Carnitine acyltransferase activities in rat liver and heart measured with palmitoyl-CoA and octanoyl-CoA

Latency, effects of K^+ , bivalent metal ions and malonyl-CoA

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1. Liver carnitine acyltransferase activities with palmitoyl-CoA and octanoyl-CoA as substrates and heart carnitine palmitoyltransferase were measured as overt activities in whole mitochondria or in mitochondria disrupted by sonication or detergent treatment. All measurements were made in sucrose/KCl-based media of 300 mosmol/litre. 2. In liver mitochondria, acyltransferase measured with octanoyl-CoA, like carnitine palmitoyltransferase, was found to have latent and overt activities. 3. Liver acyltransferase activities measured with octanoyl-CoA and palmitoyl-CoA differed in their response to changes in $[K^+]$, Triton X-100 treatment and, in particular, in their response to Mg^{2+} . Mg^{2+} stimulated activity with octanoyl-CoA, but inhibited carnitine palmitoyltransferase. 4. The effects of K^+ and Mg^{2+} on liver overt carnitine palmitoyltransferase activity were abolished by Triton X-100 treatment. 5. Heart overt carnitine palmitoyltransferase activity differed from the corresponding activity in liver in that it was more sensitive to changes in $[K^+]$ and was stimulated by Mg^{2+} . Heart had less latent carnitine palmitoyltransferase activity than did liver. 6. Overt carnitine palmitoyltransferase in heart mitochondria was extremely sensitive to inhibition by malonyl-CoA. Triton X-100 abolished the effect of low concentrations of malonyl-CoA on this activity. 7. The inhibitory effect of malonyl-CoA on heart carnitine palmitoyltransferase could be overcome by increasing the concentration of palmitoyl-CoA.

The system for transfer of long-chain fatty acid units across the mitochondrial inner membrane appears to require two pools of long-chain carnitine acyltransferase (CPT) (Fritz & Yue, 1963; Yates & Garland, 1970; Hoppel & Tomec, 1972; Harano *et al.*, 1972; Brosnan *et al.*, 1973), one of which is latent and the other overt in intact mitochondria. Generally, studies of the degree of latency of this activity have been confined to liver mitochondria. There is also evidence for the existence of a distinct medium-chain carnitine acyltransferase activity (COT) in liver and heart mitochondria (Kopeck & Fritz, 1971; Solberg, 1971). The physiological role of this activity is unknown, and it has not been investigated whether this activity also shows latency. More recently, as a result of the finding that

malonyl-CoA is a potent inhibitor of the overt form of CPT in liver mitochondria from fed rats (see review by McGarry & Foster, 1980), there has been renewed interest in the properties and control of carnitine acyltransferase. This effect has assumed particular importance in the control of ketogenesis (McGarry & Foster, 1980). At the outset of the present study it was thought to be of interest to investigate the effect of malonyl-CoA on CPT activity in mitochondria from the heart, a tissue which is regarded as having little lipogenic or ketogenic capacity, although it is active in fatty acid oxidation. Some findings from these studies are presented, together with several other observations which arose from experiments preliminary to the above study. In essence these show that liver and heart overt CPT and liver COT activities are considerably affected by various metal ions, that these ionic effects differ for each activity and also that these activities differ somewhat in the extent of their latency.

Abbreviations used: COT, carnitine acyltransferase activity with octanoyl-CoA; CPT, carnitine palmitoyltransferase (EC 2.3.1.21); Nbs₂, 5,5'-dithiobis-(2-nitrobenzoic acid).

Materials and methods

Animals

These were Sprague-Dawley rats weighing 160–180 g, bred in the Animal Colony at University College London, and maintained on GR3-EK cube diet (E. Dixon and Sons, Ware, Herts., U.K.).

Chemicals

Palmitoyl-CoA, octanoyl-CoA, malonyl-CoA and acetyl-CoA were from International Enzymes Ltd. (Windsor, Berks., U.K.). Triton X-100, L-carnitine hydrochloride, Nbs₂ and bovine plasma albumin (fraction V) were from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey, U.K.). Before use the albumin was subjected to a defatting procedure (Chen, 1967) with minor modifications (Saggerson, 1972). Oxaloacetate and CoA were from Boehringer Corp. (London) Ltd. (Lewes, Sussex, U.K.). DL-[methyl-³H]Carnitine hydrochloride was from The Radiochemical Centre (Amersham, Bucks., U.K.).

Isolation of mitochondria

All operations were at 0–2°C. Liver from a single rat or the hearts of four to six rats were collected in 0.25 M-sucrose medium containing 10 mM-Tris/HCl, pH 7.4, and 1 mM-EGTA. These were finely chopped with scissors, washed to remove blood and homogenized in the same medium containing, in addition, fatty acid-poor albumin (10 mg/ml). This was done with three or four strokes of a motor-driven Teflon pestle in a glass Potter/Elvehjem homogenizer (radial clearance 0.19 mm). The volume of the homogenate was adjusted in the proportion 10 ml of sucrose/albumin medium per g wet wt. of tissue. Centrifugation conditions for isolation and washing of the mitochondria were as described by Harper & Saggerson (1975). The mitochondrial pellets were washed once with 0.25 M-sucrose/10 mM-Tris/HCl (pH 7.4)/1 mM-EGTA. Finally, the mitochondria were resuspended in 0.3 M-sucrose/10 mM-Tris/HCl (pH 7.4)/1 mM-EGTA (2 or 4 ml of medium per g of original tissue for heart or liver mitochondria respectively). The protein contents of these stock mitochondrial suspensions, determined by the method of Lowry *et al.* (1951), were: heart, 2.35 ± 0.06 mg/ml (mean $\pm$ S.E.M. for 32 preparations); liver, 4.00 ± 0.17 mg/ml (mean $\pm$ S.E.M. for 21 preparations).

Analytical methods

CPT was measured in the direction of palmitoyl-carnitine formation from palmitoyl-CoA + carnitine. This was done at 25°C either by following spectrophotometrically the reaction of the released CoA with Nbs₂, or by following the incorporation of [³H]carnitine into palmitoylcarnitine. These are referred to as the Nbs₂-linked assay and the

radiochemical assay respectively. In all cases, reactions were performed with 50 μ l samples of mitochondria in a final volume of 1.0 ml, which contained 10 mM-Tris/HCl buffer, pH 7.4, and fatty acid-poor albumin (1.3 mg/ml). Included with these components were various proportions of sucrose and KCl such that together these always provided an osmolarity in the assay mixture of 300 mosmol/litre. Thus, when stated that [K⁺] was zero, [sucrose] was 300 mM or, for example, when [K⁺] was 40 mM, [sucrose] was 220 mM etc. The concentrations of other components are indicated in the legends to individual experiments.

The Nbs₂-linked assay was performed in a Unicam SP. 8-100 spectrophotometer with 0.1 mM-Nbs₂ present. The reaction was initiated by addition of 400 nmol of L-carnitine, which was omitted from blank cuvettes. With heart mitochondria the rate of change of A_{412} was constant after a short transient period of approx. 1 min under any of the experimental conditions. For reasons that are unclear, lags were observed with liver mitochondria before the establishment of steady rates of reaction. The lengths of these lag periods were considerably influenced by the ionic composition of the assay medium, being decreased by increasing [K⁺] and particularly by addition of low [Mg²⁺] (Fig. 1). The length of lag period seen with low [K⁺] varied considerably between mitochondrial preparations. Sonication or treatment of liver mitochondria with Triton X-100 greatly diminished the lag periods. Variations in [K⁺] or [Mg²⁺] did not alter the size of the change in A_{412} when calibrated amounts of CoA reacted with Nbs₂. These lags do not result from gradual release of latent enzyme activity (see below). Throughout, the values presented refer to the steady rates that were finally obtained after these lags. These rates of change in A_{412} were proportional to mitochondrial concentration.

For the radiochemical assay, mitochondria were preincubated for 3–4 min at 25°C with the reaction mixture before initiation of the reaction by addition of 20 μ l of water containing 400 nmol of L-carnitine + 1 μ Ci of DL-[methyl-³H]carnitine. The reaction was terminated after 4 min. During this time the reaction was proportional to mitochondrial concentration and was linear with time. Zero-time blanks were subtracted from all experimental values. The reaction was monitored by measurement of ³H incorporation either into acid-insoluble products or into butanol-soluble products. These two methods gave very similar results. For measurement of acid-precipitated palmitoylcarnitine, the reaction was stopped by addition of 1.0 ml of ice-cold 0.6 M-trichloroacetic acid followed by transfer of the assay tube into ice. After addition of 5 ml of 0.3 M-trichloroacetic acid, the tube contents were filtered under suction through a Millipore filter

Fig. 1. Time courses of *Nbs*₂-linked assays of overt CPT in liver mitochondria

Assays were performed with whole liver mitochondria and with 40 μ M-palmitoyl-CoA and 400 μ M-L-carnitine. The data are from one representative experiment. Solid lines: O, $[K^+] = 0$, $[Mg^{2+}] = 0$; ●, $[K^+] = 7.5$ mM, $[Mg^{2+}] = 0$; ■, $[K^+] = 0$, $[Mg^{2+}] = 1$ mM. Broken lines: O, $[K^+] = 25$ mM, $[Mg^{2+}] = 0$; ●, $[K^+] = 60$ mM, $[Mg^{2+}] = 0$.

Table 1. Effect of $[K^+]$ on respiratory control in liver and heart mitochondria

Experimental details are given in the text. The values are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses.

	Liver (4)		Heart (7)	
	$[K^+] = 40$ mM	$[K^+] = 120$ mM	$[K^+] = 40$ mM	$[K^+] = 120$ mM
Ratio State-3/State-2 rate of respiration	5.0 ± 0.5	6.0 ± 0.3	2.6 ± 0.2	3.0 ± 0.3
Ratio State-3/State-4 rate of respiration	4.0 ± 0.3	3.7 ± 0.4	3.3 ± 0.2	3.1 ± 0.3

(0.45 μ m pore size). The tube was washed with 5 ml of 0.3 M-trichloroacetic acid, which was also applied to the filter, followed by a final washing of the filter with 5 ml of 0.3 M-trichloroacetic acid. The filter was dissolved in 10 ml of scintillation fluid, consisting of 80 g of naphthalene + 4 g of 2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen per litre of toluene/2-methoxyethanol (3:2, v/v). For measurement of radioactivity in butanol-soluble palmitoylcarnitine, the CPT reaction was stopped by addition of 1.0 ml of ice-cold 1.2 M-HCl followed by 2.0 ml of water-saturated butanol. After mixing and brief centrifugation, the resulting butanol layer was washed with 5 ml of butanol-saturated water and 1.0 ml of this taken for scintillation counting in 10 ml of toluene containing 2,5-bis-(5-t-butylbenzoxazol-2-yl)-thiophen (4 g/litre).

COT activity was measured in the *Nbs*₂-linked assay or the radiochemical assay (by using butanol extraction of the product) used for CPT, except that octanoyl-CoA was substituted for palmitoyl-CoA. Lag periods with low $[K^+]$ were not seen in the COT assay.

Citrate synthase (EC 4.1.3.7) was assayed spectrophotometrically at 25°C and 412 nm in 1.0 ml containing sucrose/KCl mixtures (300 mosmol/litre), 10 mM-Tris/HCl, pH 7.4, 50 μ M-acetyl-CoA, 0.1 mM-*Nbs*₂ and 10 μ l of mitochon-

dria. The reaction was initiated by addition of 50 nmol of oxaloacetate, which was omitted from blank cuvettes.

Mitochondrial integrity

As explained above, all CPT and COT assays contained a sucrose/KCl mixture providing an osmolality of 300 mosmol/litre. As discussed below, the activity of CPT and COT was influenced by the concentration of K^+ in this mixture. For many experiments, this was arbitrarily fixed at 40 mM. Unless otherwise stated, CPT and COT activities refer to overt activities measured with fresh and, as far as possible, intact mitochondria. Several findings suggested that reasonable mitochondrial integrity was retained under the assay conditions, precluding the expression of latent carnitine acyltransferase activities unless the mitochondria were subjected to detergent treatment or sonication. Firstly, mitochondrial respiration was measured at 25°C with an oxygen electrode in sucrose/KCl mixtures containing 10 mM-Tris/HCl (pH 7.4), 1 mM-EGTA, 1 mM-EDTA, fatty acid-poor albumin (1.3 mg/ml), rotenone (1 μ g/ml), 1 mM-phosphate and 1.7 mM-sodium succinate. A State-2/State-3 transition was induced by addition of 0.33 mM-ADP. The final State-4 rate was also measured after consumption of this ADP. Table 1 shows that reasonable respiratory

Table 2. *Effect of $[K^+]$, $[Mg^{2+}]$ and palmitoyl-CoA on the relative latency of citrate synthase in liver and heart mitochondria*

Experimental details are given in the text. The values are means $\pm$ S.E.M. for four experiments. Total activity was measured after sonication of mitochondria for 2 min.

		Citrate synthase activity (nmol/min per mg of protein)						
		Without palmitoyl-CoA			With 40 μ M-palmitoyl-CoA			
	$[K^+]$ (mM)	$[Mg^{2+}]$ (mM)	Overt	Total	Ratio $\frac{\text{total}}{\text{overt}}$	Overt	Total	Ratio $\frac{\text{total}}{\text{overt}}$
Liver	0	0	2.4 $\pm$ 0.1	25 $\pm$ 1	10.1	2.3 $\pm$ 0.2	23 $\pm$ 2	10.2
	60	0	3.6 $\pm$ 0.3	37 $\pm$ 2	10.2	3.4 $\pm$ 0.2	34 $\pm$ 2	10.1
	120	0	4.9 $\pm$ 0.2	45 $\pm$ 1	9.2	4.7 $\pm$ 0.1	46 $\pm$ 4	9.7
	0	2.5	3.2 $\pm$ 0.2	32 $\pm$ 1	10.0	2.9 $\pm$ 0.2	29 $\pm$ 2	10.1
	60	2.5	3.9 $\pm$ 0.2	37 $\pm$ 1	9.6	3.7 $\pm$ 0.2	36 $\pm$ 3	9.6
	120	2.5	4.7 $\pm$ 0.1	39 $\pm$ 1	8.3	4.4 $\pm$ 0.1	36 $\pm$ 4	8.2
Heart	0	0	31 $\pm$ 3	337 $\pm$ 14	11.0	28 $\pm$ 3	301 $\pm$ 21	10.7
	60	0	48 $\pm$ 3	552 $\pm$ 27	11.5	44 $\pm$ 3	490 $\pm$ 37	11.3
	120	0	56 $\pm$ 5	552 $\pm$ 23	9.8	51 $\pm$ 5	495 $\pm$ 28	9.7
	0	2.5	37 $\pm$ 2	460 $\pm$ 26	12.3	34 $\pm$ 2	412 $\pm$ 27	12.1
	60	2.5	48 $\pm$ 3	558 $\pm$ 24	11.6	44 $\pm$ 4	502 $\pm$ 29	11.3
	120	2.5	52 $\pm$ 4	528 $\pm$ 41	10.1	48 $\pm$ 3	471 $\pm$ 38	9.9

control was seen over the $[K^+]$ range of 40–120 mM.

Secondly, Table 2 shows that variations in $[K^+]$ or $[Mg^{2+}]$ had little effect on the latency of citrate synthase, as indicated by the increase in this activity on sonication of liver or heart mitochondria for 2 min. Similarly, 40 μ M-palmitoyl-CoA (the concentration generally used in CPT assays) had little effect on citrate synthase latency (Table 2). A similar lack of effect of 40 μ M-octanoyl-CoA was also found (results not shown). It is therefore concluded that the concentrations of acyl-CoA substrates or the ionic mixtures used in these studies are unlikely to cause appreciable release of latent enzyme activities.

Results and discussion

Effects of metal ions on liver and heart carnitine acyltransferase activities

The Nbs₂-linked assay used here was a modification of that used by Harano *et al.* (1972). In the study of Harano *et al.* (1972) measurement of overt CPT in whole liver mitochondria was made under hypo-osmotic conditions (50 mM-Tris/HCl buffer, without K^+) in the presence of 5 mM-MgCl₂ and total activity (overt + latent) was measured after disruption with detergent (Lubrol). In a different system, Yates & Garland (1970) measured CPT without the use of Nbs₂ in a K^+ medium (approx. 80 mM-KCl, 20 mM-Tris/HCl buffer) and used sonication to gain access to latent hepatic CPT. The findings presented here show that, in a medium maintained at 300 mosmol/litre, CPT and COT activities are appreciably affected by $[K^+]$ and $[Mg^{2+}]$ and that,

Fig. 2. *Effects of K^+ on CPT activity in liver mitochondria*

Nbs₂-linked assays were performed with 40 μ M-palmitoyl-CoA and 400 μ M-L-carnitine in the absence of Mg^{2+} . The values are means $\pm$ S.E.M. for four experiments. ○, Whole mitochondria; ●, mitochondria after sonication (2 min); □, difference in CPT activity between sonicated and whole mitochondria.

in certain cases, the ionic composition of assay media may alter the apparent proportions of overt and latent activities.

Figs. 2 and 3 show that overt liver CPT and COT activities were increased when $[K^+]$ was raised from zero up to 60 or 90 mM. At higher $[K^+]$ the activities were decreased. The effect of $[K^+]$ on COT activity was more pronounced than that on CPT. Sonication increased the activities at all $[K^+]$, suggesting

Fig. 3. Effect of K^+ and Mg^{2+} on COT activity in liver mitochondria

Nbs₂-linked assays were performed with 40 μ M-octanoyl-CoA and 400 μ M-L-carnitine. The values are means $\pm$ S.E.M. for four experiments. O, Whole mitochondria; $\square$, whole mitochondria + 2.5 mM- $MgCl_2$; $\bullet$, sonicated mitochondria; $\blacksquare$, sonicated mitochondria + 2.5 mM- $MgCl_2$.

Fig. 4. Effect of K^+ on CPT activity in heart mitochondria

Nbs₂-linked assays were performed with 40 μ M-palmitoyl-CoA and 400 μ M-L-carnitine. The values are means $\pm$ S.E.M. for three experiments. O, Whole mitochondria; $\square$, mitochondria after sonication (2 min); $\bullet$, with Triton X-100 (0.4 mg/ml) added to the assays.

Fig. 5. Effect of Mg^{2+} on CPT and COT activities in liver mitochondria

Nbs₂-linked assays were performed in the presence of 40 mM- K^+ , 40 μ M-palmitoyl-CoA or 40 μ M-octanoyl-CoA and 400 μ M-L-carnitine. The values are means $\pm$ S.E.M. for four experiments. O, CPT activity with whole mitochondria; $\square$, COT activity with whole mitochondria; $\bullet$, CPT activity after treatment (2 min) of mitochondria with Triton X-100 (0.4 mg/ml).

hand it may be calculated from Fig. 3 that latent COT is increased and then decreased with increasing $[K^+]$, with a broad optimum between 30 and 90 mM- K^+ .

Fig. 4 shows that K^+ up to 60–80 mM increased overt heart CPT activity, but at higher KCl concentrations the activity was diminished. The effect of K^+ on heart CPT was considerably greater than the effect of this ion on liver CPT (compare Figs. 2 and 4).

These effects (Figs. 2–4) were not specific to K^+ or Cl^- , since both NaCl and potassium methanesulphonate had similar stimulatory/inhibitory effects on liver CPT and COT and on heart CPT (results not shown).

Overt liver CPT activity was decreased by Mg^{2+} in the presence of 40 mM- K^+ (Fig. 5), in the absence of K^+ (Table 3) and also over a wide range of $[K^+]$ from zero to 80 mM (results not shown). Effects of K^+ and Mg^{2+} on overt hepatic CPT activity were not confined to the Nbs₂-linked assay. With the radiochemical assay, an increase in $[K^+]$ from zero to 40 mM ($[Mg^{2+}] = 0$) resulted in a 65% increase in CPT ($P < 0.05$, mean of four experiments). Similarly, Mg^{2+} still inhibited CPT significantly by 12% in the radiochemical assay ($P < 0.01$, mean of four experiments). Overt heart CPT, however, was increased by Mg^{2+} . In the presence of 40 mM- K^+ ,

the presence of latent COT as well as CPT. Latent CPT (the difference between the total and the overt activity) was constant from 0 to 90 mM- K^+ but was increased appreciably by higher $[K^+]$. On the other

Table 3. Effects of K^+ , Mg^{2+} and Triton X-100 on CPT and COT activities in liver mitochondria

Nbs₂-linked assays were performed with whole mitochondria or 2 min after addition of Triton X-100 in the presence of 40 μ M-palmitoyl-CoA or octanoyl-CoA and 400 μ M-L-carnitine. The values are means $\pm$ S.E.M. for four experiments. The values in parentheses indicate the percentage change in activity on treatment with Triton X-100 (0.4 mg/ml). ***, ** indicate $P < 0.05$, < 0.01 , < 0.001 respectively for effects of K^+ , †, †† indicate $P < 0.01$, < 0.001 respectively for effects of Mg^{2+} (paired differences).

K^+ (40 mM)	Mg^{2+} (5 mM)	Carnitine acyltransferase activities (nmol/min per mg of protein)	
		COT	CPT
—	—	1.35 $\pm$ 0.03 (344 $\pm$ 6%)	5.23 $\pm$ 0.37 (+62 $\pm$ 10%)
—	+	8.86 $\pm$ 0.67†† (-21 $\pm$ 4%)	2.10 $\pm$ 0.12†† (+346 $\pm$ 43%)
+	—	5.33 $\pm$ 0.18*** (+1 $\pm$ 0%)	8.24 $\pm$ 0.63* (+23 $\pm$ 24%)
+	+	10.18 $\pm$ 0.96**† (-55 $\pm$ 3%)	3.86 $\pm$ 0.32**† (+153 $\pm$ 27%)

2.5 mM-MgCl₂ increased heart CPT by approx. 50% and 0.25 mM-MnCl₂ also slightly stimulated CPT (Fig. 6). In this Nbs₂-linked assay, higher concentrations of Mg^{2+} , or particularly Mn^{2+} , were inhibitory (Fig. 6). Other experiments, with the radiochemical assay (results not shown), indicated that these inhibitory effects of higher concentrations of Mg^{2+} or Mn^{2+} on overt heart CPT were only seen when Nbs₂ was present.

Liver overt COT activity, unlike liver CPT, but like heart CPT, was increased by Mg^{2+} (Figs. 3 and 5, Table 3). This effect was most pronounced at zero $[K^+]$ (Fig. 3, Table 3). The effects of K^+ and Mg^{2+} on COT appeared to be mutually exclusive in that no Mg^{2+} effect was seen in the presence of high $[K^+]$ and no stimulatory effect of K^+ was seen in the presence of Mg^{2+} . Mg^{2+} has similar effects on the total COT obtained on sonication of the mitochondria (Fig. 3), and it may be calculated that Mg^{2+} causes a small increase in the latent COT activity over the $[K^+]$ range of 0–90 mM.

Fig. 5 and Table 3 show that the effects of Mg^{2+} and K^+ on hepatic CPT are lost on treatment of the mitochondria with Triton X-100 (0.4 mg/ml). This detergent concentration was used since it was the lowest that totally abolishes the effect of Mg^{2+} (Fig. 7) and, on addition to the assay mixtures, appeared satisfactorily to disrupt or dissolve much of the mitochondria, rapidly resulting in stable assay conditions. As a consequence of the loss of ionic effects in the presence of detergent, the apparent ratio of latent/overt hepatic CPT activity is very dependent on the ionic conditions used for measure-

Fig. 6. Effects of Mg^{2+} and Mn^{2+} on CPT activity in heart mitochondria

Nbs₂-linked assays were performed with whole mitochondria in the presence of 40 mM- K^+ with 40 μ M-palmitoyl-CoA and 400 μ M-L-carnitine. The values are means $\pm$ S.E.M. for three experiments. O, Without Mn^{2+} and Mg^{2+} ; ●, with Mg^{2+} ; ■, with Mn^{2+} .

Fig. 7. Effect of Triton X-100 on CPT activity in liver mitochondria

Nbs₂-linked assays were performed in the presence of 40 mM- K^+ , 40 μ M-palmitoyl-CoA and 400 μ M-L-carnitine. The indicated concentrations of Triton X-100 were added 2 min before the start of the assays. The values are means $\pm$ S.E.M. for four experiments. O, $[Mg^{2+}] = 0$; ●, $[Mg^{2+}] = 2.5$ mM.

ment of the overt activity. From Table 3 it may be calculated that this ratio is: with $[K^+] = 0$ and $[Mg^{2+}] = 0$, 0.63; with $[K^+] = 40$ mM and $[Mg^{2+}] = 0$, 0.18; with $[K^+] = 0$ and $[Mg^{2+}] = 2.5$ mM, 3.51; with $[K^+] = 40$ mM and $[Mg^{2+}] = 2.5$ mM, 1.54. With $[K^+] = 40$ mM and $[Mg^{2+}] = 0$, the increase in measured CPT activity on Triton treatment is approximately the same as that after sonication (compare Table 3 and Fig. 2).

Table 3 shows that the effect of Triton X-100 on hepatic COT activity was also dependent on the ionic composition of the assays and clearly differed from the effect of the detergent on the hepatic CPT assay. Whereas the relative effect of the detergent to increase the measured CPT was greatest in the presence of Mg^{2+} , the opposite was found for the COT activity.

With the Nbs_2 -linked assay with 0–100 mM- K^+ , sonication or treatment with Triton X-100 (at an optimum concentration of 0.4 mg/ml) gave little increase in heart CPT activity over that seen with whole mitochondria (Fig. 4). In some instances increases in activity of up to 50% were observed after sonication and assay with 40 mM- K^+ . Effects of Triton X-100 were rather variable, sometimes giving small increases and sometimes small decreases in heart CPT activity. With the radiochemical assay for CPT (Table 4) it was also shown that this apparent lack of an extensive latent CPT activity cannot be attributed to the presence of Nbs_2 in assays (at 60 mM- K^+). Essentially the same results are obtained if the radiochemical assay is used with sonicated mitochondria with or without 0.5 mM-dithiothreitol for thiol-group protection (results not shown). It is therefore concluded either that a relatively large proportion of the total CPT activity in heart mitochondria is overt or that none of the range of assay conditions employed adequately measure the latent activity. Table 4 shows that the effect of high $[K^+]$ to inhibit CPT is dependent on the presence of Nbs_2 (or that inhibitory effects of

Nbs_2 are dependent on high ion concentrations). In the presence of Triton X-100, CPT was less subject to inhibition by high $[K^+]$ (see Fig. 4), or alternatively Triton X-100 protected against inhibitory effects of Nbs_2 which are enhanced when $[K^+]$ is raised.

In summary, it is shown that the three carnitine acyltransferase activities measured here are influenced considerably, but differently, by the ionic composition of assay media. In turn, the extent of these effects may be influenced by the type of assay used. At present it is not established whether these ions affect the activity of enzymes directly or do this indirectly by first perturbing membrane structure. In this respect it is noteworthy that Triton treatment abolished ionic effects on hepatic CPT. Furthermore, the inhibitory effect of malonyl-CoA on hepatic CPT is also lost on release of the enzyme from membrane association (McGarry *et al.*, 1978). It is likely that it is necessary to study the regulation of these activities *in situ* in mitochondria or at least in association with membrane structures. The findings presented here may be pertinent to such studies.

Effect of malonyl-CoA on heart mitochondria

With the Nbs_2 -linked assay, it was found (Fig. 8) that heart overt CPT activity was extremely sensitive to inhibition by malonyl-CoA. The effect of this metabolite was seen in the presence and absence of Mg^{2+} , and in both cases 50% inhibition was obtained with 1–2 μ M-malonyl-CoA. By using similar Nbs_2 -linked assays with liver mitochondria from the same population of rats, at least 10-fold higher malonyl-CoA concentrations were needed to obtain the same degree of inhibition of CPT (results not shown). In the absence of Mg^{2+} a plot of $100 \times (\text{percentage inhibition of CPT})^{-1}$ versus $[\text{malonyl-CoA}]^{-1}$ was linear ($r = 0.999$) with an intercept on the ordinate at 1.08, implying that 93% of the overt CPT activity could be inhibited by

Table 4. *Effects of K^+ , Triton X-100 and Nbs_2 on CPT activity in heart mitochondria*

Radiochemical assays were performed with 40 μ M-palmitoyl-CoA and 400 μ M-L-carnitine. The values are means $\pm$ S.E.M. for four experiments. The values in parentheses indicate the percentage effects of Nbs_2 and those in brackets the percentage effects of Triton X-100. *, **, *** indicate $P < 0.05$, < 0.01 , < 0.001 respectively.

K^+ (mM)	Triton X-100 (0.4 mg/ml)	Nbs_2 (0.1 mM)	CPT activity (nmol/min per mg of protein)	
60	—	—	11.9 $\pm$ 0.3	
	+	—	14.1 $\pm$ 0.1	[+18 $\pm$ 2%]**
135	—	—	14.2 $\pm$ 0.1	
	+	—	14.3 $\pm$ 0.4	[0 $\pm$ 1%]
60	—	+	(−31 $\pm$ 4%)**	8.3 $\pm$ 0.6
	+	+	(−14 $\pm$ 1%)***	12.1 $\pm$ 0.1
135	—	+	(−67 $\pm$ 1%)***	4.8 $\pm$ 0.1
	+	+	(+3 $\pm$ 1%)	14.8 $\pm$ 0.3
				[+211 $\pm$ 5%]***

Fig. 8. Effect of malonyl-CoA on overt CPT activity in heart mitochondria

Nbs₂-linked assays were performed with whole mitochondria in the presence of 40 mM-K⁺, 40 μM-palmitoyl-CoA and 400 μM-L-carnitine. The values are means of two experiments. ●, With 2.5 mM-MgCl₂; ○, without MgCl₂.

malonyl-CoA. In rat liver it has been concluded that only the overt form of CPT is sensitive to malonyl-CoA (McGarry *et al.*, 1978).

Fig. 9 demonstrates that 5 μM-malonyl-CoA induced a considerable sigmoidicity into the relationship between CPT activity and palmitoyl-CoA concentration. In liver mitochondria, the inhibitory effect of malonyl-CoA appears to require association of CPT with membranes (McGarry *et al.*, 1978). Inclusion of Triton X-100 in the assay of heart CPT abolished the effect of 5 μM-malonyl-CoA (Fig. 9).

Fig. 10 shows that the inhibitory effect of 5 μM-malonyl-CoA on heart CPT could not be overcome by increasing the carnitine concentration. Double-reciprocal plots suggested a hyperbolic relationship between CPT activity and [carnitine] in the presence and absence of malonyl-CoA.

After sonication or treatment of heart mitochondria with Triton X-100, considerable carnitine acetyltransferase activity was released. Commercial malonyl-CoA preparations appear to contain sufficient acetyl-CoA as a contaminant to make it impossible to investigate the effect of malonyl-CoA on CPT, measured by the Nbs₂-linked assay, after mitochondrial disruption. The same problem, however, does not apply to the radiochemical assay of the enzyme. In an experiment using this assay, treatment of intact heart mitochondria with Triton X-100 (0.4 mg/ml) increased the measured CPT

Fig. 9. Effect of palmitoyl-CoA on CPT activity in heart mitochondria

Nbs₂-linked assays were performed in the presence of 40 mM-K⁺ and 400 μM-L-carnitine either with whole mitochondria (○, ●) or with Triton X-100 (0.4 mg/ml) added to the assays (□, ■). The values are means ± S.E.M. for three experiments. ●, ■, with 5 μM-malonyl-CoA; ○, □, without malonyl-CoA.

Fig. 10. Effect of carnitine on overt CPT activity in heart mitochondria

Nbs₂-linked assays were performed with whole mitochondria in the presence of 40 mM-K⁺ and 40 μM-palmitoyl-CoA. The values are means of four experiments and are shown as double-reciprocal plots. ●, With 5 μM-malonyl-CoA; ○, without malonyl-CoA.

activity from 10.2 ± 0.5 to 13.3 ± 0.6 nmol/min per mg of protein. In the absence of Triton, 20 μM- and 200 μM-malonyl CoA decreased CPT activity by $82 \pm 2\%$ and $87 \pm 3\%$ respectively. In the presence of

Triton, the corresponding inhibitions in response to these malonyl-CoA concentrations were $26 \pm 2\%$ and $39 \pm 3\%$ (means $\pm$ s.e.m. for four experiments in each case). With intact mitochondria all the CPT activity was sedimentable by centrifugation for 2 min at $9000g_{av}$ in an Eppendorf 5412 centrifuge. After 2 min of treatment with Triton X-100 (0.4 mg/ml) none of the CPT activity was then sedimentable by this treatment (results not shown). The implication is that 'solubilization' considerably decreases, but does not abolish, sensitivity of heart CPT to malonyl-CoA. The findings of Fig. 4 discussed above suggest that detergent treatment or sonication are unlikely to release large amounts of latent CPT, which, in liver, is suggested to be insensitive to malonyl-CoA (McGarry *et al.*, 1978).

In conclusion, heart mitochondria appear to contain a CPT activity that differs in sensitivity to metal ions and in relative proportions of latent and overt forms from that found in liver. The overt form of the heart enzyme shows a high sensitivity to inhibition by malonyl-CoA. The malonyl-CoA content in heart is not known. If malonyl-CoA is present to any extent in this tissue under any normal or pathological condition, it could constitute a potent regulator of cardiac fatty acid oxidation.

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