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Quantitation of the Effect of L-Carnitine on the Levels of Acid-soluble Short-chain Acyl-CoA and CoASH in Rat Heart and Liver Mitochondria*

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The steady state levels of mitochondrial acyl-CoAs produced during the oxidation of pyruvate, α-ketoisovalerate, α-ketoisocaproate, and octanoate during state 3 and state 4 respiration by rat heart and liver mitochondria were determined. Addition of carnitine lowered the amounts of individual short-chain acyl-CoAs and increased CoASH in a manner that was both tissue- and substrate-dependent. The largest effects were on acetyl-CoA derived from pyruvate in heart mitochondria using either state 3 or state 4 oxidative conditions. Carnitine greatly reduced the amounts of propionyl-CoA derived from α-ketoisovalerate, while smaller effects were obtained on the branched-chain acyl-CoA levels, consistent with the latter acyl moieties being poorer substrates for carnitine acetyltransferase and also poorer substrates for the carnitine/acylcarnitine translocase. The levels of acetyl-CoA in heart and liver mitochondria oxidizing octanoate during state 3 respiration were lower than those obtained with pyruvate. The rate of acetylcarnitine efflux from heart mitochondria during state 3 (with pyruvate or octanoate as substrate, in the presence or absence of malate with 0.2 mM carnitine) shows a linear response to the acetyl-CoA/CoASH ratio generated in the absence of carnitine. This relationship is different for liver mitochondria. These data demonstrate that carnitine can modulate the aliphatic short-chain acyl-CoA/CoA ratio in heart and liver mitochondria and indicate that the degree of modulation varies with the aliphatic acyl moiety.

Following the discovery of carnitine acetyltransferase in mitochondria, evidence was presented that this enzyme is involved in buffering the acetyl-CoA/CoASH ratio in mitochondria (1, 2). Subsequently, it was established that carnitine can be esterified to other aliphatic short-chain acyl residues (3–5) and that tissues contain a spectrum of acylcarnitines (6). These findings led to suggestions of several roles for carnitine, including indirect roles (7–9) such as reduction of inhibitory levels of specific acyl-CoAs via conversion to acylcarnitines. The significance of such a role in branched-chain keto acyl metabolism has been debated because of the low steady state levels of branched-chain acyl-CoAs in mitochondria (10).

Many studies with isolated mitochondria using a variety of incubation conditions have shown carnitine lowers acetyl-CoA levels in heart and liver mitochondria (see Ref. 11 for review); however, other studies indicate that carnitine is both metabolically sluggish (12) and that it either does not alter or it actually increases acetyl-CoA levels (13). The latter findings are not consistent with the hypothesis that carnitine modulates acetyl-CoA levels in the mitochondrial matrix.

With the recognition that systemic carnitine deficiency is associated with some disease states which involve impaired or compromised short-chain acyl-CoA metabolism, the effect of carnitine on the metabolic fate of specific acyl-CoAs derived from different substrates has taken on additional significance (9). The effects of carnitine deficiency on physiological states are consistent with the proposal that carnitine buffers acyl-CoA/CoA ratios. However, a systematic investigation of how carnitine affects specific acyl-CoA levels in mitochondria is lacking. We demonstrated recently (14) that the addition of 0.2 mM L-carnitine promotes the efflux of considerable quantities of specific aliphatic acylcarnitines from liver and heart mitochondria during state 3 respiration in a tissue- and substrate-dependent manner. Herein we have quantitated the amounts of individual acyl-CoAs contained in heart and liver mitochondria while oxidizing different substrates in the presence and absence of carnitine, thereby determining the effect of L-carnitine on specific acyl-CoA/CoA ratios. The magnitude of matrix acetyl-CoA/CoASH ratios in the absence of carnitine correlates linearly with the rate of efflux of acetylcarnitine in the presence of carnitine for heart, but not for liver mitochondria.

MATERIALS AND METHODS

Chemicals—The following substances were obtained from the indicated sources: octanoic acid (Eastman), chloroform (J. T. Baker Chem. Co.), methanol (Mallinckrodt Chemical Works), CoA and its derivatives (P. L. Biochemicals). L-Carnitine was a gift from Sigma Tau (Rome, Italy). All other chemicals were from Sigma.

Animals and Preparation of Mitochondria—Male Sprague-Dawley rats weighing 180–300 g were used. Liver mitochondria were prepared from animals fasted for 24 h as previously described (14); heart mitochondria were prepared from fed animals according to the method of Toth *et al.* (15).

Incubation Conditions—The reactions were conducted at 25 °C and pH 7.40 on a rapidly oscillating (approximately 130 rpm) Dubnoff Metabolic Shaker. The incubation media contained in a final volume of 300 μl: 115 mM KCl, 10 mM HEPES[1], 10 mM potassium phosphate, 2 mM $MgCl_2$, 17 mM sucrose, and 3–4 mg of mitochondrial protein. Pyruvate and octanoate were 2.5 mM, 2-oxoisocaproate and 2-oxoiso-

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[1] The abbreviations used are: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high pressure liquid chromatography.

valerate were 1.0 mM, L-malate was 0.2 mM, state 3 respiration was induced by the addition of 750 μmol of ADP. The reactions were started by the addition of mitochondria and stopped after 1 min with 50 μl of 15.7% $HClO_4$ containing 2 mM dithiothreitol. The samples were centrifuged for 1 min in an Eppendorf centrifuge, and 5 M K_2CO_3 was added to bring the supernatant fluids to pH 4–5. After centrifugation, the supernatant fluids were passed through 0.2-μm cellulose membranes before chromatography. All analyses were completed within 48 h after incubation.

Separation and Quantitation of Acyl-CoAs—Separation of acyl-CoAs was performed with a Waters HPLC system using a Spherisorb S-5 ODS-2 (25 cm × 4.6 mm) column from Altex Science, Inc. (Berkeley, CA). The UV detector was operated at 254 nm and set at 0.05 sensitivity according to DeBuseyre and Olson (16), with minor modifications. A mobile phase of 220 mM potassium phosphate containing 0.05% dithioglycol (A) and 98% methanol, 2% chloroform (B) was used. The flow rate was 0.4 ml/min and the gradient was as follows: at zero time, 94% A and 6% B; at 8 min, 92% A and 8% B; at 14 min, 87% A and 13% B; at 25 min, 80% A and 20% B; at 40 min, 55% A and 45% B; at 45 min, 55% A and 45% B; and at 60 min, 94% A and 6% B. Mitochondrial extracts corresponding to approximately 0.5 mg of mitochondrial protein from liver and 1 mg from heart were injected. Fig. 1 shows the separation of standard acyl-CoAs, as well as a representative sample from liver and heart mitochondria. Identification of specific peaks was done by comparison with chromatograms of known acyl-CoA standards. Dephospho-CoA was used as an internal standard. Control experiments demonstrated a linear relationship between the amount of dephospho-CoA added (0.2–1.5 nmol) to samples and the area of the integrated peaks obtained after HPLC separation (data not shown). As shown in Fig. 1*B*, dephospho-CoA was negligible in the mitochondria extracts and is separated from other acid-soluble acyl-CoAs of liver; negligible absorbance at 254 nm in the dephospho-CoA region was also seen with extracts from heart mitochondria. Normally, 1 nmol of dephospho-CoA was used. Pilot studies show that the reproducibility is ± 8%.

Other—Protein was determined by the Coomassie Blue G-250 method of Sedmak and Grassberg (17). Statistical calculations were performed using the Student's *t* test for paired data.

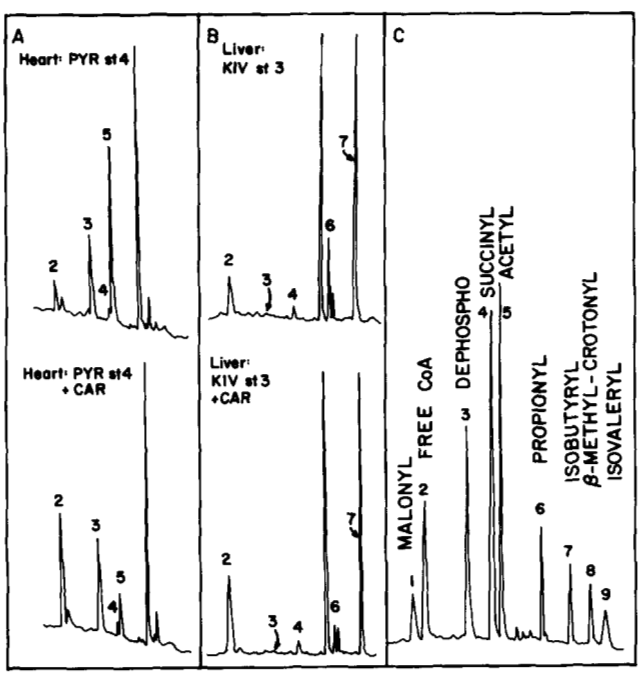

FIG. 1. **HPLC separations of standard and representative experimental acyl-CoAs.** *A*, acid-soluble acyl-CoAs of mitochondria oxidizing pyruvate in the absence (*upper*) and presence (*lower*) of 5 mM L-carnitine, no ADP, 2.5 mM [2-^{14}C]pyruvate; incubation 1 min at 25 °C. The injected volume corresponds to about 1 mg of mitochondrial protein. Dephospho-CoA (0.75 nmol). *B*, acid-soluble acyl-CoAs from rat liver mitochondria oxidizing 2-oxoisovalerate. Substrates: 1.0 mM 2-oxoisovalerate, 0.2 mM L-malate, 2.5 mM ADP; incubation 1 min at 25 °C. The injected volume corresponds to about 0.5 mg of mitochondrial protein. *KIV*, α-ketoisovalerate. *C*, separation of the standard mixture of CoA derivatives. The mixture contained 0.5–3.5 nmol of CoA derivatives. The retention times in minutes were: *1*, malonyl-CoA, 33.94; *2*, free CoA, 36.00; *3*, dephospho-CoA, 43.48; *4*, succinyl-CoA, 47.71; *5*, acetyl-CoA, 49.24; *6*, propionyl-CoA, 56.44; *7*, isobutyryl-CoA, 61.44; *8*, β-methylcrotonyl-CoA, 64.94; and *9*, isovaleryl-CoA, 67.66.

RESULTS

Previous data (14, 18) demonstrated that rat heart and liver mitochondria have the capacity to efflux considerable quantities of short-chain aliphatic acylcarnitines. In those studies, low concentrations (0.2 mM) of L-carnitine were used. Subsequent investigations demonstrated that maximum efflux rates for acetylcarnitine and other acylcarnitines require carnitine concentrations greater than 1.0 mM, especially with heart mitochondria. Therefore, for these studies, higher concentrations (usually 5.0 mM) were used, except for the studies where the acetylcarnitine efflux rates obtained previously were correlated with the acetyl-CoA/CoASH ratio. The experimental conditions used previously to quantitate the efflux of individual acylcarnitines were also used to obtain the data for Tables I–V, except that 5 mM rather than 0.2 mM L-carnitine was used to obtain the maximum effects of L-carnitine on acyl-CoA levels. The data shown in Fig. 1,*A* and *B*, are representative of the acid-soluble acyl-CoA profiles obtained with heart and liver mitochondria oxidizing specific substrates. Fig. 1*C* shows that the major water-soluble acyl-CoAs of mitochondria derived from the oxidation of individual substrates are resolved by the gradient. Fig. 1*B* also shows that dephospho-CoA, the internal standard, is very low or absent in the acid-soluble extracts of mitochondria.

The Effect of 5.0 mM L-Carnitine on CoASH and Acetyl-CoA Levels Derived from Pyruvate—The effect of carnitine on the intramitochondrial concentrations of CoASH and acetyl-CoA in rat heart and liver mitochondria oxidizing pyruvate during state 3 and state 4 conditions are given in Table I. 5.0 mM L-carnitine was used in these experiments because other studies (data presented elsewhere)[2] demonstrated that the maximum efflux of acetylcarnitine from heart mitochondria oxidizing pyruvate required 2.0–5.0 mM L-carnitine. For heart mitochondria, addition of carnitine increased both CoASH levels and reduced the amounts of acetyl-CoA, resulting in an approximately 10-fold change in the CoASH/acetyl-CoA ratio.

In contrast, experiments with liver mitochondria gave a smaller increase in the CoASH levels and a smaller decrease in the acetyl-CoA levels with an overall change in the CoASH/acetyl-CoA ratio of less than 2.

With heart mitochondria oxidizing pyruvate, the addition of carnitine consistently caused a reduction in the total acid-soluble CoA pool. The cause of this reduction was not investigated, but it may indicate that a larger percentage of the total coenzyme A pool has become associated with protein (acid-insoluble).

For the experiments in which the effect of 5.0 mM carnitine on acyl-CoA levels was investigated, high concentrations of mitochondria in a small volume (0.3 ml) were used at 25 °C. Even though samples were shaken *vigorously*, O_2 might be limiting at the end of 1 min, especially with pyruvate during state 3. Therefore, two experiments were performed to determine if this occurred. In the first, the rate of acetylcarnitine efflux with pyruvate and 5.0 mM carnitine was determined for 1 and 2 min. The rate at 2 min was identical to the rate at 1 min; the total efflux at 2 min was twice that for 1 min.

[2] *Int. J. Nutr.* (1987), in press.

In the second experiment, the rate of acetylcarnitine production from pyruvate +5.0 mM carnitine was determined using both the 0.3-ml and the 1.0-ml incubation volumes with the same total amount of mitochondrial protein. The rate of acetylcarnitine production per mg of protein for both was, within experimental error, identical. If oxygen was limiting for either of the above-mentioned experiments, flux through pyruvate dehydrogenase would be reduced, with a concomitant reduction in acetylcarnitine production. This did not occur.

The Effect of L-Carnitine on the Acyl-CoA Levels in Mitochondria Oxidizing α-Ketoisovalerate or α-Ketoisocaproate—Experiments similar to those described for Table I were performed with α-ketoisovalerate and α-ketoisocaproate in the presence of malate. Malate was added because it supports respiration, particularly state 3 with the branched-chain α-keto acid substrates, without decreasing the efflux of individual acylcarnitines (14, 18). As shown in Tables II and III, addition of carnitine to heart mitochondria oxidizing either α-ketoisovalerate or α-ketoisocaproate had little effect on the amount of CoASH, but it reduced by 60% or more the levels of propionyl-CoA and isobutyryl-CoA during state 3 respiration, thereby causing a significant change in the CoASH/propionyl- or isobutyryl-CoA ratios as shown in Table IV. In contrast, addition of L-carnitine to liver mitochondria oxidizing α-ketoisovalerate (Table II) and α-ketoisocaproate (Table III) caused a 3-fold increase in CoASH levels during state 3, as well as a large decrease in propionyl-CoA levels. Although the unknown acyl-CoA derived from α-ketoisocaproate was not identified, it most likely is β-methylglutaconyl-CoA, a carboxylation intermediate in the metabolism of α-ketoisocaproate. The levels of the unknown acyl-CoA were lower when carnitine was added to either heart or liver mitochondria during either state 3 or state 4 respiration. We previously did not detect significant amounts of any unidentified acylcarnitine when α-ketoisocaproate was the substrate for heart or liver mitochondria (14, 18); consequently, the unidentified acyl-CoA may not be a substrate or is a poor substrate for carnitine acetyltransferase.

The Effect of Carnitine on Acyl-CoA Levels during Octanoate Oxidation—The effects of carnitine on the acetyl-CoA levels in heart mitochondria oxidizing octanoate in the presence of malate are shown in Table V. The amounts of acetyl-CoA, particularly the amounts for state 3 in the absence of carnitine, were lower than for pyruvate (compare 0.05 ± 0.01 nmol/mg of protein from Table V with 1.00 ± 0.14 nmol/mg of protein from Table I). Since the small efflux of acetylcarnitine found previously during octanoate oxidation and the low level of acetyl-CoA was not expected, experiments were done to determine if the concentration of octanoate affects both the rates of acetylcarnitine production and the steady state acetyl-CoA levels in heart mitochondria. Reducing the concentration of octanoate from 2.5 to 0.5 mM and less increased production of acetylcarnitine from 2 to 6 nmol/min/mg of protein and nearly doubled the acetyl-CoA levels (compare experiments B and C of Table V). In another series of experiments with heart mitochondria, using the conditions for Table V, but 1.0 mM L-carnitine and 40 μM palmitoyl-CoA, efflux rates up to 20 and 37 nmol/min/mg of protein were obtained for state 3 and state 4, respectively. These incubation conditions gave maximum O_2 consumption rates for state 3 (data not shown) and were the only experiments utilizing 1.0 mM L-carnitine.

DISCUSSION

Effect of Carnitine on the Acetyl-CoA/CoASH Ratio—The data in Tables I and V show that 5.0 mM carnitine decreased the acetyl-CoA/CoASH ratio approximately 10-fold when either pyruvate or octanoate are substrates for heart mitochondria; smaller changes in the ratio were found for liver mitochondria. The experimental conditions used for the control acetyl-CoA levels and CoASH levels for Tables I–V (in the absence of carnitine) were the same as the ones used for the quantitation of acetylcarnitine efflux reported in a previous publication (14), except that carnitine was omitted. When the acetyl-CoA/CoASH ratios in the presence and absence of malate with either pyruvate or octanoate (no added carnitine) are plotted *versus* the efflux rate of acetylcarnitine from heart and liver mitochondria in the presence of *0.2 mM L-carnitine*, a linear relationship between the acetyl-CoA/CoASH ratio and the rate of acetylcarnitine efflux is apparent with heart mitochondria over a broad range in the magnitude of the ratio (see Fig. 2*B*). Although a linear relationship between the acetyl-CoA/CoA ratio in heart mitochondria is observed, a lag is evident in Fig. 2*A* which is most likely due to preferential utilization of acetyl-CoA for the Krebs cycle at low concentrations of acetyl-CoA. Such a preference has been shown for liver mitochondria (19). When the acetylcarnitine efflux rate is plotted *versus* the acetyl-CoA/mg of mitochondrial protein (Fig. 2*A*), different responses are evident for heart and liver mitochondria; acetylcarnitine efflux reaches a plateau with liver, but not heart mitochondria. Although the experimental conditions were not optimal for acetylcarnitine production (0.2 mM carnitine was used), the fact that acetylcarnitine production plateaus for liver mito-

TABLE I

Effect of L-carnitine on acid-soluble acyl-CoA levels in rat heart and liver mitochondria oxidizing pyruvate

The conditions are: pyruvate = 2.5 mM, ADP = 2.5 mM for state 3, incubation 1 min, 25 °C; carnitine = 5 mM. The statistical calculations were made according to Student's *t* test for paired data. *p* = in comparison to "no carnitine." *p** = in comparison to state 4 "no carnitine." Data are presented as mean ± S.E.

Conditions	*n*	CoASH	Succinyl-CoA	Acetyl-CoA	CoASH/acetyl-CoA
		nmol/mg protein			
Heart state 4	6	0.40 ± 0.12	0.09 ± 0.01	1.47 ± 0.14	0.27
Heart + carnitine	6	1.18 ± 0.20	0.10 ± 0.05	0.38 ± 0.06	3.10
		$p < 0.02$*		$p < 0.001$*	
Heart state 3	6	0.54 ± 0.17	0.08 ± 0.01	1.00 ± 0.14	0.54
Heart + carnitine	6	0.89 ± 0.20	0.07 ± 0.01	0.14 ± 0.02	6.36
		$p < 0.01$		$p < 0.001$	
Liver state 4	4	1.27 ± 0.18	0.03 ± 0.01	1.53 ± 0.04	0.82
Liver + carnitine	4	1.49 ± 0.12	0.03 ± 0.01	1.30 ± 0.07	1.17
		$0.05 < p < 0.1$*		$p < 0.01$*	
Liver state 3	4	1.28 ± 0.09	0.18 ± 0.04	1.09 ± 0.06	1.18
Liver + carnitine	4	1.44 ± 0.13	0.15 ± 0.04	0.76 ± 0.03	1.89
		$p < 0.05$		$p < 0.001$	

TABLE II

Effect of L-carnitine on acid-soluble acyl-CoA levels in rat heart and liver mitochondria oxidizing α-ketoisovalerate

The conditions are: α-ketoisovalerate = 1.0 mM, L-malate = 0.2 mM, ADP = 2.5 mM for state 3, incubation 1 min, 25 °C; carnitine = 5.0 mM. The results are expressed as mean ± S.E.

Conditions	*n*	CoASH	Succinyl-CoA	Acetyl-CoA	Propionyl-CoA	Isobutyryl-CoA
				nmol/mg protein		
Heart state 4	4	0.61 ± 0.26	0.11 ± 0.03	<0.01	0.02 ± 0.01	0.44 ± 0.11
Heart + carnitine	4	0.70 ± 0.26	0.19 ± 0.08	<0.01	0.0	0.23 ± 0.12
		$p < 0.5$				$p < 0.01$
Heart state 3	4	0.53 ± 0.19	0.02 ± 0.01	<0.01	0.26 ± 0.04	0.56 ± 0.18
Heart + carnitine	4	0.79 ± 0.28	0.06 ± 0.02	<0.01	0.0	0.17 ± 0.05
						$p < 0.01$
Liver state 4	4	0.47 ± 0.12	<0.01	0.34 ± 0.05	<0.01	1.54 ± 0.12
Liver + carnitine	4	0.77 ± 0.05	<0.01	0.35 ± 0.10	<0.01	1.21 ± 0.17
		$p < 0.02$				$p < 0.05$
Liver state 3	4	0.24 ± 0.12	<0.01	<0.01	0.29 ± 0.09	1.80 ± 0.13
Liver + carnitine	4	0.73 ± 0.09	<0.01	<0.01	0.07 ± 0.06	1.43 ± 0.14
		$p < 0.001$			$p < 0.001$	$p < 0.001$

TABLE III

Effect of L-carnitine on acid-soluble acyl-CoA levels in rat heart and liver mitochondria oxidizing α-ketoisocaproate

The conditions are: α-ketoisocaproate = 1.0 mM, L-malate = 0.2 mM, carnitine = 5.0 mM, ADP = 2.5 mM for state 3, incubation 1 min, 25 °C. The results are expressed as mean ± S.E.

Conditions	*n*	CoASH	Succinyl-CoA	Acetyl-CoA	Unknown-CoA[a]	MC[b]	Isovaleryl-CoA
				nmol/mg protein			
Heart state 4	6	0.43 ± 0.23	0.09 ± 0.03	<0.01	0.69 ± 0.12	0.17 ± 0.02	0.12 ± 0.03
Heart + carnitine	6	0.40 ± 0.22	0.13 ± 0.04	<0.01	0.50 ± 0.08	0.11 ± 0.02	0.08 ± 0.02
					$p < 0.05$		
Heart state 3	6	0.32 ± 0.14	0.01 ± 0.01	<0.01	1.02 ± 0.14	0.19 ± 0.03	0.19 ± 0.06
Heart + carnitine	6	0.47 ± 0.25	0.03 ± 0.01	<0.01	0.69 ± 0.09	0.16 ± 0.02	0.08 ± 0.02
		$p < 0.05$			$p < 0.01$		$p < 0.01$
Liver state 4	4	0.60 ± 0.12	0.04 ± 0.02	0.31 ± 0.06	0.85 ± 0.11	0.16 ± 0.03	0.70 ± 0.06
Liver + carnitine	4	0.88 ± 0.22	0.03 ± 0.02	0.26 ± 0.09	0.52 ± 0.06	0.10 ± 0.01	0.58 ± 0.08
		$p < 0.05$		$p < 0.001$	$p < 0.02$	$p < 0.001$	$p < 0.001$
Liver state 3	4	0.33 ± 0.13	0.03 ± 0.01	0.02 ± 0.01	0.58 ± 0.06	0.07 ± 0.01	0.96 ± 0.04
Liver + carnitine	4	1.00 ± 0.30	0.03 ± 0.01	0.02 ± 0.01	0.39 ± 0.06	0.07 ± 0.02	0.69 ± 0.06
		$p < 0.01$			$p < 0.1$		$p < 0.001$

[a] Unknown = β-methylglutaconyl-CoA (?). MC = β-methylcrotonyl-CoA.

TABLE IV

Effect of L-carnitine on short-chain acyl-CoA/CoASH ratio in rat heart and liver mitochondria oxidizing branched-chain α-keto acids

The amounts of short-chain acyl-CoA and CoASH used to calculate the ratios are from Tables II and III; state 3 only.

Substrate		Propionyl-CoA/CoASH	Isobutyryl-CoA/CoASH
α-Ketoisovalerate	Heart, no carnitine	0.49	1.06
α-Ketoisovalerate	Heart + carnitine	<0.01	0.22
α-Ketoisovalerate	Liver, no carnitine	1.21	7.5
α-Ketoisovalerate	Liver + carnitine	0.10	1.9
		β-Methylglutaconyl-CoA (?)/CoASH	Isovaleryl-CoA/CoASH
α-Ketoisocaproate	Heart, no carnitine	3.19	0.59
α-Ketoisocaproate	Heart + carnitine	1.47	0.17
α-Ketoisocaproate	Liver, no carnitine	1.76	2.91
α-Ketoisocaproate	Liver + carnitine	0.39	0.69

chondria (shown in Fig. 2*A*) implies that either carnitine acetyltransferase or carnitine acetylcarnitine translocase, and possibly both enzymes, were operating near their respective V_{max} values. Rat liver mitochondria contain low amounts of carnitine acetyltransferase (20). The rate of acetylcarnitine production of approximately 3.0 nmol/min/mg of protein in liver mitochondria is close to reported values for the maximal carnitine acetyltransferase activity in rat liver mitochondria. In contrast, heart mitochondria contain much larger amounts of carnitine acetyltransferase per mg of mitochondrial protein (20), consistent with the high acetylcarnitine efflux rates. The changes in acetyl-CoA/CoASH ratios in Table I are very similar to those reported by Hansford and Cohen (21) in which they showed up to a 20-fold increase in the CoASH/acetyl-CoA ratio of rabbit heart mitochondria oxidizing pyruvate when high amounts of L-carnitine were added. The steady state acetyl-CoA levels in rabbit heart mitochondria oxidizing palmitoylcarnitine reported by Hansford and Johnson (22) are also similar to those in Table V.

Although the low amounts of carnitine acetyltransferase in liver mitochondria may limit acetylcarnitine production, the data in Tables I and V show that carnitine can reduce acetyl-CoA levels in both heart and liver mitochondria and, thus, support the suggestions of others that carnitine acetyltrans-

TABLE V

Effect of L-carnitine on free, succinyl- and acetyl-CoA levels in rat heart mitochondria oxidizing octanoate

The conditions are: 0.2 mM L-malate, 5.0 mM carnitine, 2.5 mM ADP for state 3. The results are expressed as mean ± S.E. For experiments A and B, octanoate was 2.5 mM, and for experiment C, it was 0.5 mM.

Experiment	Conditions	*n*	CoASH	Succinyl	Acetyl	CoASH/acetyl-CoA
				nmol/mg protein		
A	State 4	3	0.77 ± 0.21	0.06 ± 0.02	0.64 ± 0.10	1.2
	State 4 + carnitine	3	1.02 ± 0.39	0.12 ± 0.04	0.07 ± 0.01	14.6
B	State 3	3	0.76 ± 0.17	0.04 ± 0.02	0.05 ± 0.01	13.4
	State 3 + carnitine	3	0.72 ± 0.07	0.06 ± 0.01	0.04 ± 0.01	18.0
C	State 3	3	0.80 ± 0.01	0.05 ± 0.01	0.10 ± 0.02	8.0
	State 3 + carnitine	3	0.60 ± 0.11	0.05 ± 0.02	0.02 ± 0.01	30.0

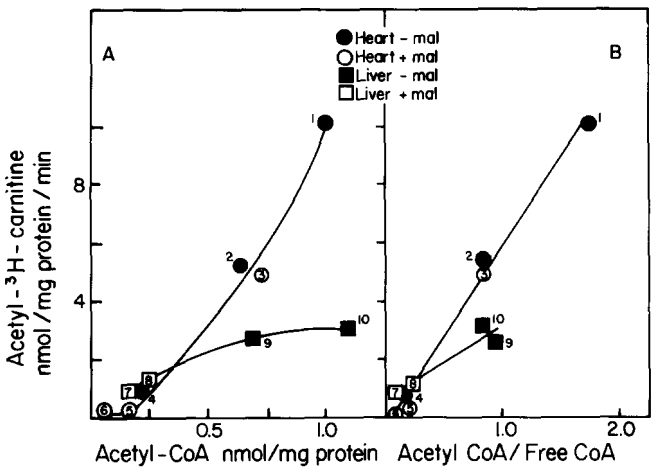

FIG. 2. **Effect of acetyl-CoA levels and the acetyl-CoA/CoASH ratio on acetylcarnitine efflux during state 3 oxidation by heart and liver mitochondria.** Data from Table I (pyruvate) and Table IV (2.5 mM octanoate), the acetyl-CoA/CoASH ratios in the *absence* of L-carnitine, were plotted against the acetyl-L-carnitine efflux promoted by addition of 0.2 mM L-carnitine. Acetylcarnitine production rates were obtained primarily from Table 4 of Ref. 14, and also from unpublished data. The *open symbols* represent data obtained in the presence of malate (*mal*), and the *closed symbols* represent data obtained in the absence of malate. The substrates for the numbered symbols were: *1*, 2.5 mM pyruvate, no malate; *2*, 0.5 mM octanoate, no malate; *3*, 2.5 mM pyruvate, +malate; *4*, 2.5 mM octanoate, no malate; *5*, 0.5 mM octanoate, +malate; *6*, 2.5 mM octanoate, +malate; *7*, 2.5 mM pyruvate, +malate; *8*, 2.5 mM octanoate, +malate; *9*, 2.5 mM octanoate, no malate; *10*, 2.5 mM pyruvate, no malate.

ferase buffers or at least modulates the acetyl-CoA/CoASH ratio in mitochondria (1, 2).

The low amounts of acetyl-CoA found in heart mitochondria oxidizing 2.5 mM octanoate during state 3 agree with previous data (14). When 40 μM palmitoyl-CoA was oxidized in the presence of 1.0 mM carnitine, much larger acetylcarnitine efflux rates were obtained, but they were not nearly as large as the efflux rates with pyruvate as substrate.

Although acetylcarnitine is derived from the β-oxidation of long-chain fatty acids (22, 23), the perfused heart systems used to investigate acetylcarnitine production contain a pyruvate precursor such as glucose (24). Recent studies by French *et al.* (25) indicate that acylcarnitines can be derived from glucose in rat heart and liver. Our data indicate that the oxidation of pyruvate could be a major source of acetylcarnitine, particularly in myocardium. In this respect, it is of interest that high levels of carnitine acetyltransferase occur in some tissues that oxidize carbohydrates rather than fatty acids, *i.e.* sperm (26), some insect flight muscles (27), and *Torulopsis bovina* (28).

Our data do not support conclusions that carnitine is metabolically sluggish (12, 13). This conclusion was based on two lines of evidence, one of which was discussed above. Injected L-carnitine caused large changes in blood acetylcarnitine without changing the steady state acetyl-CoA levels in mitochondria isolated from the animal's liver mitochondria. The failure of elevated blood carnitine to alter the steady state levels of mitochondrial acetyl-CoA is not surprising when one considers that mitochondria have a very large capacity to regenerate acetyl-CoA. Blood carnitine must cross at least two membrane barriers and must enter at least two metabolic pools (uptake from blood to cytosol and passage from cytosol to the mitochondrial matrix) before affecting matrix acetyl-CoA levels via carnitine acetyltransferase. If the rates of either of the transport processes are slower than the capacity of the mitochondria to maintain the acetyl-CoA pool, then negligible effects of elevated blood carnitine on the steady state levels of acetyl-CoA would be anticipated. The uptake rate of carnitine in isolated liver cells has a $V_{max} \cong 2.4$ nmol/min/mg of protein (29), and the V_{max} of carnitine acetyltransferase in liver mitochondria is approximately 5 nmol/min/mg of protein. Analyses of the data in Tables 1 and 2 of Ref. 12 indicate that blood carnitine is metabolically very active. For fed rats, the plasma acid-soluble (short-chain) acylcarnitines rose from 19 ± 8 to 360 ± 100 μM 1 min after carnitine injection, then it declined to 60 ± 70 μM in 5 min, followed by an increase. A similar pattern was observed with fasted rats. The rise and fall and subsequent rise of blood short-chain acylcarnitines might be expected if carnitine rapidly entered and exited a major organ with concomitant equilibration with the intracellular acetyl-CoA pool, followed by a slower uptake of carnitine and possible acetylcarnitine by another major organ. It is known that carnitine uptake in muscle is much slower than in liver (11).

Effect of Carnitine on Branched-chain Acyl-CoA and Propionyl-CoA Levels—Carnitine (5.0 mM) reduced the levels of propionyl-CoA to a much greater extent than it reduced the amounts of 4-carbon and 5-carbon branched-chain acyl-CoAs. This effect on propionyl-CoA levels is consistent with clinical studies of impaired propionate metabolism where carnitine therapy has been used to eliminate excess propionate as propionylcarnitine (30). HCO_3^- was not added in these studies; thus, carboxylation of propionyl-CoA and other acyl-CoAs was likely limited. The smaller reduction of branched-chain acyl-CoA levels by carnitine is expected when one considers that the branched-chain acyl-CoAs are poorer substrates for mitochondrial carnitine acetyltransferase than either acetyl-CoA or propionyl-CoA; and acetylcarnitine is a better substrate for the carnitine/acylcarnitine translocase than branched-chain acylcarnitines, such as isobutyrylcarnitine (31). The expected consequence of these considerations is that carnitine should have less of an impact on the intramitochondrial concentrations of the branched-chain acyl-CoAs than on acetyl-CoA or propionyl-CoA levels. Regardless, carnitine did alter the individual acyl-CoA/free CoA ratios (see Table

IV), but the degree of modulation was different for heart and liver and was dependent on the oxidative state and the branched-chain α-keto acid used as substrate.

The acyl-CoA profiles obtained with heart and liver mitochondria oxidizing α-ketoisocaproate are similar to those reported by others for these tissues (35, 36), except methylmalonyl-CoA was not found in our extracts. The accumulation of this intermediate and the products derived from it are dependent on the presence of considerable quantities of bicarbonate, a substrate that was not added to our incubation media. Thus, these studies may be representative of physiological settings in which a specific carboxylase activity is diminished or absent, presumably producing elevated levels of specific acyl-CoAs that can be lowered by available carnitine.

The stimulation of branched-chain α-keto acid and/or branched-chain amino acid oxidation by carnitine is well documented (4, 5, 7, 32–34). The limited effect of carnitine on branched-chain acyl-CoA levels may be sufficient to account for the stimulation of branched-chain amino acid metabolism by carnitine via secondary or indirect rather than direct means.

The Effects of Carnitine on Short-term Metabolic Regulation—Since these studies were performed in the absence of added HCO_3^-, the steady state levels of the acyl-CoAs that are substrates for specific carboxylase enzymes should be more representative of situations where carboxylase activity is limited or impaired. Alterations of the acetyl-CoA/CoASH, propionyl-CoA/CoASH, and branched-chain/CoASH ratios by carnitine has implications for short-term metabolite control, since most of the acyl-CoAs studied can exert allosteric effects on specific matrix enzymes subject to short-term metabolite control. In addition to the well established inhibition of pyruvate dehydrogenase by acetyl-CoA (37, 38), propionyl-CoA and isobutyryl-CoA (38) are inhibitors of pyruvate dehydrogenase and their precursor, α-ketoisovalerate, can inhibit both glucose and urea synthesis by liver cells (38). Pyruvate dehydrogenase is also limited by isovaleryl-CoA (39), an intermediate in leucine catabolism. Other studies have shown that isobutyryl-CoA forms an inhibitory complex with α-ketoglutarate dehydrogenase (40) and the precursor of isobutyryl-CoA, α-ketoisovalerate, inhibits both pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Isovaleryl-CoA can also inhibit pyruvate carboxylase (see Fig. 12-2 of Ref. 41). A more comprehensive discussion of the regulatory effects of branched-chain acyl-CoAs in the mitochondria matrix has been presented by Williamson *et al.* (42). Our data support the conclusions of others about the effect of carnitine on branched-chain amino acid metabolism in muscle (43, 44).

It is difficult to determine whether the concentrations of carnitine used herein (0.2 mM for Fig. 2 and 5.0 mM for Tables I–V) represent concentrations that occur in either heart or liver. L-Carnitine levels in liver are generally between 200–400 and 700–2000 μmol/kg in heart. However, due to bound water and potential binding to macromolecules, the concentration free in solution may be quite different than the total carnitine of the tissue. Clearly, 0.2 mM carnitine is below normal physiological levels in heart, but it may represent the lower range of normal in liver, while 5.0 mM may be close to normal for heart, but high for liver. For both tissues, the use of high carnitine (5.0 mM) may be within the clinical or pharmacological range that occurs in humans receiving L-carnitine therapy.

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