitine acylated |
|---|---|---|---|---|---|
| | Carnitine | Short-chain acylcarnitine | Long-chain acylcarnitine | Total carnitine | |
| Skeletal muscle | | | | | |
| Control (6) | 573±17 | 239±18 | 78±10 | 889±19 | 36±2 |
| Ciprofibrate-fed (6) | 582±13 | 160+23* | 43±4** | 785±19*** | 26±3* |
| POCA-fed (5) | 627+28 | 194±22 | 83±3 | 904±31 | 31±3 |
| Plasma | | | | | |
| Control (6) | 28±2 | 4.7±0.7 | 1.7±0.1 | 33.5±1.5 | 17±1 |
| Ciprofibrate-fed (9) | 13.7±2.1** | 3.4±0.8 | 1.2±0.1* | 18.0±1.8** | 23±3 |
| POCA-fed (5) | 18.1±0.9** | 1.8±0.4 | 1.1±0.1** | 21.6±0.6** | 14±2 |

**Table 3. Estimated contents of carnitine and acylcarnitine esters in skeletal muscle, extracellular fluid and liver in rats given ciprofibrate or POCA for 5 days**

The total carnitine (free and acylated) of liver was determined (Table 1) and that of skeletal muscle and extracellular fluid was estimated from measured concentrations (Table 1) and body weights, assuming that muscle is 40% of body weight and that extracellular fluid is 15%. The total carnitine content (sum of free plus acylated forms) is expressed in μmol, as means ± S.E.M. ($n = 5$ or 6): $*P < 0.05$; $***P < 0.005$.

| Tissue carnitine (all forms) | Controls | Ciprofibrate-fed | POCA-fed |
|---|---|---|---|
| Skeletal muscle | 73.0±2 | 61.8±1.5 | 71.6±0.3 |
| Extracellular fluid | 1.01±0.06 | 0.54±0.05*** | 0.70±0.10 |
| Liver | 2.24±0.06 | 15.9±0.8*** | 3.72+0.21* |
| Sum | 76.2 | 78.2 | 76.0 |
| Urinary output (μmol/day per rat) | 0.17±0.03 | 0.34±0.04 | 0.21±0.02 |

**Table 4. Activities of enzymes in livers from rats given ciprofibrate or POCA for 5 days**

Experimental details are given in the Materials and methods section. The activities are expressed as units/g wet wt., as means ± S.E.M. for $n$ observations. Significance of differences from means: $*P < 0.05$; $***P < 0.005$.

| Enzyme | Activity | | |
|---|---|---|---|
| | Control | Ciprofibrate-fed | POCA-fed |
| CAT | 0.67±0.04 ($n = 9$) | 18.7±1.0*** ($n = 10$) | 3.67±0.74*** ($n = 5$) |
| Acetyl-CoA hydrolase | 1.49±0.04 ($n = 9$) | 3.56±0.11*** ($n = 10$) | 2.53±0.07*** ($n = 5$) |
| plus 1 mM-ADP (1) | 0.75±0.04 ($n = 5$) | 1.09±0.03*** ($n = 5$) | – |
| plus 2 M-ATP (2) | 3.60±0.10 ($n = 5$) | 8.38±0.16*** ($n = 5$) | – |
| (2)−(1) | 2.85±0.07 | 7.29±0.14*** | – |
| Octanoyl-CoA hydrolase | 1.32±0.09 ($n = 9$) | 5.87±0.73*** ($n = 10$) | 2.53+0.06*** ($n = 5$) |
| Palmitoyl-CoA hydrolase | 0.69±0.02 ($n = 9$) | 1.76±0.17*** ($n = 10$) | 0.93+0.03*** ($n = 5$) |
| 3-Hydroxybutyryl-CoA dehydrogenase | 69.3±0.02 ($n = 5$) | 99.2±0.8*** ($n = 5$) | – |
| Glutamate dehydrogenase | 135±7 ($n = 9$) | 103±10* ($n = 10$) | 116±5 ($n = 5$) |
| Citrate synthase | 15.9±0.4 ($n = 9$) | 11.3±0.7* ($n = 10$) | 14.6±0.8 ($n = 5$) |

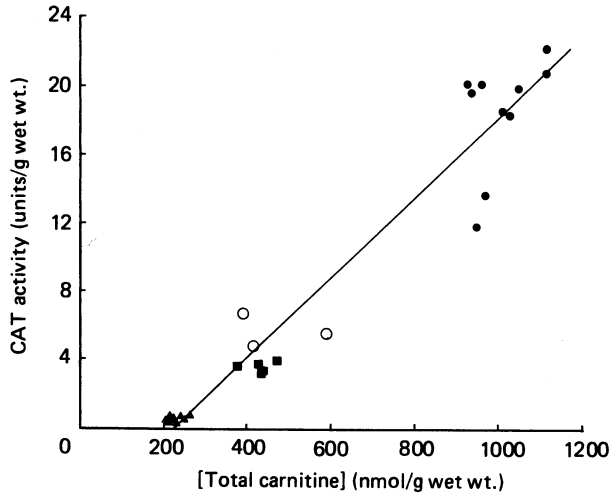


**Fig. 1. Relation between hepatic carnitine content and CAT activity**

Hepatic carnitine (free plus acylated) is expressed as nmol/g wet wt., and CAT activity is expressed as units/g wet wt. Data from control rats (▲), POCA-fed rats (■), ciprofibrate-fed rats (●) and guinea pigs (○) are from the experiments in Tables 1 and 4.

carnitine esters (controls, 148±32, and ciprofibrate-fed, 318±40 nmol/24 h; means±S.E.M., $P < 0.01$), but not of free carnitine (controls, 23±3, and ciprofibrate-fed 19±4, nmol/24 h). POCA had no significant effect on excretion of either free carnitine (35±8 nmol/24 h) or acylcarnitine (179±22 nmol/24 h) (Table 3).

### Effects of ciprofibrate and POCA on enzyme activities in liver

The activity of CAT increased 28-fold and 6-fold (per g wet wt.) after ciprofibrate and POCA treatment respectively (Table 4), and the total liver activity increased 46-fold in livers from ciprofibrate-treated rats, after allowance for hypertrophy. The activities of acyl-CoA hydrolases assayed with acetyl-CoA, octanoyl-CoA and palmitoyl-CoA as substrates were also increased by ciprofibrate and POCA treatment (Table 4); the increases were greater with ciprofibrate than with POCA. In rat liver, acetyl-CoA hydrolases occur in the cytoplasm and the mitochondrial matrix [20]. The increased acetyl-CoA hydrolase activity in livers from ciprofibrate-fed rats was predominantly due to the cytoplasmic ATP-dependent enzyme. The concentrations of acyl-CoA esters were increased by ciprofibrate and POCA (Table 1), despite elevated acyl-CoA hydrolase activity. The activity of hydroxyacyl-CoA dehydrogenase with acetoacetyl-CoA as substrate, which measures both the peroxisomal and mitochondrial enzymes [23], was greater after ciprofibrate treatment. By contrast, the activities of glutamate dehydrogenase and citrate synthase, which only occur in the mitochondrial matrix, were lower (Table 4). Acyl-CoA oxidase activities, determined by Dr. P. McNamee, were increased 10-fold and 3-fold per g wet wt. after ciprofibrate treatment and POCA treatment respectively (results not shown), in agreement with earlier studies [4,7].

### Correlation between hepatic CAT and [carnitine]

The activity of hepatic CAT strongly correlated ($r = 0.976 \pm 0.047$; $P < 0.00001$) with [total carnitine] in liver for all treatments (Fig. 1, Table 1). The correlation coefficients for CAT activity versus [carnitine] and [short-chain acyl-carnitine] were respectively 0.971±0.051 and 0.918±0.085 ($P < 0.00001$, $n = 24$).

### Effects of ciprofibrate on enzyme activities in cultured hepatocytes

Hepatic changes caused by administration of ciprofibrate may be caused by direct effects of the drug or secondary changes in concentrations of circulating hormones or metabolites [24]. In monolayer-cultured hepatocytes, ciprofibrate caused similar increases in CAT and acyl-CoA hydrolase activities (Table 5) as *in vivo* (Table 4), indicating that changes in hepatic enzyme activities (Table 4) are caused directly.

## DISCUSSION

### Relation between mitochondrial and peroxisomal β-oxidation

Chronic administration of fibrate drugs causes a massive increase (up to 10-fold) in the number of liver peroxisomes and the capacity for peroxisomal β-oxidation in rats [25,26]. The capacity for mitochondrial β-oxidation is also increased 2–3-fold, so that the absolute increase is comparable with that in the peroxisomes as assessed by the capacity for acetyl-CoA formation [6,27]. [CoA] [6,8], [carnitine] [9,10] and the fatty-acid-binding Z-protein are increased [28–30]. Fibrate drugs may cause

**Table 5. Enzyme activities in hepatocytes cultured with ciprofibrate for 4 days**

Hepatocytes isolated from rats maintained on standard diet were cultured for 4 days with the concentrations of ciprofibrate indicated. The medium was changed daily, and enzyme activities are expressed as munits/mg of protein, as means±S.E.M. of results from duplicate flasks from four cultures. Significance of differences from controls: $^{**}P < 0.01$; $^{***}P < 0.005$.

| Enzyme | [Ciprofibrate] (ng/ml)... | Activity 0 | 10 | 100 | 1000 |
|---|---|---|---|---|---|
| CAT | | 1.02±0.27 | 1.28±0.30 | 3.26+0.47** | 14.1±0.47*** |
| Acetyl-CoA hydrolase | | 2.65±0.04 | 2.79±0.09 | 4.68±0.25*** | 8.59±0.56*** |
| Octanoyl-CoA hydrolase | | 4.9±1.6 | 5.4±1.7 | 7.1±1.8 | 15.0±2.5** |
| Palmitoyl-CoA hydrolase | | 2.4±1.0 | 2.6±1.2 | 2.6±1.0 | 4.7±1.4 |
| Glutamate dehydrogenase | | 1006±294 | 1035±748 | 963±243 | 859±351 |

co-ordinated changes in rodent liver, which increase the capacity for fatty acid oxidation and which may be an exaggerated physiological response to the ingestion of some natural very-long-chain fatty acids such as erucate ($C_{22:1}$) [31,32]. POCA causes a limited (about 3-fold) increase in peroxisomes and in peroxisomal $\beta$-oxidation, similar to that caused by erucate [7]. POCA (as POCA-CoA), however, is a very potent inhibitor of CPT I [5] and hence of mitochondrial oxidation of long-chain fatty acids; and induction of peroxisomes may be due to POCA/POCA-CoA itself, or because of this inhibition. Curiously, simultaneous administration of bezafibrate and POCA to fasted, but not fed, rats limits peroxisomal proliferation to about the extent caused by POCA alone [33]. However, the mechanisms by which ciprofibrate and POCA produce their effects, and why these differ quantitatively, are unknown.

### Effect of ciprofibrate and POCA on hepatic enzyme activities

Administration of ciprofibrate or of POCA for only 5 days caused large increases in the activities of CAT and of acyl-CoA hydrolases in rat liver. Ciprofibrate caused larger changes than POCA (Table 4). Maximum increases occur after administration of ciprofibrate or POCA for 10–14 days [34].

### Role of carnitine as an acyl-group buffer

Hepatic [carnitine] increases during the fed-to-fasted transition in the rat [7,35]. Plasma [carnitine] and [acylcarnitine] are also higher during fasting and diabetes [36], suggesting increased export by the liver. Increased tissue [carnitine] increases the buffering capacity for acyl groups and may facilitate maintenance of the [CoA]/[carnitine] ratio within limits compatible with normal metabolism [37].

Both ciprofibrate and POCA caused a similar increase (2–3-fold) in total liver [CoA] (Table 1). However, ciprofibrate caused a much larger increase in total liver [carnitine] than did POCA (Table 1). Both drugs increased the activity of CAT, and there was a highly significant correlation between CAT activity and [total carnitine] and [acylcarnitine] for all conditions (Fig. 1), but not between CAT and [CoA]. This correlation also holds for guinea-pig liver, which has higher CAT activity [38] and [total carnitine] than control rat liver (Table 1).

The mitochondrial inner membrane is impermeable to CoA and acyl-CoA esters, with separate pools of CoA plus acyl-CoA esters in the mitochondrial matrix and other compartments. Measured [CoA], [carnitine], [acyl-CoA] and [acyl-carnitine] are the sums of these (Tables 1 and 2). However, the mitochondrial and cytosolic compartments are connected by a carnitine/acylcarnitine exchange carrier in the mitochondrial inner membrane [3] (assumed to have a low control strength), so that at equilibrium the ratios of [acylcarnitine]/[carnitine] will be equal, and hence the [CoA]/[acyl-CoA] ratios will also be equal. Further, it has been suggested that peroxisomes are permeable to small water-soluble molecules [39], so that the acylation status of the major pools of CoA and carnitine in the hepatocyte may be related. The reactions catalysed by the carnitine acyltransferases have two substrates and two products, so that the equilibrium constant, defined as [carnitine][acyl-CoA]/[CoA][acyl-carnitine], which has a value of 0.6 [40], is dimensionless. At equilibrium, therefore, the ratios of the reactants are independent of their absolute concentrations. It has been proposed that a reaction is at near-equilibrium if the mass-action ratio does not differ by more than a factor of 5 from the equilibrium constant [41]. The apparent mass-action ratios for the reactions catalysed by CAT (and by other carnitine acyltransferases acting on short-chain esters) were in the range 1.0–2.2 (Table 1), suggesting that these were not too far displaced from equilibrium *in vivo* (allowing for any zonal differences [42]) (Table 1). This explains the high [short-chain acylcarnitine] in liver after administration of ciprofibrate. Since [carnitine] is greater than [CoA], the [CoA]/[carnitine] ratio is low when the [acylcarnitine]/[acyl-CoA] ratio is high at near-equilibrium. However, even in control animals the reactions catalysed by CAT appear to be near equilibrium. Tissue [CoA], [carnitine], [short-chain acyl-CoA] and [short-chain acylcarnitine] (Table 1) are in the range of, or greater, than the $K_m$ values for pigeon breast-muscle CAT: CoA, 37–60 $\mu$M; short-chain acyl-CoA, 11–44 $\mu$M; carnitine, 100–174 $\mu$M; and short-chain acyl-carnitine, 350–455 $\mu$M [40]. By contrast, the mass-action ratio for the reaction catalysed by CPT was strongly displaced from equilibrium (Table 1), and was not apparently changed by POCA, even though POCA-CoA strongly inhibits CPT I [5]. This latter equilibrium may be influenced by binding of long-chain acyl-CoA and carnitine esters to cell proteins.

### Mechanism of the increase in liver carnitine concentrations

Administration of ciprofibrate increases liver [total carnitine] from 0.23 to about 1 $\mu$mol/g wet wt. However these values are only about 2.4% and 18% of the estimated skeletal-muscle [total carnitine] respectively (Table 3). Ciprofibrate increased the proportion of carnitine that was acylated in liver and plasma, but decreased it in muscle (Table 2). Plasma [total carnitine] was lowered by 46% and by 35% respectively, by administration of ciprofibrate or POCA (Table 2). This decrease cannot be explained by increased urinary excretion or uptake by muscle (Table 3). Increased hepatic [carnitine] may be due to greater retention. Muscle [total carnitine] was lowered by 15% by ciprofibrate. The estimated sum of [total carnitine] in muscle, liver and the extracellular fluid (Table 3) was the same in all three groups. Although this may be a coincidence, it suggests redistribution of carnitine between tissues after administration of ciprofibrate. There was no apparent simple relation between plasma and tissue [carnitine] and [acylcarnitine]. Ciprofibrate increased the amount of carnitine excreted in the urine; this was only a tiny fraction (0.2–0.5%) of the total tissue content (Table 3).

Carnitine is synthesized from methionine and lysine, which are converted into $\gamma$-butyrobetaine in muscle [1]. In the rat only the liver converts $\gamma$-butyrobetaine into L-carnitine, which is then exported to other tissues [1]. Our results differ from those of a previous study on rats given 30 mg of clofibrate orally for 14–21 days, in which no decreases in muscle or plasma [carnitine] were found [43]. However, it is difficult to compare results obtained with different compounds and dose schedules.

There was a strong correlation between [long-chain acyl-CoA] and the activity of palmitoyl-CoA hydrolase in livers of rats treated with various peroxisomal

proliferators [44]. The correlations between liver [carnitine] and CAT activity (Fig. 1) may be analogous. However, ciprofibrate increased CAT activity in hepatocytes cultured in the absence of the carnitine precursor γ-butyrobetaine (Table 5). Hepatocyte [carnitine] declines in culture [14], in both the presence and the absence of ciprofibrate (results not shown), presumably because of lack of precursor. Therefore, ciprofibrate may cause parallel rather than interdependent changes in [carnitine] and CAT activity.

## Conclusions

Three conclusions are drawn from this study: first, that the reactions catalysed by the short-chain carnitine acyltransferases, but not by the carnitine palmitoyltransferases, are not too far displaced from equilibrium in rat liver both before and after modification of liver metabolism by administration of ciprofibrate and POCA; second, that retention of carnitine by the liver, and hence redistribution of carnitine between tissues, may be an important factor in the increase in hepatic carnitine caused by ciprofibrate and by POCA; third, that hepatic CAT activity and [carnitine] may be closely related.

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