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INHIBITION OF RAT PEROXISOMAL PALMITOYL-CoA LIGASE BY XENOBIOTIC CARBOXYLIC ACIDS

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Abstract—ATP-dependent coenzyme A (CoA) ligases catalyse the formation of the acyl-CoA thioesters of xenobiotic carboxylic acids and the formation of xenobiotic-CoAs has been implicated as being a causative factor in peroxisomal proliferation. In this study we have demonstrated using rat liver peroxisomes that the formation of palmitoyl-CoA is inhibited by a variety of xenobiotic carboxylic acids. Palmitoyl-CoA formation exhibited biphasic kinetics indicative of two isoforms, a high affinity (K_{m1} 2.3 μM) low capacity form and a low affinity (K_{m2} 831 μM) high capacity form. These forms were differentially inhibited by a range of xenobiotics. However, it would appear that the low affinity component may not contribute to any major extent to the formation of xenobiotic-CoAs *in vivo*. At a concentration of 1 mM, greater than 20% inhibition of the high affinity form was observed with the 2-arylpropionates, ibuprofen, naproxen, benoxaprofen, fenoprofen, indoprofen, ketoprofen, tiaprofenic acid and cicloprofen, the hypolipidaemics, nafenopin and ciprofibrate, and the herbicides, silvex and 2,4,5-trichlorophenoxyacetate. Valproic acid, clofibric acid, salicylic acid and 2,4-dichlorophenoxyacetate were non-inhibitory at all concentrations studied (0.1–2.5 mM). Analysis of the type of inhibition established that only nafenopin (K_i 430 μM) and ciprofibrate (K_i 97 μM) were competitive inhibitors of palmitoyl-CoA formation suggesting that they bind at the active site and thus potentially function as alternative substrates for the peroxisomal ligase. Notably, clofibric acid which has previously been shown to form clofibroyl-CoA in peroxisomes did not interact with the palmitoyl-CoA ligase thereby suggesting that activation is mediated via an alternative peroxisomal CoA ligase. In addition, the xenobiotic inhibitors of the peroxisomal palmitoyl-CoA ligase differed from those previously reported for the equivalent microsomal enzyme suggesting that the organellar forms may be functionally distinct. This study establishes that numerous xenobiotic carboxylic acids interact with the peroxisomal palmitoyl-CoA ligase; however, it would appear that relatively few function as alternative substrates. The toxicological ramifications of peroxisomally mediated xenobiotic-CoA formation and the identification of other peroxisomal xenobiotic-CoA ligase(s) remain to be elucidated.

Oxidation of fatty acids necessitates their prior activation to acyl coenzyme A (CoA†) thioesters and the CoA ligases (E.C 6.2.1.1–3) responsible for this activation are localized in several subcellular compartments. The long chain fatty acid CoA ligase (E.C 6.2.1.3) initially demonstrated in rat liver microsomes has subsequently been found to exist in both the mitochondrial and peroxisomal outer membrane [1–3] and recent studies have suggested that these three organellar forms are identical [4, 5]. The peroxisomal long chain CoA ligase, first observed in rat liver by Shindo and Hashimoto [2], shows optimal affinity for palmitic acid (C16) and is situated in the peroxisomal membrane, facing the cytosolic side [6]. *In vivo*, peroxisomes oxidize a wide spectrum of fatty acids, fatty acid derivates, xenobiotics and bile acid intermediates.

Xenobiotic carboxylic acids are known to be alternative substrates for both the hepatic mitochondrial and microsomal CoA ligases and the formation of novel xenobiotic-CoA esters by peroxisomes has been implicated as a prerequisite for the proliferating activity of a variety of sulphur- and oxy-substituted fatty acid analogues [7]. Previous studies have demonstrated that hypolipidaemic drugs such as clofibric acid and nafenopin are also activated to CoA esters by the microsomal long chain ligase and it has been suggested that the thioester derivative plays a causative role in the induction of peroxisomal proliferation and alterations in lipid metabolism [8, 9]. Additionally, a variety of other xenobiotics containing a carboxylic acid moiety e.g. ibuprofen, fenoprofen [10, 11] and 3-phenoxybenzoic acid [12] are activated to acyl-CoA thioesters by the microsomal enzyme. A recent study has demonstrated the existence of two isoforms of rat microsomal long chain fatty acid CoA ligase with activity of the high affinity form showing inhibition by various 2-arylpropionate enantiomers [13]. Given the apparent inter-organellar homology of this ligase a comparable profile of xenobiotic activation should be observed with peroxisomes. This is an important consideration in view of the fact that alteration of the functional capacity of this enzyme in peroxisomes may perturb lipid metabolism.

The aims of this study were firstly to establish the presence of either single or multiple forms of peroxisomal palmitoyl-CoA ligase and secondly to characterise the xenobiotic substrate profile of the enzyme(s). Inhibition of palmitoyl-CoA formation

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† Abbreviations: CoA, coenzyme A; 2,4,5-T, 2,4,5-trichlorophenoxyacetate; 2,4-D, 2,4-dichlorophenoxyacetate.

was studied in the presence of putative substrates using representative peroxisomal proliferators (nafenopin, clofibric acid and ciprofibrate), racemic 2-arylpropionates (tiaprofenic acid, cicloprofen, indoprofen, ketoprofen, fenoprofen and benoxaprofen) and the *R* and *S* enantiomers of ibuprofen and naproxen, phenoxyherbicides [silvex, 2,4,5-trichlorophenoxyacetate (2,4,5-T) and 2,4-dichlorophenoxyacetate (2,4-D)], valproic acid, and salicylic acid.

MATERIALS AND METHODS

Materials

Xenobiotic carboxylic acids were obtained from the following sources: ketoprofen (May and Baker, U.S.A.); fenoprofen and benoxaprofen (Eli Lilly, U.S.A.); cicloprofen (E.R. Squibb, U.S.A.); indoprofen (Farmitalia Carlo Erba, Italy); tiaprofenic acid (Roussel, France); *S* naproxen (Sigma Chemical, Co, St Louis, MO, U.S.A.); *R* naproxen (Syntex Research, Palo Alto, CA, U.S.A.); *R* and *S* ibuprofen (Boots Co., Nottingham, U.K.); clofibric acid, 2,4,5-T, 2,4-D, silvex, valproic acid and salicylic acid (Sigma); nafenopin (Ciba-Geigy, Australia) and ciprofibrate (Sterling Drug Inc, U.S.A.). [^{14}C]-Palmitic acid was obtained from Amersham Radiochemicals (Australia) and Nycodenz from BDH Chemicals (Melbourne, Australia). All other chemicals used were of the highest analytical purity and purchased from various commercial sources.

Methods

Male outbred Sprague–Dawley rats (200–300 g) were used throughout this study. All animals were housed under standard conditions of light and temperature and allowed food and water *ad lib*.

Preparation of peroxisomes. The liver was removed from each animal under sodium pentobarbitone anaesthesia (60 mg/kg); placed in ice-cold 0.25 M sucrose, 10 mM Tris–HCl (pH 7.4); blotted dry; weighed; minced and homogenized (two strokes) in a 30-mL Potter–Elvehjem hand-held homogenizer with a teflon pestle at 4°. The homogenate was centrifuged using a Beckman JA-20 rotor (700 *g*, 10 min, 4°). The pellet, containing nuclear cellular debris was discarded and the supernatant centrifuged (4°) at an integrated *g*-force of 24,000 *g*/min. The supernatant was removed, kept on ice, the pellet suspended in sucrose buffer (25% original liver weight/mL) and the 24,000 *g*/min centrifugation repeated. The pellet obtained represented the nuclear and heavy mitochondrial fraction as previously described by Leighton *et al.* [14]. The supernatants from both centrifugations were combined and re-centrifuged (4°) at an integrated *g*-force of 305,000 *g*/min (Beckman Ti50.2 rotor). The supernatant was discarded and the pellets resuspended in sucrose–Tris buffer (20 mL/pellet) and the centrifugation step repeated. The pellets (representing the light mitochondrial fraction) were resuspended in sucrose–Tris buffer (0.8 mL each) and homogenized (two strokes in a 10 mL Potter–Elvehjem homogenizer, 1000 rpm). Using Beckman ultra centrifuge tubes (28.5 mL), approximately 30–35 mg of the protein was carefully applied to a Nycodenz gradient (comprising 20, 30, 40 and 50% w/v solutions) containing 1 mM tetra sodium EDTA (4 mL, pH 7.3) which had previously been allowed to diffuse at 4° for 24 hr. Following application of the protein to the Nycodenz, the gradient was centrifuged (4°) for 2 hr 50 min (105,000 *g* in a Beckman Ti50.2 rotor). The fractions were collected as 2-mL aliquots diluted 1:5 in sucrose–Tris buffer (approximately eight aliquots in all) and recentrifuged at 175,000 *g* for 35 min. The pellets were collected, resuspended in sucrose–Tris buffer (0.5 mL) and gently homogenized in 1-mL tubes with a hand-held micro teflon pestle. The fractions were stored at −70° until use. (Preliminary studies had indicated that the enzyme was stable under these conditions for 2 months.)

Enzyme assays. The stored fractions were analysed for the following marker enzymes: catalase (peroxisomes), according to the method of Cohen *et al.* [15], glucose-6-phosphatase (microsomes) [16], and glutamate dehydrogenase (mitochondria) [17]. Protein was determined according to the procedure of Lowry *et al.* [18].

[^{14}C]*Palmitoyl CoA ligase activity*. Palmitoyl CoA ligase activity was measured as described previously with minor modifications [3]. Preliminary studies established the linearity of palmitoyl-CoA formation with respect to protein concentration and duration of incubation. The standard incubation medium (0.2 mL) contained Tris–HCl (150 mM, pH 7.4), $MgCl_2$ (6.2 mM), Triton X-100 (0.05%), EDTA (2 mM), ATP (2.5 mM), CoA (reduced) (0.6 mM), dithiothreitol (1 mM) and [^{14}C]palmitic acid 0.25–720 μM (7 mCi/mmol). The medium was pre-warmed (1 min, 37°) and the reaction initiated by the addition of 20 μL enzyme protein (25 μg/mL). The incubation was terminated after 10 min and [^{14}C]palmitoyl-CoA extracted and quantified as described previously [3].

Inhibition of [^{14}C]*palmitoyl-CoA formation*. The possible inhibitory effects of the 2-arylpropionates benoxaprofen, ibuprofen (*R* and *S*), naproxen (*R* and *S*), indoprofen, ketoprofen and fenoprofen [all dissolved in a minimal volume of NaOH (2 M) and titrated to pH 8.0], cicloprofen and tiaprofenic acid (10% w/v dimethyl sulphoxide), 2,4,5-T, 2,4-D and silvex (10% w/v dimethyl sulphoxide), clofibric acid, nafenopin, ciprofibrate, valproic acid, and salicylic acid (in 0.1 M Tris–HCl pH 8.0 containing 0.5% w/v Triton X-100) on palmitoyl-CoA formation were assessed using concentrations of 0.1, 1.0 and 2.5 mM xenobiotic and 1.0 and 720 μM [^{14}C]palmitic acid. In the cases of racemic drugs the concentrations stated applied to the individual enantiomers.

When >40% inhibition was observed at 1 mM xenobiotic concentration, full kinetic studies were performed to determine the type of inhibition and the apparent K_i value. [^{14}C]Palmitic acid (0.5, 1.5 and 4 μM) was incubated either in the absence or presence of *R* and *S* ibuprofen (10–125 μM), *R* and *S* naproxen (10–100 μM), nafenopin (10–250 μM), ciprofibrate (10–50 μM), 2,4,5-T (25–250 μM), and silvex (25–250 μM).

Data analysis. Values for the Michaelis–Menten parameters apparent K_m and V_{max} were initially obtained from Eadie–Hofstee plots of the data [19].

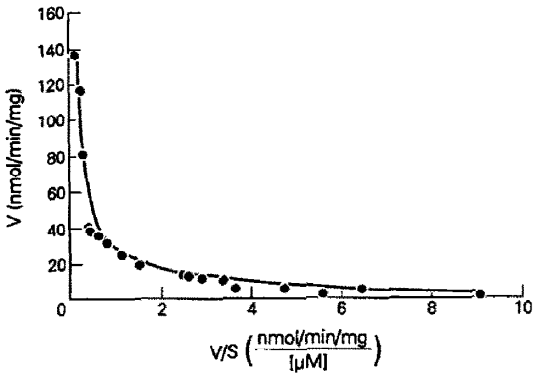

Fig. 1. A representative Eadie–Hofstee plot of palmitoyl-CoA ligase activity in rat hepatic peroxisomes. Palmitoyl-CoA ligase activity was determined by quantifying palmitoyl-CoA formation over a substrate range of 0.25–720 μM palmitic acid. The computer fitted regression curve was derived from the experimental data points (•).

Table 1. Michaelis–Menten parameters for palmitoyl-CoA formation in rat liver peroxisomes

N	K_{m1} (μM)	V_{max1} (nmol/mg/min)	K_{m2} (μM)	V_{max2} (nmol/mg/min)
1	2.82	29.3	1429	284
2	3.30	24.3	1408	362
3	2.11	16.1	563	196
4	1.96	15.6	617	221
5	1.28	14.1	287	154

Determination of palmitoyl-CoA ligase activity and calculation of the apparent K_m and V_{max} values were undertaken as described in Materials and Methods.

The values are tabulated as individual results from each rat.

These values were then used as initial estimates in MKMODEL, an extended least-squares regression modelling programme [20]. Analysis of the data from the inhibition studies was carried out using the method of Dixon [21] and the type of inhibition verified by secondary plot analysis [22].

RESULTS

Similarly to a previous study [23], contamination of the peroxisomal fraction with microsomes and mitochondria as determined by the marker enzymes glucose-6-phosphatase and glutamate dehydrogenase was 11 ± 3 and 9 ± 2%, respectively (mean ± SD, N = 5). Palmitoyl-CoA formation in peroxisomes exhibited biphasic kinetics in the five rat livers studied (Fig. 1). The mean of the apparent K_{m1} and V_{max1} for the high affinity component was 2.29 ± 0.7 μM and 19.88 ± 5.9 nmol/mg/min, respectively, while the apparent K_{m2} was 860.8 ± 468.9 μM and V_{max2} 243.4 ± 72.7 nmol/mg/min (mean ± SD, N = 5). The presence of two activities was not explained by contamination with other organelles because the maximum contribution to the high and low affinity enzymic processes was 1.4 and 5.8 nmol/mg/min, respectively, which was considered negligible in relation to total peroxisomal palmitoyl-CoA ligase activity. A further consideration however in interpreting the kinetics of lipid metabolizing enzymes is the effect of micellar formation with increasing substrate concentration. The higher concentrations of palmitic acid used in this study (50–720 μM) are within the range reported by numerous investigators studying long chain ligase activity (50 μM–1 mM). In addition, a previous study [24] has reported no evidence of micellar formation at a concentration of 55 μM palmitic acid. In this study, biphasic kinetics were evident at at concentration of 10 μM and seven additional concentrations studied betweeen 10 and 50 μM suggested the presence of a second isoform catalysing palmitoyl-CoA formation. This was confirmed in that the data was best fitted to a two enzyme system, as evidenced by the even distribution of the residuals around zero across the substrate range examined. The Michaelis–Menten parameters determined using MKMODEL are shown in Table 1.

A number of xenobiotics were screened for inhibitory effects on palmitoyl-CoA formation using two palmitic acid concentrations, i.e. 1.0 and 720 μM. At a substrate concentration of 1 μM the high affinity form of the enzyme predominated at a ratio of 22:1 while at 720 μM the low affinity component predominated at a 5:1 ratio. At palmitic acid concentrations of either 1 or 720 μM and an inhibitor concentration of either 100 μM, 1 or 2.5 mM, valproic, salicylic and clofibric acid and 2,4-D all caused <4% inhibition of both forms of the enzyme. In contrast, inhibition of the high affinity form was observed to varying degrees by the remaining xenobiotics (Fig. 2). The peroxisomal proliferators ciprofibrate and nafenopin were both inhibitory with the latter compound exhibiting profound inhibition at all three concentrations. Notably, none of the inhibitors achieved greater than 20% inhibition of the high affinity form until a concentration of 1 mM was used and the majority required a concentration of 2.5 mM to exert greater than 50% inhibition. Benoxaprofen, ketoprofen, indoprofen, fenoprofen, cicloprofen and tiaprofenic acid are racemates and hence it is impossible to attribute the inhibitory effect to either one or both of the enantiomers.

A similar pattern of inhibition of the low affinity form (although markedly weaker) was observed. This may in part be a reflection of the presence of approximately 20% of the high affinity enzyme (Fig. 3). Kinetic studies of the high affinity component of palmitoyl-CoA formation indicated that only nafenopin and ciprofibrate were competitive inhibitors (Table 2) while *R* and *S* ibuprofen, *R* and *S* naproxen, and 2,4,5-T and silvex displayed mixed type inhibition (K_i > 340 μM). Representative Dixon plots for *R* ibuprofen and nafenopin are shown in Fig. 4.

DISCUSSION

The results of this study suggest the involvement

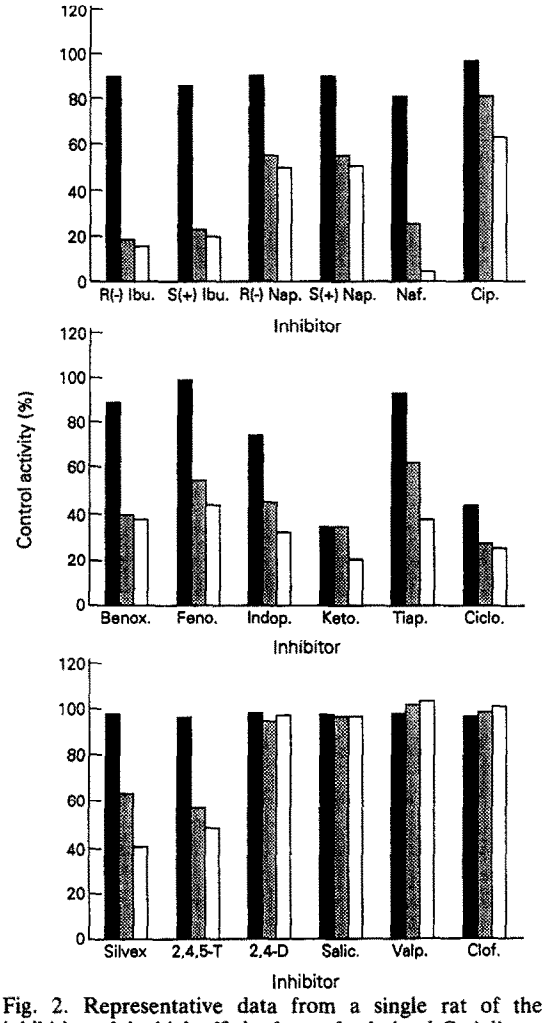

Fig. 2. Representative data from a single rat of the inhibition of the high affinity form of palmitoyl-CoA ligase by xenobiotics containing a carboxylic acid moiety. Inhibition of palmitoyl-CoA formation was determined in the presence of 1 μM palmitic acid and either 0.1 (■), 1.0 (▨) or 2.5 mM (□) xenobiotic. In the case of racemates these concentrations applied to the individual enantiomers: Ibu (ibuprofen), Nap (naproxen), Naf (nafenopin), Cip (ciprofibrate), Benox (benoxaprofen), Feno (fenoprofen), Indop (indoprofen), Keto (ketoprofen), Tiap (tiaprofenic acid), Ciclo (cicloprofen), Salic (salicylic acid), Valp (valproic acid) and Clof (clofibric acid).

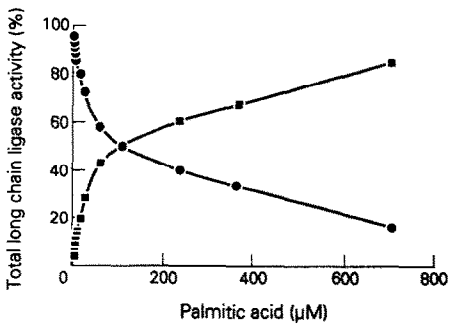

Fig. 3. Plot of the percentage contribution of the high and low affinity isoforms to overall palmitoyl-CoA ligase activity. Using the Michaelis–Menten equation for a two enzyme system and the mean kinetic data from the five rats studied the contribution of the high (•) and low (■) affinity long chain ligase isoforms towards total palmitoyl-CoA formation was calculated.

Table 2. Type of inhibition and apparent K_i values for inhibition of the high affinity process of palmitoyl-CoA formation in rat liver peroxisomes.

Compound	Inhibition	K_i (μM)
Nafenopin	Competitive	390 ± 71
Ciprofibrate	Competitive	95 ± 14
(−) *R* Ibuprofen	Mixed	460 ± 158
(+) *S* Ibuprofen	Mixed	380 ± 109
(−) *R* Naproxen	Mixed	650 ± 138
(+) *S* Naproxen	Mixed	540 ± 125
2,4,5-T	Mixed	3000 ± 1000*
Silvex	Mixed	2900 ± 868*

Data are presented as means ± SD (N = 2*, 3).
The type of inhibition and apparent K_i values were determined from Dixon plots as described in Materials and Methods.

of two forms of rat liver peroxisomal long chain ligase in the formation of the acyl CoA thioester of palmitic acid. In all livers studied, high affinity, low capacity and low affinity, high capacity components were observed. The contribution of each form to total palmitoyl-CoA formation was calculated from the Michaelis–Menten equation for a two enzyme system using mean kinetic data from the five rat livers studied. It is apparent that the low affinity component contributes 4.3% of the activity towards total palmitoyl-CoA formation at a concentration of 1 μM and is therefore unlikely to contribute to any major extent to the formation of xenobiotic-CoAs *in vivo*. Previous investigators have shown using isolated hepatocytes that the incorporation of fenoprofen via fenoprofen-CoA into triglycerides proceeds via a high and a low affinity enzymatic process; however, the authors concluded that the low affinity process occurred at fenoprofen concentrations not clinically relevant to man [25]. The existence of a very long chain CoA ligase in peroxisomes has been postulated [6, 26]; however, it is beyond the scope of this study to speculate on a possible relationship between the low affinity enzyme for the activation of palmitic acid as demonstrated in this study and the presence of a very long chain ligase.

There was relatively little variability in the kinetic parameters for palmitoyl-CoA formation by hepatic peroxisomes in the five rat livers studied. The apparent K_{m1} values varied less than 3-fold while the V_{max1} values varied approximately 2-fold. The apparent K_{m1} for the high affinity peroxisomal process as determined in this study is similar in

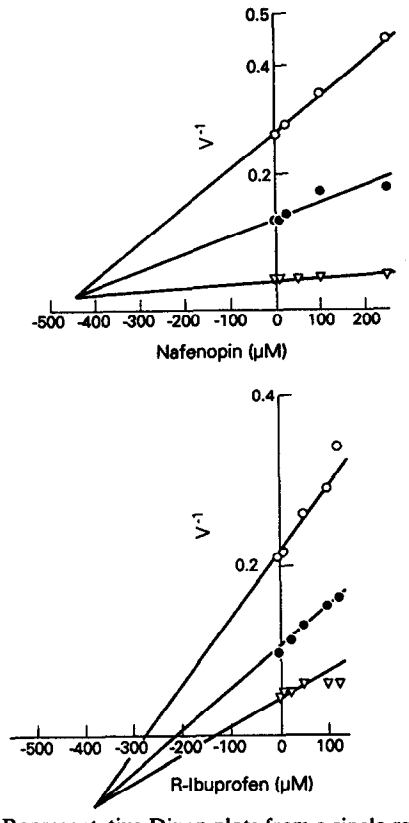

Fig. 4. Representative Dixon plots from a single rat of the inhibition of the high affinity peroxisomal long chain ligase by nafenopin and *R* ibuprofen. Regression lines were fitted to the inhibition data obtained using palmitic acid 0.5 (○), 1.5 (●) and 4 μM (▽), and either nafenopin (10–250 μM) or *R* ibuprofen (10–125 μM).

magnitude to the apparent K_m previously reported for a rat microsomal long chain ligase [13, 27]. However, the values for the low affinity component markedly differ between the organelles (apparent K_{m2} 0.86 and 0.51 mM and V_{max2} 243 and 58.5 nmol/mg/min for peroxisomes and microsomes, respectively) [13].

Selected xenobiotic carboxylic acids were screened for inhibitory effects on palmitoyl-CoA formation. All but four of the compounds, 2,4-D, valproic, clofibric and salicylic acid showed come degree of inhibition of either one or both of the isoforms. Valproic and salicylic acid are known to undergo activation by the mitochondrial matrix medium chain ligase [28, 29] and therefore it was not unexpected that a lack of an inhibitory effect on the peroxisomal long chain ligase was observed. Bronfman *et al.* [8] and Lygre *et al.* [9] have demonstrated that the rat hepatic microsomal long chain ligase catalyses the formation of clofibroyl-CoA. However, in this study no substantial inhibition of peroxisomal-mediated palmitoyl-CoA formation was observed with clofibric acid. The failure of clofibric acid to inhibit the peroxisomal ligase at concentrations as high as 2.5 mM suggests that it may not act as an alternative substrate for the long chain ligase. Aarsland and Berge [7] have shown that the rate of formation of clofibroyl and fenofibroyl-CoA in the post nuclear fraction of enzyme-induced animals was <10% of the rate relative to palmitoyl-CoA synthesis. It is apparent that peroxisomes mediate clofibroyl-CoA formation; however, the results of this study suggest that this process is unlikely to be catalysed by the long chain (palmitoyl) CoA ligase. The phenoxy herbicides 2,4-D, 2,4,5-T and silvex undergo amino acid conjugation following activation to CoA thioesters [30–32] by the mitochondrial medium chain ligase and it is interesting to note that the latter two compounds inhibited palmitoyl-CoA formation in peroxisomes, albeit relatively weakly ($K_i > 2$ mM).

Studies investigating the interaction of non-steroidal arylpropionates with intermediary metabolism have reported the incorporation of fenoprofen and ibuprofen into triglycerides, a step preceded by the formation of a CoA intermediate [25, 33]. Thioester formation is catalysed by the microsomal long chain ligase [11] and is stereospecific for the *R* enantiomers [25, 33]. All of the racemic non-steroidal arylpropionic acids used inhibited both forms of the peroxisomal long chain ligase but the *R* and *S* enantiomers of ibuprofen and naproxen were mixed inhibitors of the high affinity component ($K_i > 340$ μM). Evidence of mixed inhibition of the peroxisomal ligase would suggest that there are two or more sites on the enzyme with binding at one influencing the affinity of the substrate at the second site. A two site hypothesis has been proposed previously as an explanation for the observation that 1-pyrenedecanoic acid inhibits palmitic acid activation but not *vice versa* [26]. This study contrasts with that of Knights and Jones [13] who demonstrated competitive inhibition of the microsomal long chain ligase with the *R* enantiomer of ibuprofen and non-competitive inhibition with both enantiomers of naproxen. In addition, the K_i values as reported in this study are an order of magnitude greater for the peroxisomal ligase than for the microsomal enzyme.

Of all the xenobiotics screened only nafenopin and ciprofibrate competitively inhibited palmitoyl-CoA formation suggesting that they bind at the active site thus potentially giving rise to xenobiotic acyl-CoA thioesters. Both compounds are substrates for the microsomal ligase [8] and activation to CoA intermediates correlates with their pharmacological potency: ciprofibrate > nafenopin > clofibric acid. In this study the K_i value was 97 and 430 μM for ciprofibrate and nafenopin, respectively, indicating that ciprofibrate has a greater affinity for the peroxisomal ligase compared with nafenopin.

Recently, Suzuki *et al.* [5] isolated complementary DNAs encoding rat long chain CoA ligase. Based on limited sequence homology, the authors concluded from their study that the enzyme from the three different organelles may be identical. Paradoxically, it has also been postulated that the microsomal and mitochondrial forms differ [34]. It is apparent from this study that in relation to the conjugation of carboxylic acids with CoA the substrate specificity of the peroxisomal long chain ligase differs from that reported for the equivalent microsomal enzyme,

thus suggesting that these two organellar forms may be functionally distinct. The diversity of the kinetics observed (competitive and mixed) would suggest that numerous xenobiotics interact with the peroxisomal enzyme but relatively few would appear to function as alternative substrates. In many cases, the concentration at which an interaction was observed would not be physiologically relevant. Evidence of competitive inhibition of palmitoyl-CoA formation by nafenopin and ciprofibrate suggests that peroxisomes may play a role in addition to that of microsomes in the activation of these hypolipidaemics to their respective acyl-CoA thioesters. Recent studies have confirmed that peroxisomes catalyse the formation of nafenopin-CoA (data unpublished). These data support a relationship between activation to a CoA thioester intermediate and peroxisomal proliferation. However, the data does not support a role for the peroxisomal palmitoyl-CoA ligase in the formation of clofibroyl-CoA but rather suggests the involvement of an as yet unidentified clofibroyl-CoA ligase.

In general terms, the results of this study highlight several points germane to the conjugation of xenobiotics with CoA. Firstly, xenobiotics exhibit regiospecificity in relation to CoA conjugation which is not fully explained by an interaction with long chain fatty acid CoA ligase (E.C. 6.2.1.3). Secondly, the xenobiotic-CoA derivative may either cause direct interference with metabolic pathways depending on the site of activation (i.e. microsomes or peroxisomes) or exert indirect effects via sequestration of compartmental pools of CoA. The consequential toxicity of xenobiotic-CoA formation in peroxisomes has not been elucidated and further studies are aimed at identifying and characterizing peroxisomal xenobiotic-CoA ligase(s).

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