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THE EFFECTS OF 2{5(4-CHLOROPHENYL)PENTYL}OXIRANE-2-CARBONYL-CoA ON MITOCHONDRIAL OXIDATIONS

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Abstract—2{5(4-Chlorophenyl)pentyl}oxirane-2-carbonyl-CoA (POCA-CoA) was prepared from 2{5(4-chlorophenyl)pentyl}oxirane-2-carboxylate (POCA) and characterised chromatographically. POCA-CoA does not inhibit citrate cycle oxidations or effect oxidative phosphorylation by rat liver mitochondria. POCA-CoA at low (μM) concentrations, but not free $POCA^-$, specifically inhibits palmitoyl-CoA oxidation at the stage of carnitine palmitoyltransferase I (CPT I) situated on the outer face of the inner mitochondria membrane. Palmitoyl-carnitine oxidation was not inhibited by POCA-CoA. POCA-CoA inhibits palmitoyl-CoA oxidation in liver mitochondria from fed rats more strongly than it does in mitochondria from fasted rats, similarly to the inhibition by malonyl-CoA [E. D. Saggerson and C. A. Carpenter, *FEBS Lett.* **129**, 225 (1981)]. Palmitoyl-CoA, by contrast with palmitoyl-carnitine, is not quantitatively oxidised to acetoacetate by liver mitochondrial fractions, presumably due to competing palmitoyl-CoA hydrolase activity. In the presence of POCA-CoA the amount oxidised is decreased further because the slower rate of oxidation allows more palmitoyl-CoA to be hydrolysed to palmitate. The oxidation of palmitoyl-CoA, but not that of palmitoyl-carnitine, was strongly decreased in washed liver and muscle mitochondrial fractions from POCA-fed animals. $POCA^-$ inhibited the oxidation of {U-^{14}C}palmitate in cultured human fibroblasts, and caused small increases in $^{14}CO_2$ production from {1-^{14}C}pyruvate and {U-^{14}C}glucose. Inhibition of β-oxidation at the stage of CPT I by POCA-CoA can explain the powerful hypoketonaemic and hypoglycaemic effects of POCA in fasted normal and fasted diabetic animals [H. P. O. Wolf, K. Eistetter and G. Ludwig, *Diabetologia* **22**, 456 (1982)].

The metabolic effects of inhibitors of long-chain fatty acid oxidation are important because of the relationship between fatty acid oxidation, gluconeogenesis, glucose utilisation and glycaemia [1]. Wolf, *et al.* recently introduced ethyl 2{5(4-chlorophenyl)pentyl}oxirane-2-carboxylate (POCA) which has powerful hypoketonaemic and hypoglycaemic activities in fasted normal and diabetic rats [2–4]. The pathway for the mitochondrial oxidation of long-chain fatty acids first involves conversion to their acyl-CoA esters outside the mitochondrial matrix. The acyl-CoA esters are then converted to acyl-carnitine esters by reaction with carnitine, catalysed by carnitine palmitoyl-transferase I (CPT I) {EC 2.1.1.21} situated on the outer face of the inner mitochondrial membrane. Acyl-carnitine esters enter the matrix on a specific carrier [5] where they are reconverted to acyl-CoA esters by CPT II on the inner face of the inner membrane. Acyl-CoA esters are then converted to acetyl-CoA by β-oxidation. In this paper we provide evidence that POCA-CoA, thought to be formed *in vivo* from POCA, is a potent inhibitor of β-oxidation at the stage of CPT I. It is also shown that the specific activity of CPT I in liver and muscle mitochondrial fractions from rats fed a POCA-containing diet is lower than that in the controls. Further, POCA-CoA inhibits CPT I [6] and β-oxidation in liver mitochondria from fed rats more strongly than in those from fasted rats; and inhibits CPT I equally strongly in skeletal muscle mitochondria from fed and fasted rats [6]. Preliminary accounts of some of this work have already appeared [7, 8], and some chronic metabolic effects of feeding normal rats diets containing POCA are described in the accompanying paper [9].

Abbreviations: POCA, ethyl 2{5(4-chlorophenyl)pentyl}oxirane-2-carboxylate; $POCA^-$, 2{5(4-chlorphenyl)pentyl}oxirane-2-carboxylate; POCA-CoA, 2{5(4-chlorophenyl)pentyl}oxirane-2-carbonyl-CoA; POCA-carnitine, 2{5(4-chlorophenyl)pentyl}oxirane-2-carbonyl-(L)-carnitine; 2-TDGA, methyl 2-tetradecyloxirane-2-carboxylate; 2-TDGA-CoA, 2-tetradecyloxirane-2-carbonyl-CoA; CoASH, coenzyme A (free form); -CoA, coenzyme A (esterified form); CoA, coenzyme A (sum of all forms); CPT I, carnitine palmitoyl-transferase I; CPT II, carnitine palmitoyltransferase II; COT, carnitine octanoyltransferase; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; EDTA, ethylenediamine-tetra-acetate; EGTA, ethanedioxybis-(ethylamine)-tetra-acetate.

MATERIALS AND METHODS

Chemicals. POCA, 2-TDGA and sodium salts of $POCA^-$, 2[3(3-trifluromethylphenyl)propyl]-oxirane-2-carboxylate, 2(5-phenylpentyl)oxirane-

2-carboxylate and 2[4(3-chlorophenoxyl)butyl]-oxirane-2-carboxylate were gifts from the BYK Gulden Lomberg Chemische Fabrik GmbH, Konstanz, F.R.G. Most enzymes and cofactors used were obtained from Boehringer Corp. (London) or Sigma (London). (U-^{14}C)Glucose, (1-^{14}C)pyruvate and (U-^{14}C)palmitate were obtained from Amersham International. Fetal calf serum, minimum essential medium, HEPES buffer, sodium bicarbonate, trypsin and glutamate for tissue culture were obtained from Flow Laboratories Ltd., Irvine, U.K.

The sources of other materials used are given elsewhere [10, 11].

Preparation of acyl-CoA and acyl-carnitine esters. Palmitoyl-carnitine and octanoyl-carnitine were prepared and characterised as described previously [12]. POCA-CoA and other CoA esters of 2-oxirane-2-carboxylates were prepared using *N*-hydroxysuccinamide esters [6, 13] and characterised by ester bond contents [14]. One peak in the expected position for POCA-CoA was seen with reversed phase HPLC [6]. POCA$^-$ was liberated by alkaline hydrolysis of POCA-CoA and characterised (as its methyl ester) by GLC [6].

Determination of protein. Protein was determined by the method of Lowry *et al.* [15].

Animals. Male albino Wistar rats from a local inbred strain were used.

Chronic administration of POCA to rats. Tissues were obtained from the animals that had been fed normal low fat diets containing 0.05% or 0.2% POCA for 12 weeks, or a high fat diet containing 0.05% or 0.1% POCA for 4 weeks, and used in the chronic toxicity study described in the preceding paper [9].

Preparation of mitochondrial fractions from liver and muscle. Liver mitochondrial fractions were prepared in 0.3 M mannitol, 5 mM HEPES, 0.1 mM EGTA, pH 7.2 as previously described [10]. Mitochondrial fractions were prepared from muscle after homogenisation for 5 s with a Polytron homogeniser in 0.25 M sucrose, 10 mM Tris–HCl, 0.1 mM EGTA as previously described [16].

Polarographic measurements of mitochondrial oxidation. Oxygen uptake by mitochondria was recorded polarographically at 30° in a final volume of medium containing 120 mM KCl, 2.5 mM phosphate, 10 mM HEPES, 5 mM $MgCl_2$ and 1 mM EDTA, pH 7.2 [10], using various substrates as indicated in the tables and figures. For measurements of β-oxidation either 20 μM palmitoyl-CoA plus 0.5 mM L-carnitine, or 20 μM acylcarnitines were used in the presence of 0.4 mM ADP, 5 mM malonate and defatted bovine serum albumin (6 mg), so that acyl-groups transferred to intramitochondrial CoA were quantitatively oxidised to acetoacetate and the oxygen uptake recorded was a direct measure of the flux through β-oxidation [17].

Spectrophotometric assay of mitochondrial succinate and glycerol 3-phosphate oxidation, and of β-oxidation. The mitochondrial oxidation of 10 mM succinate, 20 mM DL-glycerol 3-phosphate, 20 M palmitoyl-CoA plus 0.5 mM L-carnitine or of 20 μM acylcarnitine was followed by recording the reduction of $Fe(CN)_6^{3-}$ as final electron acceptor at 425–275 nm using a Hitachi 557 spectrophotometer in the dual-wavelength mode, at 30° in 3.0 ml of 130 mM KCl (liver mitochondria) or 300 mM mannitol (muscle mitochondria), and 10 mM HEPES, 2.5 mM phosphate 0.5 mM ADP, 0.1 mM EDTA, 4.5 mg of defatted bovine serum albumin, 1 mM KCN, 3 μg of rotenone and 0.5 mM $K_3Fe(CN)_6$. This assay measures directly the flux through the appropriate flavoprotein dehydrogenase [18].

Effects of pre-incubation with POCA$^-$ on oxidations in cultured human fibroblasts. The fibroblast line used was established from a forearm skin biopsy from a normal volunteer and maintained with appropriate subculturing in Eagles minimum essential medium, containing 10% fetal calf serum and 10 mM HEPES, pH 7.4 in 25 cm^2 Falcon flasks [19]. The cells were grown to subconfluence and then detached from the Falcon flasks by trypsinisation. Cells (0.37×10^7) were added to small flasks fitted with a centre well and a rubber cap and incubated with 5 or 50 μM POCA$^-$ for 24 hr. The medium was decanted and the cells were washed with Gey and Gey medium [20]. They were then incubated for 1 hr in a final volume of 1.0 ml of Gey and Gey medium containing 2% defatted bovine serum albumin, 1 mM L-carnitine and {U-^{14}C}palmitate (0.37 μCi), 1 mM {1-^{14}C}pyruvate (0.1 μCi) or {U-^{14}C}glucose (9.26 μCi) in an atmosphere of 95% O_2/5% CO_2 with shaking (180 strokes/min). This change of medium was necessary because of the uncertainty about the concentrations of metabolites in Eagles medium. Pre-incubation with POCA$^-$ was to allow time for the intracellular formation of POCA-CoA and it was also known that the inhibitory effects of POCA persisted in mitochondrial fractions from POCA-fed animals (Table 3). After 30 or 60 min the reaction was stopped by injection of 1.0 ml of 5% $HClO_4$ through the rubber cap. Then 0.75 ml of 1 N hyamine hydroxide was injected into the centre well and the flasks were shaken for a further 4 hr to collect $^{14}CO_2$ formed in the hyamine. The hyamine hydroxide solution (0.4 ml), and the supernatant fraction of the homogenate obtained by centrifugation in an Eppendorf microcentrifuge for 2 min after addition of $HClO_4$ to incubations when palmitate was the substrate (1.0 ml), were counted for radioactivity. Appropriate substrate blanks were run. The results are expressed as the percentage of added radioactivity recovered as $^{14}CO_2$ or as ^{14}C-labelled products.

RESULTS

Effects of POCA-CoA in vitro *on the oxidation of succinate and of glutamate plus malate measured polarographically*

Addition of POCA-CoA at concentrations of 100 μM to liver mitochondrial fractions from rats fed a normal diet had no effects on the state 3 and state 4 rates [21] of oxidation with 10 mM succinate or with 10 mM glutamate plus 1 mM malate as substrate (Table 1). This indicated that there was no uncoupling of oxidative phosphorylation, inhibition of the citrate cycle or of the respiratory chain. Further, the lack of effect on the state 3 rates indicated that POCA-CoA did not inhibit the adenine nucleotide translocator, by contrast with inhibition by palmitoyl-CoA [22].

Table 1. Oxidations by liver mitochondrial fractions from rats fed a standard diet containing POCA

Substrate	Diet		
	Control	0.05% POCA-fed	0.2% POCA-fed
10 mM Succinate			
Rate of oxidation	152 ± 9	140 ± 5	160 ± 13
RCR	3.50 ± 0.19	3.32 ± 0.2	2.86 ± 0.41
($n = 4$)			
10 mM Glutamate plus 1.0 mM malate			
Rate of oxidation	104 ± 7	156 ± 12*	127 ± 9*
RCR	3.46 ± 0.25	3.36 ± 0.38	3.52 ± 0.68
($n = 4$)			
20 μM Palmitoyl-CoA plus 0.5 mM carnitine			
Rate of oxidation	50 ± 3	28 ± 2*	10 ± 5*
ΔO	100%	39 ± 14%*	37 ± 11%*
($n = 3$)			
20 μM Palmitoyl-carnitine			
Rate of oxidation	58 ± 7	90 ± 12*	98 ± 24*
ΔO	100%	107 ± 7%	103 ± 8%
($n = 3$)			

Oxidations were measured polarographically as described in the Materials and Methods section and rates are expressed as ng atoms O/min per mg of protein as means ± S.E.M. RCR is the respiratory control index, the ratio of the rate stimulated by 10 μM ADP to that when all the ADP is phosphorylated [21]. ΔO is the amount of oxygen consumed during a pulse of oxidation of palmitoyl-carnitine by mitochondria from POCA-fed rats expressed as percentage of that by the controls. With mitochondria from control rats about 80% of the theoretical oxygen uptake was obtained with 20 μM palmitoyl-CoA and 95% with palmitoyl-carnitine. Significance of differences from controls: * $P < 0.001$.

Inhibition of palmitoyl-CoA oxidation by POCA-CoA measured polarographically

Low concentrations of POCA-CoA strongly inhibited the state 3 rate of oxidation of 20 μM palmitoyl-CoA plus 0.5 mM carnitine, with 50% inhibition by 0.3 μM POCA-CoA (Fig. 1). The oxidation of 20 μM palmitoyl-carnitine or of 20 μM octanoyl-carnitine was not inhibited. This localised the inhibition of β-oxidation at CPT I on the outer face of the inner mitochondrial membrane [7]. It was also shown that using mitochondria uncoupled with

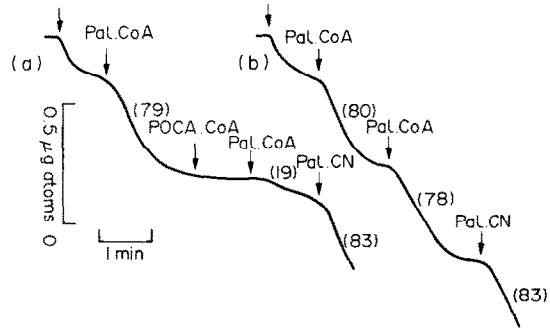

Fig. 1. Inhibition of the oxidation of palmitoyl-CoA by POCA-CoA. Oxygen uptake by liver mitochondrial fractions was recorded as described in the Materials and Methods section, in a medium containing 0.5 mM carnitine and 0.4 mM ADP. Mitochondria (about 10 mg of protein) were added where indicated by unlabelled arrows. Other additions were made as shown; 20 μM palmitoyl-CoA (Pal-CoA), 20 μM palmitoyl-carnitine (Pal-CN), 1 μM POCA-CoA. The rates of oxygen uptake (ng atoms O/min per mg of protein) are given in parentheses. The traces illustrate the smaller proportion of palmitoyl-CoA oxidised when the rate of oxidation is slower.

50 μM 2,4-dinitrophenol and 5 mM arsenate that free 1.0 mM $POCA^-$ does not inhibit. These results suggest that POCA-CoA is the inhibitor formed *in vivo* by the metabolism of POCA [12].

With our conditions, uninhibited mitochondria quantitatively oxidise palmitoyl-carnitine to acetoacetate [23]. Washed mitochondria fractions from POCA-fed rats also oxidised palmitoyl-carnitine quantitatively (Table 1). A competing reaction for the oxidation of palmitoyl-CoA is deacylation by mitochondrial palmitoyl-CoA hydrolase (EC 3.1.3.2) which is located outside the matrix. The size of the pulse of oxygen uptake for the oxidation of 20 μM palmitoyl-CoA by uninhibited mitochondria was always less than the theoretical 14 oxygen atoms/mol and depended on when the reaction was stated by 0.4 mM carnitine. The longer the interval between the addition of carnitine following that of palmitoyl-CoA, the smaller the size of the pulse compared with that obtained when carnitine was added first (not shown, see also Fig. 1), as already noted by Bremer and Norum [24]. In the presence of POCA-CoA, or with mitochondrial fractions from POCA-fed rats, the oxygen uptake for palmitoyl-CoA oxidation was less than that for uninhibited mitochondria (for the same protein concentration) when the reaction was started with palmitoyl-CoA. These results can be explained by palmitoyl-CoA hydrolase having more time to act on palmitoyl-CoA because of the slower rate of β-oxidation. Further, with mitochondrial fractions from POCA-fed rats palmitoyl-CoA hydrolase activity may be increased by analogy with the increase caused by clofibrate [25]. There was no indirect evidence for hydrolysis of POCA-CoA as the extent of inhibition of palmitoyl-CoA oxidation was apparently independent of the length of pre-incubation of mitochondria with POCA-CoA for up to 10 min (not shown). The

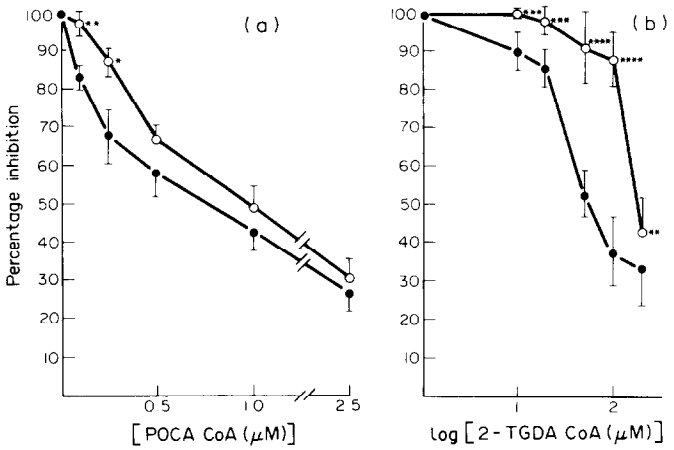

Fig. 2. Comparison of the inhibition of oxidation of palmitoyl-CoA by POCA-CoA and TDGA-CoA in liver mitochondrial fractions from fed and 48 hr fasted rats. Oxidations were measured spectrophotometrically as described in the Materials and Methods section. Mitochondria (about 3 mg of protein) were added to medium containing 10 mM oxaloacetate, 20 μM palmitoyl-CoA and different concentrations of POCA-CoA or 2-TDGA-CoA as shown in the abscissa. After 1 min the reaction was started by the addition of 0.5 mM carnitine and the rate of $Fe(CN)_6^{3-}$ reduction recorded. The rates are expressed as percentages of the control rates; (●), fed; (○), fasted. Significance of differences from the controls; * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$.

importance of palmitoyl-CoA hydrolysis, if any, in intact cells is not known.

The CoA esters of four other 2-oxiranecarboxylic acids, 2{3(3-trifluoromethylphenyl)propyl}oxirane 2-carbonyl-CoA, 2{4(3-chlorophenoxy)butyl}-oxirane-2-carbonyl-CoA, 2(5-phenylpentyl)-oxirane-2-carbonyl-CoA and 2-TDGA-CoA also inhibited palmitoyl-CoA, but not palmitoylcarnitine oxidation, the concentrations giving 50% inhibition with the conditions used being 1.8, 0.03, 0.1 and 10 μM respectively.

Inhibition of palmitoyl-CoA oxidation by POCA-CoA and by 2-TDGA-CoA measured spectrophotometrically

β-Oxidation of long-chain acyl-CoA esters is inhibited by the physiological effector malonyl-CoA more strongly in liver mitochondria from fed rats than from 24 hr fasted rats [26]. Similarly, both POCA-CoA and 2-TDGA-CoA also inhibited palmitoyl-CoA oxidation more strongly in mitochondrial fractions from fed than from fasted rats (Fig. 2). It was also confirmed that with our experimental conditions malonyl-CoA (5–20 μM) inhibited palmitoyl-CoA oxidation more strongly in liver mitochondria from fed than from fasted rats (not shown).

Oxidations by liver mitochondrial fractions from POCA-fed rats

(*a*) *Polarographic measurements, low fat diet.* The rate of succinate oxidation per mg of mitochondrial protein was not significantly changed by POCA-feeding, although there was a 30–50% increase in the rate of oxidation of 10 mM glutamate plus 1 mM malate (Table 1). There was no uncoupling of oxidative phosphorylation (Table 1). The rate of palmitoyl-CoA oxidation was decreased by about

Table 2. Oxidations by liver mitochondrial fractions from rats fed a high fat diet containing POCA

Substrate	Control	Diet 0.05% POCA-fed	0.2% POCA-fed
10 mM Succinate			
Rate	44, 76	56, 72	65, 49
RCR	2.76, 4.45	4.00, 3.92	2.95, 2.70
10 mM Glutamate plus 10 mM malate			
Rate	40, 57	48, 66	60, 43
RCR	6.08, 7.53	4.47, 5.88	4.31, 2.55
20 μM Palmitoyl-CoA			
Rate	6.5, 1.0	5.1, 4.9	3.3, 4.3
20 μM Palmitoyl-carnitine			
Rate	40, 53	36, 61	40, 42

Oxidations were measured polarographically as described in the Materials and Methods section, and rates are expressed as ng atoms/min O per mg protein for individual livers. RCR is the respiratory control index, the ratio of the rate stimulated by 10 M ADP to that when all the ADP is phosphorylated.

Table 3. The effects of feeding POCA for 12 weeks on oxidations by liver and muscle mitochondrial fractions

Substrate	Rate of oxidation					
	Liver mitochondria			Muscle mitochondria		
	Control	0.05% POCA-fed	0.2% POCA-fed	Control	0.05% POCA-fed	0.2% POCA-fed
10 mM Succinate	370 ± 9	312 ± 59	347 ± 75	396 ± 30	463 ± 87	380 ± 56
40 mM DL-Glycerol 3-phosphate	17 ± 4	21 ± 4	28 ± 8*	152 ± 27	183 ± 47	200 ± 29‡
20 μM Palmitoyl-CoA plus 0.5 mM carnitine	22 ± 4	7.5 ± 1.9‡	5.8 ± 0.8‖	9.0 ± 2.4	4.5 ± 1.4§	4.0 ± 0.8§
20 μM Palmitoyl-carnitine	38 ± 8	59 ± 8*	53 ± 5†	12 ± 3.7	14 ± 2.6	14 ± 2.3

Oxidations were assayed spectrophotometrically as described in the Materials and Methods section. Rates are expressed as nmol $Fe(CN)_6^{3-}$ reduced/min per mg of protein, and given as means ± S.E.M. ($n = 6$). For the β-oxidation of fatty acyl-groups only the flux through the acyl-CoA dehydrogenase step is recorded [18]. Significance of differences from the controls: * $P < 0.05$; † $P < 0.025$; ‡ $P < 0.01$; § $P < 0.005$; ‖ $P < 0.001$.

44% after feeding 0.05% POCA and by 80% after feeding 0.2% POCA, together with large decreases in the total amount of oxygen consumed (Table 1). POCA-feeding also increased the rate of palmitoyl-carnitine oxidation by 65–70% with no effect on the amount of oxygen consumed (Table 1).

(*b*) *Polarographic measurements, high fat diet.* The rates of succinate and of glutamate plus malate oxidation by liver mitochondrial fractions from rats fed a high fat diet containing POCA were about 40% of those of mitochondria from animals on a standard diet, while the rate of palmitoyl-carnitine oxidation was about half. Oxidative phosphorylation was not impaired. Preparations from all groups on the high fat diet had high apparent palmitoyl-CoA hydrolase activities and it was not possible to record a meaningful rate of palmitoyl-CoA oxidation (Table 2).

(*c*) *Spectrophotometric measurements.* The ability of mitochondrial fractions from POCA-fed animals on the low fat diet to oxidise palmitoyl-CoA was impaired (Table 3). There was a 75% decrease in the specific activity of palmitoyl-CoA oxidation by liver mitochondrial fractions, and about a 45% decrease by skeletal muscle mitochondrial fractions. POCA-feeding also caused a 40–50% increase in the rate of palmitoyl-carnitine oxidation in liver mitochondrial fractions (Table 3). Succinate dehydrogenase activity was unchanged. There was an increase in glycerol 3-phosphate dehydrogenase activity both in liver and muscle which was significant with the 0.2% POCA-containing diet (Table 3).

Effects of POCA on oxidations by cultured human fibroblasts

$^{14}CO_2$ production from {U-^{14}C}palmitate is a poor indicator of the flux through β-oxidation in these cells since about 30 times as much radioactivity was found in the acid-soluble fraction of control incubations as in CO_2. Pre-incubation of fibroblasts with 5 μM or with 50 μM $POCA^-$ inhibited the subsequent formation of $^{14}CO_2$ from {U-^{14}C}palmitate by 10-fold and 15-fold respectively, and the formation of acid soluble ^{14}C-labelled products (citrate, acetate, etc.) was strongly inhibited (Fig. 3(a)). By contrast, the formation of $^{14}CO_2$ from {1-^{14}C}pyruvate was stimulated by 0.24-fold and by 0.4-fold by 5 μM and by 50 M POCA respectively (Fig. 3(b)), and $^{14}CO_2$ formation from {U-^{14}C}glucose was increased markedly by 2.5-fold and 5-fold respectively (Fig. 3(c)).

DISCUSSION

The carnitine-dependent oxidation of palmitoyl-CoA is strongly inhibited by POCA-CoA while that of palmitoyl-carnitine is unaffected. This unambiguously locates the site of inhibition as CPT I on the outer face of the inner mitochondrial membrane, since this enzyme is bypassed by palmitoyl-carnitine which enters the mitochondrial matrix by means of a specific carrier [5] where palmitoyl-CoA is reformed by the action of CPT II. It has been confirmed directly that CPT I is inhibited by POCA-CoA using both a radioisotopic assay [26] and spectrophotometrically by coupling CoASH formed to NADH production [27]. Free $POCA^-$ (1 mM) had

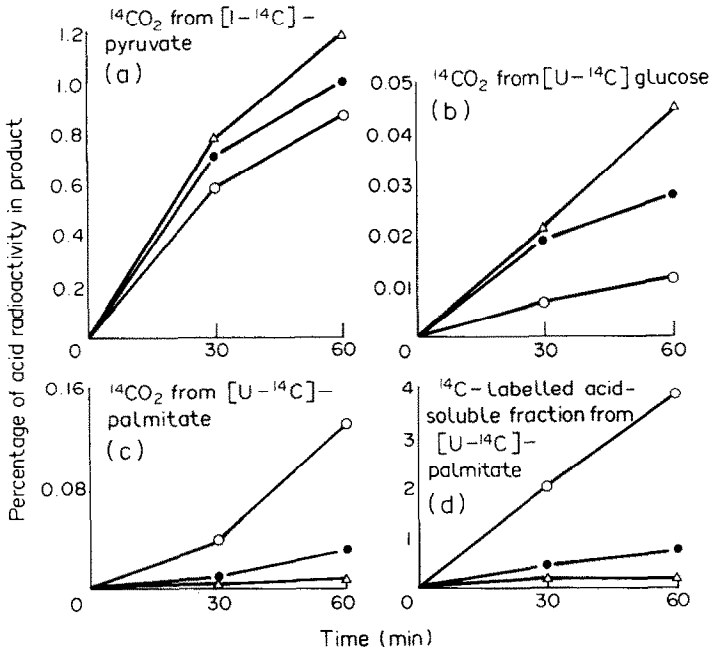

Fig. 3. Effects of $POCA^-$ on oxidations by cultured human fibroblasts. Fibroblasts were grown and incubated as described in the Materials and Methods section: (○), control; (●), 5 μM $POCA^-$; (△), 50 μM $POCA^-$. (a) $^{14}CO_2$ formed from {1-^{14}C}pyruvate, (b) $^{14}CO_2$ formed from {U-^{14}C}glucose, (c) $^{14}CO_2$ formed from 1 mM {U-^{14}C}palmitate, (d) acid-soluble ^{14}C-labelled products formed from 1 mM {U-^{14}C}palmitate.

no effect on CPT I activity [6] nor on the oxidation of palmitoyl-CoA. No formation of POCA-carnitine was detected when POCA-CoA was incubated with carnitine and liver mitochondria [6] so that POCA-carnitine is unlikely to contribute to the inhibitory effects of $POCA^-$ on β-oxidation. The specific activity of palmitoyl-CoA oxidation was much less in washed liver and muscle mitochondrial fractions from POCA-fed animals than from control animals consistent with inhibition *in vivo* by POCA-CoA (Tables 1 and 3). (A much less likely explanation is that there are smaller amounts of CPT I (but not of CPT II) in livers of POCA-fed animals.) The CoA esters of three other substituted 2-oxiranecarboxylates and 2-TDGA-CoA also inhibit the oxidation of palmitoyl-CoA, but not of palmitoylcarnitine. This confirms the earlier interpretations of the mechanism of action of 2-TDGA [28] although TDGA-CoA was the least potent inhibitor.

CPT I and CPT II have not been well characterised. There is also evidence for the existence of COT in some tissues whose preferred substrates are medium-chain acyl-CoA esters, but which is thought to have some activity with palmitoyl-CoA [29]. The presence of separate COT was not confirmed in beef heart [30, 31]. COT, if present in mitochondria, may also be inhibited by POCA-CoA by analogy with malonyl-CoA [29]. Another complication is that the sensitivity of liver CPT I, but not skeletal muscle CPT I, to inhibition by malonyl-CoA is decreased by fasting for 24 hr [32]. Similarly, POCA-CoA and 2-TDGA-CoA inhibit palmitoyl-CoA oxidation more strongly in liver mitochondrial fractions from fed than from fasted rats (Fig. 2), although with our conditions POCA-CoA is about an order of magnitude more potent as an inhibitor. Further, CPT I activity is inhibited more strongly by POCA-CoA in liver mitochondria from fed normal or diabetic rats than from fasted normal rats. CPT I activity is inhibited to the same extent in skeletal muscle mitochondria from fed and fasted rats [6].

The inhibition of liver CPT I by POCA-CoA may be partly competitive with respect to palmitoyl-CoA (D. M. Turnbull and H. S. A. Sherratt, unpublished work) and may resemble that by malonyl-CoA [6], although it is difficult to apply conventional kinetic analysis to this system [6]. The apparent inhibition of CPT I (50–75%) in washed mitochondrial fractions from POCA-fed animals (Tables 1 and 3) implies a very tight binding of POCA-CoA. Yet the oxidation of palmitoyl-CoA in liver mitochondria from control animals was partly resistant (about 20%) to inhibition *in vitro* by very high concentrations of POCA-CoA (20 μM). Further, inhibition of liver and muscle CPT I does not increase as much with increasing POCA-CoA concentration as expected for simple hyperbolic binding [6]. This suggests that CPT I usually exists in at least two molecular forms with different sensitivities to inhibition and that both the relative and absolute amounts of these forms vary with the nutritional state. It is also possible that at least one form shows negative co-operativity with respect to POCA-CoA binding.

The results with fibroblasts indicate that long-chain fatty acid oxidation in human tissues is sensitive to inhibition by POCA-CoA (Fig. 3). The small

increase in pyruvate oxidation after pre-incubation with $POCA^-$ was presumably due to an activation of pyruvate dehydrogenase {EC 1.2.4.1} secondary to inhibition of fatty acid oxidation, similar to that which occurs in rat hearts perfused with media containing $POCA^-$ [33]. The larger stimulation of $^{14}CO_2$ production from {U-^{14}C}glucose than from {1-^{14}C}pyruvate in fibroblasts could be due to greater stimulation of the pentose phosphate pathway than of pyruvate oxidation in the presence of $POCA^-$.

The pharmacological effects of POCA [3] may be essentially explained as a consequence of the inhibition of long-chain fatty acid oxidation at the stage of CPT I by POCA-CoA, although the extent of inhibition *in vivo* is not known. Inhibition of long-chain fatty acid oxidation following administration of POCA would be expected to occur in all tissues, by contrast with that by the natural effector malonyl-CoA which may only occur in tissues which synthesise fatty acids *de novo* [34].

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