Comparative Biochemistry of β -Oxidation

AN INVESTIGATION INTO THE ABILITIES OF ISOLATED HEART MITOCHONDRIA OF VARIOUS ANIMAL SPECIES TO OXIDIZE LONG-CHAIN FATTY ACIDS, INCLUDING THE $C_{22:1}$ MONOENES

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1. Rates of acylcarnitine oxidation by isolated heart mitochondria from various animal species were measured polarographically, and by using a spectrophotometric assay [see Osmundsen & Bremer (1977) *Biochem. J.* **164**, 621–633]. Polarographic measurements do not give a correct guide to abilities to β -oxidize very-long-chain acylcarnitines, in particular $C_{22:1}$ fatty acylcarnitines. 2. No significant species differences were detected in the abilities to β -oxidize various $C_{22:1}$ fatty acylcarnitines. Significant species differences were, however, detected when rates of β -oxidation were correlated with rates of respiration brought about by very-long-chain acylcarnitines. We concluded that some aspects of oxidative metabolism (possibly the oxidation of tricarboxylic acid-cycle intermediates) are inhibited by very-long-chain fatty acids in some species (e.g. the rat and the cat), but not in others (e.g. the pig and the rabbit). 3. It is proposed that the pattern of variation of rates of oxidation of various acylcarnitines (as measured spectrophotometrically) of various chain lengths can be used as a guide to the chain-length specificities of the acyl-CoA dehydrogenases of β -oxidation (EC 1.3.99.3).

The feeding of oils containing large amounts of $C_{22:1}$ fatty acids has in some animal species caused pronounced, although transient, cardiac- and skeletal-muscle lipidosis (Roquelin *et al.*, 1971; Beare-Rogers *et al.*, 1972). Because nutritionally valuable sources of fats contain appreciable concentrations of $C_{22:1}$ fatty acids, e.g. partially hydrogenated marine oils, which contain brassidic acid (*trans*- $C_{22:1(9)}$) and cetoleic acid (*cis*- $C_{22:1(11)}$) (see Lambertsen *et al.*, 1971), it would be of value to know whether there are species differences in metabolic handling of these acids by isolated heart mitochondria.

Species differences in β -oxidation have been little studied, since enzyme-fractionation work described by Beinert (1963) indicated that such differences exist. This work gave evidence of an acyl-CoA dehydrogenase (EC 1.3.99.3) with optimal specificity for very-long-chain acyl-CoA compounds (>16 carbon atoms) that was present in extracts from pig heart and liver mitochondria, but not in similar preparations from ox. Such enzymic differences have been confirmed by Hall *et al.* (1976). To study whether there are species differences in the β -oxidation system we have chosen to measure the rates of oxidation of a broad spectrum of acylcarnitines, with carbon-chain lengths ranging from 4 to 22 carbon atoms. It is not unreasonable to expect that differences in

specificities of β -oxidation enzymes might be reflected in differences in the acylcarnitine oxidation 'spectra', i.e. plots of rates of oxidation against acylcarnitine carbon-chain length.

Experimental

Animals and tissues

Male Wistar albino rats (150–200g) and male Balb/c albino mice (20–30g) were purchased from Vætrinær Møllergårds Avlsstasjon, Havdrup, Denmark. Male cats (about 2kg) and male rabbits (about 2–3kg) were obtained from a local supplier. Frogs (*Rana temporaria*) were obtained from T. Gerrard and Co., East Preston, W. Sussex, U.K. Monkey (*Cercopithecus aetiops*) hearts were supplied by Statens Seruminstitut, Copenhagen, Denmark. These hearts were air-freighted from Copenhagen, during which time they were stored in a medium containing 50mM-Tris, 220mM-mannitol and 1mM-EGTA, pH 7.8, and kept at 1–3°C. Because of the air transport these hearts were 7–8 h old by the time they could be used. Hearts from pig, ox and sheep were collected from freshly killed animals at a local abattoir, and transported to the laboratory in the above medium.

Materials

Rotenone (grade II), *cis*-oxaloacetic acid (grade 1), cytochrome *c* (type III) and Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Cetolic acid, brassidic acid and erucic acid (*cis*-C_{22:1(9)}) were obtained from Nu-Chek Preparations Inc., Elysian, MN, U.S.A. All other fatty acids were purchased from E. Merck, Darmstadt, Germany, and were of analytical grade. Saturated acylcarnitines were prepared as described by Bremer (1968), and unsaturated acylcarnitines as described by Christopherson & Bremer (1972). Acylcarnitines were analysed for fatty acid content by g.l.c. with a column of 10% EGSS-X on Supelcoport 100/120, and were all found to contain less than 0.5% free acid. All other reagents were of analytical grade or of the highest purity available.

Measurement of acylcarnitine oxidation by isolated mitochondria

Polarographic measurements of rates of acylcarnitine oxidation were carried out by using a Clark-type oxygen electrode. The assay medium contained 300mM-mannitol, 10mM-Hepes, 1mM-EGTA, 1.5mg of defatted bovine serum albumin/ml, 2mM-ADP, 7mM-P_i and 2mM-malate, pH7.2. The addition of P_i to a concentration of 7mM was made because preliminary experiments showed that this concentration, in most species, had a significant suppressive effect on the endogenous malate oxidation, without interfering with the oxidation of acylcarnitines.

Rates of oxidation of acylcarnitines were also measured by using a spectrophotometric assay that selectively measures the flux through the acyl-CoA dehydrogenases of β -oxidation. In this procedure O₂ is replaced by Fe(CN)₆³⁻ as the final acceptor of reducing equivalents. The reduction of ferricyanide can be followed by recording the decrease in A₄₂₀ with time, and by using 475nm as the reference wavelength. These assays were carried out by using an Aminco DW-2 spectrophotometer, operated in the dual-beam mode. A detailed description of this assay has been given elsewhere (Osmundsen & Bremer, 1977). The assay medium was similar to that used in the polarographic assay, except that malate was excluded. In addition the medium contained 10 μ M-cytochrome *c*, 1mM-KCN, 0.5mM-K₃Fe(CN)₆, 10mM-oxaloacetate and 6 μ g of rotenone/ml. Each assay contained about 1mg of mitochondrial protein.

Acylcarnitines were as a routine included in the assay mixtures to a concentration of 20 μ M, except butyrylcarnitine and hexanoylcarnitine, which were used at concentrations of 120 and 40 μ M respectively. With long-chain acylcarnitines increased concen-

trations did not increase their rates of oxidation. Decreasing their concentrations invariably led to decreased rates of oxidation. All assays were carried out at 25°C.

Preparation of mitochondria

Heart mitochondria from all species investigated were isolated in a medium containing 300mM-mannitol, 10mM-Hepes and 1mM-EGTA, pH7.2. The isolation medium was also supplemented with 20i.u. of heparin/ml of medium. With these modifications mitochondria from abattoir animals were prepared as described by Green & Ziegler (1963), the heavy mitochondrial fraction being used in all assays. Mitochondria from other species were isolated as described by Osmundsen & Bremer (1977), except that a Waring blender had to be used for the initial homogenization of hearts from the larger animals (cat, rabbit and monkey). After the final centrifugation all mitochondrial samples were immediately resuspended in a small volume of the homogenization medium, to a final concentration of 30–50mg of protein/ml.

The intactness of the mitochondrial preparations was checked with some species (the rat, the mouse and the pig) by measuring the State-3 (+3mM-ADP) and State-4 (no added ADP) rates of palmitoylcarnitine oxidation. The State-3 rate of oxidation was about 3 times faster than the State-4 rate.

Determination of mitochondrial protein

The mitochondrial protein concentrations were measured by using the assay of Gornall *et al.* (1949), with Precimat (Boehringer Mannheim G.m.b.H., Mannheim, Germany) protein standards.

Statistical analysis

Student's *t* test was the statistical test used to determine the significance of differences between the means (Armitage, 1971).

Results

Methodology

Because the polarographic assays were carried out with malate in the incubation medium, an appreciable fraction of the measured O₂ uptake (about two-thirds) was due to the oxidation of tricarboxylic acid-cycle intermediates. Spectrophotometric measurements of acylcarnitine-dependent reduction of ferricyanide, however, provide a direct measurement of the flux through the rate-limiting acyl-CoA dehydrogenase stage of β -oxidation under conditions facilitating optimal rates of β -oxidation, i.e.

Fig. 1. Effect of assay method on measurements of rates of oxidation of long-chain acylcarnitines

The oxidation of cetoleoyl-carnitine (filled columns) and palmitoyl-carnitine (open columns) was measured spectrophotometrically (a) and polarographically (b) as described in the Experimental section, by using the same preparations of heart mitochondria from mouse, rat and cat. The results presented show mean rates of oxidation based on measurements from four mouse and rat heart mitochondrial preparations, and two cat heart mitochondrial preparations. The shaded columns represent endogenous rates of oxidation. The levels of significance obtained when comparing each rate of oxidation with the corresponding rates of endogenous oxidation are indicated.

the oxaloacetate-facilitated entry of acetyl groups into the tricarboxylic acid cycle, as well as re-oxidation of NADH generated by the reaction sequence (see Osmundsen & Bremer, 1977). We consider it unlikely that any components of the assay may have inhibitory effects on β -oxidation-related enzymes, as this assay can give higher rates of β -oxidation than a polarographic assay, under otherwise similar conditions (see Osmundsen & Bremer, 1977).

As the polarographic assay measures the rate of β -oxidation plus the tricarboxylic acid cycle (under present assay conditions), whereas the spectrophotometric assay is selective for β -oxidation, we have investigated the abilities of mitochondria of different species to oxidize very-long-chain acylcarnitines by using both assay systems. This approach should make it possible to detect any effects that these acylcarnitines may have on, e.g., the oxidation of tricarboxylic acid-cycle intermediates.

Fig. 1 shows results obtained from measurements

of rates of oxidation of cetoleoyl-carnitine and palmitoyl-carnitine by heart mitochondria from mouse, rat and cat. With all three species significant increases in rates of oxidation were observed on addition of the acylcarnitine with the spectrophotometric assay. Monitored by the polarographic assay, however, cetoleoyl-carnitine did not cause significant stimulation of respiration above the endogenous rate with mouse heart mitochondria, and the stimulation given with rat heart mitochondria was just statistically significant (see Fig. 1 for levels of significance). By contrast, cat heart mitochondria gave a statistically significant decrease in rates of respiration when exposed to cetoleoyl-carnitine (see Fig. 1). Both erucoyl- and brassido-yl-carnitine gave results similar to cetoleoyl-carnitine (results not shown). These data indicate that a $C_{22:1}$ fatty acid has species-selective effects on metabolic sequences not directly related to β -oxidation.

Oxidation of very-long-chain acylcarnitines by isolated heart mitochondria from various species

The absolute rates of acylcarnitine oxidation varied severalfold between species: for palmitoyl-carnitine from 16 (sheep) to 56 nmol of $Fe(CN)_6^{3-}$ reduced/min per mg of protein (mouse), and from 44 (sheep) to 206 ng-atoms of O/min per mg of protein (monkey). The data presented have therefore been expressed as percentages of the rates given by palmitoyl-carnitine, to facilitate inter-species comparisons. The mean relative rates for individual acylcarnitines for various species are presented in Table 1. No significant species differences were detected in rates of β -oxidation of very-long-chain monounsaturated acylcarnitines, as indicated by the small value for s.d. for the spectrophotometric data in Table 1. This was also the case for relative rates of combined oxidation of equimolar concentrations of a $C_{22:1}$ fatty acylcarnitine and palmitoyl-carnitine (see Table 1).

The polarographic measurements, by contrast, show significant higher standard deviations for the inter-species mean relative rates of oxidation of the three $C_{22:1}$ fatty acylcarnitines compared with those obtained from spectrophotometric measurements ($P = 0.02$). The standard deviations obtained for the three other (shorter) acylcarnitines [stearoyl-carnitine ($C_{18:0}$), oleoyl-carnitine (*cis*- $C_{18:1(9)}$) and gadeoyl-carnitine (*cis*- $C_{20:1(11)}$)] are not significantly different from those obtained with the spectrophotometric assay ($P = 0.34$). The same is true for standard deviations of the combined rates of oxidation of a $C_{22:1}$ fatty acylcarnitine plus palmitoyl-carnitine ($P = 0.24$).

The results presented in Fig. 2, showing relative rates of oxidation obtained from oleoyl-, brassido-yl- and cetoleoyl-carnitine with heart mitochondria

Table 1. Mean relative rates of acylcarnitine oxidation for mitochondria from different species

The mean relative rates of oxidation by mitochondria from various species observed with stearylacarnitine (SC; $C_{18:0}$), oleoylcarnitine (OC; $cis-C_{18:1}$), gadeoylcarnitine (GC; $cis-C_{20:1}$), erucoylcarnitine (EC; $cis-C_{22:1(9)}$), brassidoylecarnitine (BC; $trans-C_{22:1(9)}$) and cetoleoylcarnitine (CC; $cis-C_{22:1(11)}$) are shown. The rates of oxidation of palmitoylcarnitine (PC; $C_{16:0}$) were set at 100%. The rates of combined oxidation of equimolar concentrations (20 μM) of a $C_{22:1}$ acylcarnitine added 1 min before the addition of PC are expressed on a similar basis, and are presented on the right-hand side of the Table. The tabulated values represent the mean values from measurements with isolated heart mitochondria from mouse (four preparations), rat (four preparations), cat (two preparations), pig (four preparations), sheep (two preparations) and monkey (two preparations; polarographic data not included). The numbers in parentheses represent standard deviations for the tabulated means. Results obtained with the spectrophotometric and polarographic assays (see the Experimental section for assay details) are presented.

Assay method	Relative rates of acylcarnitine oxidation (%)									
	PC	SC	OC	GC	EC	BC	CC	PC+EC	PC+BC	PC+CC
Spectrophotometric	100	96 (4)	82 (2)	74 (8)	41 (5)	49 (6)	39 (5)	68 (12)	85 (6)	68 (12)
Polarographic	100	93 (4)	91 (7)	70 (10)	36 (14)	39 (14)	36 (14)	72 (5)	89 (7)	67 (15)

Fig. 2. Oxidation of monounsaturated long-chain acylcarnitines by isolated heart mitochondria from various species

The oxidation, of oleoylcarnitine (OC; $C_{18:1}$), brassidoylecarnitine (BC; $trans-C_{22:1(9)}$) and cetoleoylcarnitine (CC; $cis-C_{22:1(11)}$) were measured polarographically (shaded columns) and spectrophotometrically (open columns) as described in the Experimental section. The values presented in the histogram represent mean relative rates of oxidation within each species (the rate of oxidation of palmitoylcarnitine being taken as 100%) obtained by using heart mitochondria from rat (four preparations), cat (two preparations), pig (four preparations) and rabbit (four preparations). The levels of significance indicated refer to differences between spectrophotometrically and polarographically measured relative rates. For each species each pair of columns, starting from the left-hand side, represents relative rates obtained with oleoylcarnitine, brassidoylecarnitine and cetoleoylcarnitine. The levels of significance are shown above each pair (i.e. polarographically and spectrophotometrically measured) of columns.

from rat, cat, pig and rabbit, show the source of the large standard deviations obtained with polarographic assays. It is noteworthy in these data that in some species (rat and cat) the rates of respiration are

significantly and markedly lower than the corresponding rates of β -oxidation (the latter having been measured spectrophotometrically). With pig and rabbit, however, the two rates are mainly not significantly different (levels of significance are indicated in Fig. 2), and never markedly different.

Relation between rates of β -oxidation and rates of respiration with different very-long-chain acylcarnitines

The results plotted in Fig. 3 represent the ratios between relative rates of respiration relative rates of β -oxidation (as measured spectrophotometrically) from three monounsaturated acylcarnitines. These data show that this ratio remains constant for pig heart mitochondria, but mitochondria from mouse, rat and cat give ratios that decrease progressively with increasing carbon-chain lengths. The ratio obtained from gadeoylcarnitine with rat mitochondria is significantly different from the value obtained with pig mitochondria (see Fig. 3 for levels of significance). The ratios obtained with brassidoylecarnitine from rat, mouse and cat mitochondria were all significantly different from the ratio observed for pig mitochondria (see Fig. 3).

It is also noteworthy that the ratios given by mitochondria from rat, mouse and cat approach the value given by pig mitochondria when after exposure for 1 min to brassidoylecarnitine (20 μM) an equimolar concentration of palmitoylcarnitine is added to them.

Heart mitochondria from rabbit and sheep gave ratios similar to those of the pig (results not shown). Cetoleoyl- and erucoyl-carnitine gave ratios very similar to those obtained with brassidoylecarnitine (results not shown).

Oxidation of acylcarnitines of various carbon-chain lengths by heart mitochondria from various species

Because the spectrophotometric assay selectively measures the acyl-CoA flux through the acyl-CoA

Fig. 3. Effect of chain length of monounsaturated acylcarnitines on ratios between relative rates of oxidation measured polarographically and spectrophotometrically

The ratios between mean relative rates of oxidation as measured polarographically and spectrophotometrically (see the Experimental section for details of assays) have been calculated from measurements obtained with isolated mitochondria. The rates of oxidation of oleoylcarnitine (C_{18:1}), gadeoylcarnitine (C_{20:1}) and brassidoylecarnitine (*trans*-C_{22:1(9)}) were determined with heart mitochondria from rat (four preparations), mouse (four preparations), cat (two preparations) and pig (four preparations). The values plotted represent the mean ratios ($\pm$ s.d.). The points to the right-hand side, connected to the others by broken lines, represent the mean ratios obtained from the combined rates of oxidation of equimolar concentrations (20 μ M) of brassidoylecarnitine and palmitoylecarnitine (the latter having been added 1 min after the C_{22:1} acylcarnitine). The significances of the difference of the ratios obtained with mitochondria from mouse (○), rat (●) and cat (□) compared with the corresponding ratios obtained with pig (▲) were measured and are indicated.

Fig. 4. β -Oxidation 'spectra' of ox and pig heart mitochondria

The rates of oxidation of various saturated acylcarnitines with carbon-chain lengths from 4 to 22 carbon atoms were measured spectrophotometrically as described in the Experimental section. The plotted data represent the mean relative rates of oxidation (the rate given by palmitoylecarnitine being taken as 100%) ($\pm$ s.d.) based on measurements on mitochondria from two ox hearts (▲) and two pig hearts (○). The data points shown at C₂₂ represent results obtained with cetoleoylcarnitine (*cis*-C_{22:1(11)}). The significances of the difference of the corresponding data sets from the two species were measured and are shown above the relevant symbols.

dehydrogenases in intact mitochondria, graphs based on data obtained with this assay have been used to illustrate variation in rates of β -oxidation as a function of acylcarnitine carbon-chain length, i.e. β -oxidation 'spectra'. Results obtained in these experiments with mitochondria from pig, ox, monkey, rat and the frog are presented in Figs. 4 and 5. To facilitate inter-species comparisons the results are expressed in terms of relative rates of oxidation (the rate given by palmitoylecarnitine being taken as 100%). Although it would have been desirable to include saturated acylcarnitines containing more than

20 carbon atoms, this was not feasible because of the problems related to the synthesis of extremely long saturated acylcarnitines.

Fig. 4 shows that there are significant differences between the 'spectra' obtained with pig and ox mitochondria. This is noteworthy in view of the known differences in acyl-CoA dehydrogenase composition between these species (see Hall *et al.*, 1976). These differences are expressed primarily by the significantly higher rates given by ox mitochondria over the chain

Fig. 5. β -Oxidation 'spectra' of isolated mitochondria from rat heart, monkey heart and frog heart

Rates of acylcarnitine oxidation were measured spectrophotometrically as described in the legend of Fig. 4. The plotted mean relative rates of oxidation were obtained by measurements from three rat heart mitochondrial preparations (○), two monkey heart preparations (■) and two frog heart preparations (▲). The error bars indicate s.d.

lengths from 8 to 14 carbon atoms (see Fig. 4 for levels of significance).

Discussion

β -Oxidation of very-long-chain acylcarnitines by isolated heart mitochondria from various species

The data in Table 1 show that gadeoylcarnitine was always oxidized at rates that were markedly faster than those of the $C_{22:1}$ fatty acylcarnitines ($P < 0.001$). Surprisingly, the *trans*- $C_{22:1}$ fatty acylcarnitine (brassidoylecarnitine) was always oxidized at rates that were about 10% faster than the corresponding *cis*-isomer (erucoylecarnitine) ($P = 0.0005$). This finding is surprising as *trans*-fatty acids are as a rule oxidized slower than their corresponding *cis*-isomers (Hsu & Kummerow, 1977). No significant differences were found between relative rates of oxidation of cetoleoylcarnitine and erucoylecarnitine ($P = 0.14$).

The inhibitory effect of the $C_{22:1}$ fatty acylcarnitines on the oxidation of palmitoylecarnitine is illustrated by the columns showing the spectrophotometric inter-species mean rates of combined oxidation (see Table 1). These results show that brassidoylecarnitine in all species gave a combined rate of oxidation that was 15–20% higher than that of the two other $C_{22:1}$ fatty acylcarnitines ($P = 0.00003$ for cetoleoylcarnitine and $P = 0.003$ for erucoylecarnitine). No significant differences were observed in this respect between cetoleoylcarnitine and erucoylecarnitine ($P = 0.36$).

Species differences with respect to effects of very-long-chain acylcarnitines on oxidative metabolism

Fig. 1 points to the existence of species differences in metabolic responses to the $C_{22:1}$ fatty acylcarnitines. The data presented here, however, strongly suggest that the cause of these differences is not in the β -oxidation system itself. This point is further illustrated by the data presented in Fig. 2, which show that the large standard deviation of the polarographic inter-species mean relative rates of $C_{22:1}$ fatty acylcarnitine oxidation (see Table 1) are indeed caused by dramatic species variation in respiratory responses to these acylcarnitines. These data (Fig. 2) show that the spectrophotometrically measured rates of β -oxidation in some species (rat and cat) are almost 100% faster than the rates of respiration brought about by the same acylcarnitine. With other species (pig and rabbit) the rates are identical or nearly so (see Fig. 2 for levels of significance). Erucoylecarnitine gave results not significantly different from those with cetoleoylcarnitine (results not shown). Of other species investigated, mouse gave results similar to rat and cat, whereas sheep heart mitochondria showed properties similar to those of pig heart mitochondria (results not shown).

The decrease in ratios of relative rates of oxidation (measured polarographically and spectrophotometrically) obtained with mitochondria from mouse, rat and cat as the chain length of the monounsaturated acylcarnitines increased from 18 to 22 carbon atoms (see Fig. 3) may be taken to indicate that, in these species, decreased rates of β -oxidation are accompanied by proportionally larger decreases in rates of respiration. This relative inhibition of respiration may be caused by an inhibited rate of oxidation of tricarboxylic acid-cycle intermediates brought about by the very-long-chain acylcarnitines. For other species, e.g. pig (and rabbit and sheep; results not shown), a decreased rate of β -oxidation is accompanied by an almost proportional decrease in rates of respiration. Therefore in these latter species the $C_{22:1}$ acylcarnitine causes no apparent extra inhibition of the rate of oxidation of tricarboxylic

acid-cycle intermediates. The ratios of relative rates therefore remain constant (about 1.0) as the carbon-chain lengths are increased from 18 to 22 carbon atoms.

This apparent inhibitory effect is also specific to the very-long-chain acylcarnitines ($C_{20:1}$ and $C_{22:1}$), as the ratios obtained with oleoylcarnitine do not show any significant species differences (see Fig. 3). The sites of inhibition have not been characterized, but 'malic' enzyme (EC 1.1.1.40) or pyruvate dehydrogenase (EC 1.2.4.1) might conceivably be involved.

The addition of an equimolar concentration of palmitoylcarnitine to mitochondria that were pre-exposed to brassidoylecarnitine gives final ratios of relative rates that are markedly and significantly ($P = 0.001$) higher than those obtained in the presence of brassidoylecarnitine alone (for mitochondria from mouse, rat and cat). This would suggest that palmitoylcarnitine is competitive with brassidoylecarnitine as far as this apparent inhibition is concerned, and that palmitoylcarnitine itself (like oleoylcarnitine) does not exert a similar effect.

Correlation of data obtained in vivo with results obtained from the present investigation in vitro

Friend *et al.* (1975) have reported that feeding of oils containing $C_{22:1}$ fatty acids to pigs causes little or no cardiac lipidosis. Similarly, Kramer *et al.* (1975) found that pigs did not appear to suffer from cardiac lipidosis after feeding on a $C_{22:1}$ fatty acid-containing diet, and they state that rats given proportional quantities of $C_{22:1}$ fatty acids in their diet developed marked cardiac lipidosis. These results correlate with our finding that there is no apparent decrease in the oxidation of tricarboxylic acid-cycle intermediates after the exposure of pig heart mitochondria to a $C_{22:1}$ acylcarnitine. With rat heart mitochondria, however, the presence of a $C_{22:1}$ acylcarnitine appears to exert this effect (see Fig. 3). It is tempting therefore to suggest that this phenomenon, which involves a central pathway of cardiac energy metabolism, is in some way associated with the development of cardiac lipidosis after dietary intake of $C_{22:1}$ fatty acids.

Species difference in β -oxidation 'spectra'

The data presented in Fig. 4 show that ox heart mitochondria are able to oxidize short- and medium-chain acylcarnitines relatively faster than do pig heart mitochondria. It is possible that this difference may reflect differences in acyl-CoA dehydrogenase composition described by Hall *et al.* (1976) (i.e. the absence of an enzyme with optimal specificities for very-long-chain acyl-CoA esters in ox mitochondria).

Monkey heart mitochondria gave a β -oxidation 'spectrum' similar to that of pig heart mitochondria in that it is devoid of a well-defined maximum rate of oxidation. Now the relative rate of oxidation obtained with arachidoylecarnitine ($C_{20:0}$) is significantly larger than the corresponding relative rate obtained with ox heart mitochondria ($P = 0.01$). Also the relative rates obtained with acylcarnitines containing from 12 to 14 carbon atoms are significantly smaller than those obtained with ox heart mitochondria ($P = 0.003$). It is therefore likely that both pig and monkey have acyl-CoA dehydrogenases of similar composition, both being dissimilar to that of ox heart mitochondria.

Rat and frog heart mitochondria, by contrast, show β -oxidation 'spectra', with well-defined maximum rates of oxidation (see Fig. 5). The relative rates obtained for rat heart mitochondria with acylcarnitines containing 10 to 14 carbon atoms are significantly different from those obtained with pig heart mitochondria ($P = 0.04$) with the same acylcarnitines. They are not, however, significantly different from those obtained with ox heart mitochondria ($P = 0.24$), except that the maximum rate of oxidation occurs at the C_{10} acylcarnitine with rat heart mitochondria, and at C_{12} acylcarnitine with ox heart mitochondria. Rat heart mitochondria are also similar to ox heart mitochondria in that the relative rates of oxidation obtained with arachidoylecarnitine are not significantly different ($P = 0.35$), but there is a significant difference between rat and pig mitochondria in this respect ($P = 0.02$).

Frog heart mitochondria show a very marked maximum rate of oxidation with decanoylecarnitine (see Fig. 5), and are in this respect similar to rat heart mitochondria. As the chain lengths increase beyond 10 carbon atoms there is a progressive and marked decrease in rates of oxidation. This decrease is more marked for stearoyl- and arachidoylecarnitine in frog heart mitochondria than with rat heart mitochondria ($P = 0.0005$ and $P = 0.02$ respectively). These results therefore suggest that frog heart mitochondria, although able to oxidize short- and medium-chain acylcarnitines at comparatively high rates, oxidize the longer-chain acylcarnitines at rates that are relatively smaller than those obtained with any of the other species investigated.

It seems reasonable to conclude from these data that the use of the spectrophotometric assay has revealed species differences in the composition of the β -oxidation enzymes, probably residing in the acyl-CoA dehydrogenases. The apparent absence of species differences in the handling of very-long-chain monounsaturated acylcarnitines may in this context appear contradictory. It is, however, possible that the resulting acyl-CoA esters are poor substrates for any acyl-CoA dehydrogenase, irrespective of chain-length specificities.

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