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DIFFERENCES BETWEEN HUMAN, RAT AND GUINEA PIG HEPATOCYTE CULTURES

A COMPARATIVE STUDY OF THEIR RATES OF β-OXIDATION AND ESTERIFICATION OF PALMITATE AND THEIR SENSITIVITY TO *R*-ETOMOXIR

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Abstract—Rat hepatocyte cultures have higher rates of β-oxidation of palmitate and lower rates of esterification to glycerolipid than human or guinea pig hepatocytes. The *R*-enantiomer of etomoxir (sodium 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate), a hypoglycaemic compound and inhibitor of carnitine palmitoyltransferase I, inhibited palmitate β-oxidation in all three species, but the sensitivity to inhibition was highest in human hepatocytes and lowest in rat hepatocytes. The concentration causing half-maximal inhibition was approximately: 0.1 μM in human; 1 μM in guinea pig and 10 μM in rat hepatocytes. In human and in guinea pig hepatocytes the inhibition of β-oxidation by *R*-etomoxir was associated with an increase in the esterification of palmitate but in rat hepatocytes *R*-etomoxir lowered the total rate of palmitate metabolism. The *S*-enantiomer of etomoxir had no significant effect on β-oxidation or esterification of palmitate in any of the three species. It is concluded that there are significant differences between human, rat and guinea pig hepatocytes, not only in the relative partitioning of palmitate between β-oxidation and esterification, but also in the sensitivity to an inhibitor of carnitine palmitoyltransferase I.

Rat hepatocytes are used much more frequently in studies of intermediary metabolism and its modification by drugs and toxins than hepatocytes from other species. However, rat hepatocytes are not necessarily a good model for human metabolism [1–3]. In this study we compare the rates of β-oxidation and esterification of palmitate in human, rat and guinea pig hepatocyte cultures and show that rat hepatocytes have the highest rate of β-oxidation and lowest rate of esterification. Further, we found that β-oxidation is most sensitive to inhibition by the hypoglycaemic compound etomoxir (2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate‡) [4] in human hepatocytes and least sensitive in rat hepatocytes. It is also shown for the first time that only the *R*-form of *rac*-etomoxir (*RS*-etomoxir) inhibits β-oxidation.

MATERIALS AND METHODS

Materials. The *R*- and *S*-enantiomers of etomoxir and *RS*(*rac*)-etomoxir were a generous gift from Dr H. P. O. Wolf, Byk Gulden Chemische Fabrik GmbH (Konstanz, Germany). They were resolved from *rac*-etomoxir and had no more than 2% cross contamination with the other enantiomer; with for 12.5 mg/mL solutions in water an alpha[D]R+ of +39.7 ± 0.2° and alpha[D]S− of −43.8 ± 0.2° which were equal within experimental error.

[U-^{14}C]Palmitate was from Amersham International (Amersham, U.K.). Collagenase (type IV) and biochemicals were from the Sigma Chemical Co. (Poole, U.K.).

Animals. Male Wistar rats (body wt: 250–300 g) were bred in the Medical School, University of Newcastle upon Tyne; and male Dunkin–Hartley guinea pigs (body wt: 260–320 g) were from Bantin and Kingman, Hull, U.K.

Hepatocyte cultures. Hepatocytes were isolated from rats and guinea pigs fed *ad lib.* by the conventional collagenase perfusion technique [5]. Human hepatocytes were isolated from liver sections (100–150 g) from kidney donors (female: 6, 63 and 66 years old; male: 59 years old) as described previously [5]. After isolation, the hepatocytes were suspended in Minimum Essential Medium containing fetal bovine serum (5%, v/v) and 4–6 hr was allowed for hepatocyte attachment for the rat and guinea pig hepatocytes and 12 hr for attachment of human hepatocytes. After cell attachment the medium was changed to serum-free Minimum Essential Medium containing 10 nM dexamethasone phosphate [6], and the studies were performed after 16 hr culture in serum-free medium.

Palmitate metabolism. The medium was replaced by fresh Minimum Essential Medium containing 1 mM [U-^{14}C]palmitate (0.5–0.8 Ci/mol); 0.5 mM L-carnitine; and 20 mg/mL defatted bovine serum albumin, and the concentrations of *R*- or *S*-etomoxir indicated, and the cultures were incubated for 3 hr.

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‡ Abbreviations: etomoxir, sodium 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate; 2-TDGA, tetradecyl-2-oxiranecarboxylate; etomoxir-CoA; 2-[6-(chlorophenoxy)hexyl]oxirane-2-carbonyl-CoA.

Table 1. Effects of *R*- and *S*-etomoxir on β-oxidation of palmitate

Hepatocytes: (N) Additions:	Human (3)	Rat (4)	Guinea pig (5)
	(nmol palmitate/hr/mg protein)		
Control	50.7 ± 1.5 (100)	100.6 ± 4.6 (100)	64.5 ± 5.8 (100)
S-Etomoxir 0.1 μM	50.2 ± 1.2 (99)	95.8 ± 4.2 (95)	60.2 ± 5.7 (93)
1 μM	49.2 ± 2.2 (97)	93.3 ± 3.0 (93)	57.9 ± 4.2 (90)
10 μM	45.0 ± 1.7 (89)	98.5 ± 2.9 (98)	61.5 ± 4.8 (95)
R-Etomoxir 0.1 μM	24.1 ± 5.2 (48)*	92.4 ± 5.7 (92)	50.1 ± 3.5 (78)
1 μM	12.6 ± 2.6 (25)†	72.9 ± 4.4 (73)*	29.5 ± 3.1 (46)*
10 μM	6.6 ± 1.0 (13)†	48.1 ± 3.9 (48)†	19.6 ± 2.7 (30)†

Human, rat and guinea pig hepatocyte cultures were incubated with 1 mM [U-^{14}C]palmitate and 0.5 mM carnitine as described in Materials and Methods. Rates of β-oxidation of palmitate are expressed as nmol of palmitate converted to acid-soluble metabolites per hr per mg of cell protein. Values are means ± SEM for the number of hepatocyte preparations indicated. Values in parentheses indicate the rates expressed as percentages of the respective controls.
Statistics: * $P < 0.05$; † $P < 0.005$ relative to the respective control.

Rates of palmitate metabolism were linear with time during this interval. On termination of the incubation the medium was collected for determination of palmitate [7], an aliquot was deproteinized with perchloric acid (3%, w/v, final concentration) for determination of acid-soluble metabolites [6]. The hepatocyte monolayer was washed with 150 mM NaCl and extracted [6] for determination of total triacylglycerol [8]. The cellular lipid was extracted [9] and its radioactivity determined. Protein was determined by an automated Lowry method [10].

Expression of results. The rate of β-oxidation of palmitate was determined from the incorporation of [U-^{14}C]palmitate into acid-soluble metabolites [6] and the rate of esterification was determined from the incorporation of radioactivity into cellular lipid and are expressed as nmol of palmitate incorporated per hr per mg of cell protein. The cellular triacylglycerol content determined at the end of the incubation is expressed as nmol of triacylglycerol per mg of protein and the rate of fatty acid uptake was determined from the difference in fatty acid concentration at the beginning and end of the incubation. All results are expressed as means ± SEM for the number of hepatocyte preparations indicated. Statistical analysis was by the Student's *t*-test.

RESULTS

Differences in palmitate metabolism

In human and guinea pig hepatocytes there was a greater partitioning of palmitate towards esterification as opposed to β-oxidation compared with rat hepatocytes (Tables 1 and 2). In the rat, the rate of β-oxidation was approximately 2-fold higher than the rate of esterification while in human and guinea pig hepatocytes rates of esterification were higher than rates of β-oxidation (Tables 1 and 2). In absolute terms the rates of β-oxidation were 2-fold higher in the rat compared with human or guinea pig. The cellular triacylglycerol content, was 40% higher in guinea pig compared to rat hepatocytes ($P < 0.01$) but it was 4-fold higher in human hepatocytes compared to rat ($P < 0.005$; Table 3). We have shown that the rates of total palmitate metabolism are similar in rat and human hepatocytes [11, 12]. The rate of total palmitate metabolism in guinea pig hepatocytes is also similar to that of the rat (Table 4), indicating that there are no differences between these species in the overall rates of palmitate metabolism but there are differences in the partitioning of palmitate between β-oxidation and esterification.

Effects of R- *and* S-*etomoxir*

The *R*-enantiomer of etomoxir inhibited β-oxidation in all three species at concentrations of 1–10 μM, but in human hepatocytes it also inhibited β-oxidation at a concentration of 0.1 μM (Table 1). The concentration of *R*-etomoxir causing half-maximal inhibition was highest in rat hepatocytes and lowest in human hepatocytes (EC_{50} approximately: 0.1 μM, human; 1 μM, guinea pig; 10 μM, rat). In human and guinea pig hepatocytes the inhibition of β-oxidation by *R*-etomoxir was associated with a significant increase in the rate of esterification (Table 2) as well as with an increase in the cellular triacylglycerol content (Table 3). In rat hepatocytes there were small (not statistically significant) trends of increased esterification and cellular triacylglycerol content at high concentrations of *R*-etomoxir (Tables 2 and 3), but there was also a decrease in the total rate of palmitate metabolized at 10 μM etomoxir (Table 4).

The *S*-enantiomer of etomoxir, unlike the *R*-enantiomer, had no effect on either β-oxidation of palmitate or esterification in any of the three species, except for a small inhibition (11%) in human hepatocytes at 10 μM, which may be due to a small contaminant (<2%) of the *R*-isomer. In two other experiments on rat hepatocytes we compared the effects of the *rac*-etomoxir. The inhibition by *rac*-etomoxir (20 μM) was the same as that by the *R*-enantiomer (10 μM) indicating that the inactive *S*-enantiomer does not compete with the *R*-enantiomer.

Table 2. Effects of *R*- and *S*-etomoxir on esterification on palmitate to glycerolipid

Hepatocytes: (N)	Human (3)	Rat (4)	Guinea pig (5)
Additions:	(nmol palmitate/hr mg protein)		
Control	81.3 ± 7.1 (100)	47.6 ± 5.6 (100)	86.7 ± 6.9 (110)
S-Etomoxir 0.1 μM	82.1 ± 6.9 (101)	45.9 ± 2.0 (96)	90.2 ± 2.6 (104)
1 μM	85.1 ± 5.7 (105)	44.3 ± 1.5 (93)	85.7 ± 2.4 (99)
10 μM	81.3 ± 10.8 (100)	45.5 ± 1.8 (96)	93.0 ± 3.0 (107)
R-Etomoxir 0.1 μM	114.8 ± 9.2 (141)	49.1 ± 4.4 (103)	78.8 ± 7.1 (91)
1 μM	128.0 ± 13.1 (157)*	51.6 ± 8.0 (108)	115 ± 10 (133)
10 μM	139.8 ± 11.4 (172)*	54.8 ± 9.2 (115)	122 ± 9 (141)*

For experimental details see Table 1. Rates of palmitate are expressed as nmol of palmitate esterified into cellular lipid per hr per mg of cell protein. Values are means ± SEM for the number of hepatocyte preparations indicated. Values in parentheses indicate the rates expressed as a percentage of the respective controls.

Statistics: * $P < 0.05$ relative to the respective controls.

Table 3. Effects of *R*- and *S*-etomoxir on the cellular triacylglycerol content

Hepatocytes: (N)	Human (3)	Rat (3)	Guinea pig (3)
Additions:	(nmol triacylglycerol/mg protein)		
Control	577 ± 24	124 ± 9	173 ± 3
S-Etomoxir 0.1 μM	592 ± 74	123 ± 11	176 ± 6
1 μM	604 ± 91	118 ± 9	169 ± 6
10 μM	617 ± 102	119 ± 9	175 ± 7
R-Etomoxir 0.1 μM	625 ± 31	123 ± 9	179 ± 7
1 μM	736 ± 12*	129 ± 11	202 ± 4*
10 μM	730 ± 6*	134 ± 11	206 ± 2*

For experimental details see Table 1. The cellular triacylglycerol content was determined on termination of the 3 hr incubation with palmitate. Values are means ± SEM for the number of hepatocyte preparations indicated.

Statistics: * $P < 0.05$.

Table 4. Effects of *R*- and *S*-etomoxir on total palmitate metabolism

Hepatocytes: (N)	Rat (3)	Guinea pig (3)
Additions:	(nmol palmitate/hr/mg protein)	
Control	213 ± 9 (100)	249 ± 9 (100)
S-Etomoxir 0.1 μM	219 ± 13 (103)	249 ± 15 (100)
1 μM	221 ± 8 (104)	242 ± 12 (97)
10 μM	221 ± 12 (104)	248 ± 15 (100)
R-Etomoxir 0.1 μM	217 ± 9 (102)	245 ± 13 (98)
1 μM	207 ± 13 (97)	243 ± 16 (98)
10 μM	179 ± 14 (84)*	236 ± 13 (95)

For experimental details see Table 1. Total palmitate metabolism was determined from the decrease in palmitate concentration (determined enzymically) during incubation with 1 mM palmitate. Values are means ± SEM for the number of hepatocyte preparations indicated. Values in parentheses indicate the rates expressed as a percentage of the respective controls.

Statistics: * $P < 0.05$ relative to the control.

DISCUSSION

The vast majority of studies on the effects of inhibitors of carnitine palmitoyltransferase on hepatocyte metabolism have used rat hepatocytes. There is consequently little information on possible species differences in the effects of these inhibitors. The present study shows: first, that there are species differences in the relative partitioning of palmitate between β-oxidation and esterification but not in the overall rate of palmitate metabolism; secondly, that there are species differences in the sensitivity of β-oxidation to inhibition by *R*-etomoxir; and thirdly it establishes that the *S*-enantiomer of etomoxir does not affect β-oxidation.

Species differences in the partitioning of palmitate between β-oxidation and esterification in hepatocyte cultures

Under physiological conditions the partitioning of palmitate between β-oxidation and esterification is influenced by the endocrine state and particularly by the concentrations of glucagon and epinephrine. The present findings of significant differences between rat, guinea pig and human hepatocyte cultures in the relative partitioning of palmitate between β-oxidation and esterification raise the question whether these differences reflect true physiological species differences or are the result of differences in control mechanisms which may be amplified by the culture conditions. There are indications that suggest that they reflect true species differences. Firstly, studies on ketogenesis from fatty acids in isolated mitochondria have shown that rates of ketogenesis are lower in guinea pig than in rat mitochondria per mg of protein, indicating an inherent lower capacity for ketogenesis in guinea pig compared with rat liver [13, 14]. Secondly, when we determined rates of ketogenesis and β-oxidation of palmitate in rat and guinea pig hepatocyte cultures on 4 successive days of culture starting from the day of isolation, there were similar differences between rat and guinea pig on successive days (results not shown) suggesting that this difference is independent of the age of the culture. Thirdly, the species differences were not abolished when hepatocytes from rat, guinea pig and human liver were cultured with maximally effective concentrations of glucagon or epinephrine ([11, 12] and D. Tosh and L. Agius, unpublished results). Glucagon (1–100 nM) or epinephrine (10 μM) increased ketogenesis in all three species. Although the percentage stimulation of ketogenesis by glucagon and epinephrine was greater in human than in rat or guinea pig hepatocytes these differences were smaller than the differences in their basal rates of ketogenesis. The inhibition of β-oxidation by etomoxir was associated with an inhibition of total palmitate metabolism in rat hepatocytes but with an increase in palmitate esterification in human and guinea pig hepatocytes. This could be explained by the higher capacity for palmitate esterification in the latter species when palmitate is diverted to glycerolipid during inhibition of β-oxidation.

Stereochemistry of inhibition of β-oxidation

Etomoxir and 2-TDGA are hypoglycaemic 2-oxiranecarboxylates which impair gluconeogenesis secondarily to inhibition of β-oxidation of long-chain fatty acids. They are converted to their CoA esters which inhibit carnitine palmitoyltransferase I [4]. Only the *R*-enantiomer of 2-TDGA-CoA inhibits carnitine palmitoyltransferase I in rat liver mitochondria [15]. The present study establishes that only the *R*-enantiomer of etomoxir inhibits palmitate β-oxidation and the sensitivity differs in different species, it does not, however, define the mechanism of these species differences. This may be due to differences in the sensitivity of carnitine palmitoyltransferase I to inhibition by *R*-etomoxir-CoA, or to differences in the intracellular concentrations of *R*-etomoxir-CoA and palmitoyl-CoA because of differences in the activities of palmitoyl-CoA synthase with palmitate and *R*-etomoxir, and of palmitoyl-CoA hydrolase with palmitoyl-CoA and *R*-etomoxir-CoA. Regardless of the mechanism, the greater sensitivity of human hepatocytes to etomoxir correlates with the greater hypoketonaemic and hypoglycaemic potency of etomoxir in man than in rats [4, 16]. By contrast with the specific inhibition of β-oxidation by *R*-etomoxir, both the *R*- and the *S*-forms are equally active as inhibitors of *de novo* fatty acid and cholesterol biosynthesis in rat hepatocytes [17].

Conclusions

These species differences in the rates of esterification of palmitate, and in β-oxidation and its sensitivity to inhibition by *R*-etomoxir highlight the potential problems of extrapolating from studies on animal models to human metabolism.

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