β-Hydroxy Fatty Acid Production by Ischemic Rabbit Heart

DISTRIBUTION AND CHEMICAL STATES

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ABSTRACT β-Hydroxymyristate, -palmitate, and -stearate were produced by and accumulated in isolated rabbit heart when perfused ischemically for 2–10 min by the nonrecirculating Langendorff technique with 0.75 mM palmitate and 0.16 mM albumin. Tissue fractionation into mitochondria and cytosol showed that by 2 min of ischemia 44% of β-hydroxypalmitate and 38% β-hydroxystearate was located in the cytosol; this percentage increased to >50% by 5 min of ischemia. Lipid fractionation studies showed that by 10 min these two β-hydroxy fatty acids were distributed approximately as 60% acylcarnitine, 20% acyl-coenzyme A (CoA), and 20% free fatty acids. All three chemical forms of β-hydroxypalmitate were found in both the mitochondria and the cytosol. After 10 min of ischemia β-hydroxypalmitoyl-CoA and β-hydroxystearoyl-CoA constituted at least 16% of the incremental long-chain acyl-CoA, whereas β-hydroxypalmitoylcarnitine and β-hydroxystearoylcarnitine constituted ~8% of the incremental long-chain acylcarnitine. These data suggests that myocardial β-hydroxyacyl-CoA oxidation is limited during ischemia. Substrate accumulates and is transferred to the cytosol where it accumulates primarily as β-hydroxyacylcarnitine.

INTRODUCTION

Ischemia is known to depress cardiac fatty acid oxidation, apparently by inhibition of β-oxidation per se rather than by impairment of fatty acid activation or transfer (1, 2). FAD-linked acyl-coenzyme A (CoA) oxidation and NAD-linked β-hydroxy fatty acyl-CoA oxidation are two possible loci for inhibition of β-oxidation. Disproportionate suppression of the latter step is supported by several studies showing β-hydroxypalmitate production by rotenone-inhibited mitochondria oxidizing palmitoylcarnitine (3, 4). Our recent report of the accumulation of β-hydroxy fatty acids in isolated, perfused rabbit hearts during ischemia or hypoxia shows significant depression of β-hydroxy fatty acyl-CoA oxidation in the intact tissue (5).

Ischemic or hypoxic hearts promptly accumulate long-chain acyl-CoA and acylcarnitine esters (6, 7). Recent investigations suggest that these long-chain fatty acid esters may be deleterious to the ischemic myocardium (7, 8). However, no studies have identified the acyl moieties of these accumulated long-chain fatty acyl esters. In fact, most studies have used exogenous long-chain fatty acids that are normal constituents of the intracellular fatty acid pools (7, 8). Because β-hydroxy fatty acids also accumulate during ischemia it is important to determine their contribution to the pools of accumulated carnitine and CoA esters.

Herein, we report the results of our studies of the intracellular distribution and chemical state(s) of long-chain β-hydroxy fatty acids produced by isolated ischemic rabbit heart.

METHODS

Fatty acid-free bovine serum albumin, NAD, NADH, lactate, pyruvate, alcohol dehydrogenase, lactate dehydrogenase, malic dehydrogenase, carnitine acetyl transferase, citrate synthase, and *N*-ethylmaleimide were obtained from Sigma Chemical Co., St. Louis, Mo., boron trifluoride-methanol reagent from Applied Science Laboratories, Inc., State College, Pa., the silylating reagent, *N,O-bis*(trimethylsilyl) acetamide, and palmitate from Supelco, Inc., Bellefonte, Pa.,

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neutral alumina AG7 and Dowex 1-X10 from Bio-Rad Laboratories, (Richmond, Calif.), and [1-^{14}C]acetyl-CoA from New England Nuclear (Boston, Mass.). Thin-layer chromatography plates, precoated with Silica Gel G, were from Analtech Inc., Newark, Del.; the plates were prewashed in methanol, air-dried, and activated at 115°C for 40 min. All solvents were reagent grade and were redistilled.

Reference methyl esters of nondeuterated and doubly α-deuterated β-hydroxystearate, -palmitate, and -myristate were synthesized and purified in the laboratory as previously described (5).

Perfusion. Hearts from male New Zealand White rabbits were perfused by the non-recirculating Langendorff technique without pacing in a water-jacketted apparatus with 60 cm water pressure and unrestricted flow rates as previously described (5). The perfusion medium was a modified Krebs-Ringer-bicarbonate buffer containing 2.5 mM calcium, 10 mM glucose, and 0.5 mM EDTA, pH 7.4, which was equilibrated with O_2:CO_2 (95:5) at 37°C before use. The initial 15-min perfusion was followed by a 20-min unrestricted perfusion (25–30 ml/min) with perfusate containing 0.75 mM palmitate complexed with 1% albumin (9) to establish aerobic steady state fatty acid oxidation. Severe ischemia was effected by reducing the flow rate to 1–3 ml/min for 2–10 min, which caused a two-to fivefold increase in the effluent lactate-to-pyruvate ratio. Throughout the perfusion, coronary effluent was collected and measured at 2–5 min intervals.

Estimation of tissue levels of metabolites. The perfusion was stopped by instantly freezing the empty heart with liquid N_2-cooled Wollenberger clamps. Duplicate portions of frozen tissue were powdered and extracted in cold 6% (wt/vol) perchloric acid and centrifuged at 0°C. The supernatants were neutralized with K_2CO_3, recentrifuged, and used for assay of tissue content of acid-soluble CoA, acetyl-CoA, free carnitine and acid-soluble carnitine. The precipitates were washed with 1% perchloric acid and used for assay of tissue content of long-chain acyl-CoA or acylcarnitine.

Precipitates to be analyzed for acyl-CoA were dispersed in hydrolysis buffer, brought to pH 11–12 with KOH, and incubated at room temperature for 30 min. The samples were then acidified with concentrated HCl and 17.5% $HClO_4$ to precipitate any residual protein, centrifuged and neutralized with 1.2 M K_2CO_3. The hydrolyzed acyl-CoA samples were assayed for CoA in a Beckman Acta II Spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) according to the enzymatic cycling method of Veloso and Veech (10). Duplicate aliquots of the original acid-soluble extracts were assayed by the above method for free and acetyl-CoA; after brief treatment with *N*-ethylmaleimide (11), other aliquots were assayed for acetyl-CoA by the same method. Free CoA was calculated as the difference between free plus acetyl-CoA readings and acetyl-CoA readings.

Precipitates to be assayed for acylcarnitine were dispersed in 2 ml H_2O, brought to pH 11–12 with KOH and incubated at 37°C for 1 h. Aliquots of the original acid-soluble extract, to be assayed for acid-soluble carnitine, were similarly hydrolyzed. Both hydrolysis mixtures were then acidified and neutralized as in the previous paragraph. Other aliquots of the original acid-soluble extract were assayed directly for free carnitine. Acid-soluble carnitine, free carnitine, and acylcarnitine were analyzed for carnitine content by the radioisotopic assay of McGarry and Foster (12). Acetylcarnitine (including short chain acylcarnitines) was calculated as the difference between the acid-soluble and free carnitine values.

Aliquots of coronary effluent and powdered tissue were assayed for pyruvate and lactate levels as detailed previously (5). An aliquot of heart powder was dessicated to obtain tissue wet weight-to-dry weight ratio.

Gas chromatography mass spectrometry (GC/MS)[1] *analysis.* Portions of frozen tissue with known weights of added deuterated β-hydroxymyristate, -palmitate, and -stearate were saponified, extracted, methylated, chromatographed and silylated in preparation for GC/MS analysis as described by Moore et al. (5). Samples were analyzed on a Hewlett-Packard 5992A GC/MS (Hewlett-Packard Co., Palo Alto, Calif.), using a 6-ft. column of 3% SE-30, temperature programmed from 160 to 240°C. The instrument was operated in the select ion monitoring program for the M-15 m/e signals for β-hydroxymyristate, -palmitate, and -stearate and their doubly α-deuterated analogues. The retention times under these conditions for the methyl esters of β-hydroxymyristate, -palmitate, and -stearate were 5.9, 7.8, and 9.6 min, respectively. The ratios of the GC/MS-integrated peak areas for the two signals of each β-hydroxy fatty acid were applied to a standard curve to calculate the content of each β-hydroxy fatty acid (5).

Tissue fractionation. In experiments designed to determine tissue distribution of β-hydroxy fatty acid, the perfused hearts were rapidly flushed with 20 ml ice-cold isotonic saline containing 10 mM KCN. A 1-g apical section was excised, weighed, quickly minced and suspended in isolation media (0.18 M KCl, 0.1 mM EGTA and 0.5% fatty acid-free bovine serum albumin, pH 7.2). Mitochondria were rapidly released by use of Polytron tissue processor (13) and immediately centrifuged at 0°C at a relative centrifugal force of 754 for 10 min to separate the pellet of unbroken cells and heavy fragments. The supernatant was then centrifuged for 10 min at a relative centrifugal force of 8,714 to separate mitochondria from the cytosol. This method resulted in a mitochondrial fraction that represented 20% of total cardiac mitochondria as assessed by distribution of citrate synthase activity (14). Only 7.8% of the citrate synthase activity was isolated in the cytosolic fraction; this was similar to that observed by Idell-Wenger et al. (15) for the ischemic rat heart. Lactate dehydrogenase distribution was used as a cytosolic marker (16); 74% of the lactate dehydrogenase activity was recovered in the cytosolic fraction and 1.8% in the mitochondrial fraction. Enzyme distributions were identical for freshly isolated and ischemically perfused hearts. The three fractions (mitochondria, cytosol, and low-speed pellet) were either saponified in 1 N KOH/30% MeOH with deuterated reference β-hydroxy fatty acid for assay of total β-hydroxy fatty acid content or were extracted for acyl-CoA, acylcarnitine, and free fatty acids as described below.

Acyl-CoA extraction for β-hydroxy fatty acid analysis. Powdered frozen tissue (0.1–0.4 g) or appropriate aliquots of tissue fractions were extracted with isopropanol: 0.05 M potassium phosphate, pH 7.2 (1:1) as detailed by Mancha et al. (17) and chromatographed on a 2.5-in. pasteur pipette column of neutral alumina AG7. The sample was applied in 8 ml $CHCl_3$/MeOH (1:2); free fatty acids were eluted by 5 ml ether; contaminating acyl-carnitines were eluted with 10 ml $CHCl_3$:MeOH:0.1 M sodium acetate (4:4:1); and the acyl-CoA fraction was quantitatively eluted with 15 ml 0.1 M potassium phosphate/MeOH (1:1) (11). The acyl-CoA fraction was dried under N_2 and, after addition of deuterated β-hydroxy fatty acid, was saponified for subsequent GC/MS analysis of acyl-CoA β-hydroxy fatty acid content.

Acylcarnitine and free fatty acid extraction for β-hy-

[1] *Abbreviation used in this paper*: GC/MS, gas chromatography/mass spectrometry.

droxy fatty acid analysis. Powdered frozen tissues (0.2–0.8 g) or appropriate aliquots of tissue fractions were dispersed in 0.4 M $HClO_4$ and extracted with chloroform and methanol according to the method of Bligh and Dyer (18). The chloroform layer was concentrated and applied to a silica gel G plate with reference palmitic acid and palmitoylcarnitine. The plate was sequentially developed in two systems: petroleum ether/ether/formic acid (60:40:2) and chloroform/methanol/0.1 M sodium acetate (50:40:10) to separate the acylcarnitines from free fatty acids (19). After identification of reference palmitic acid (R_F = 1) and palmitoylcarnitine (R_F = 0.4 − 0.5) with rhodamine G spray, the gel was collected from 6 horizontal strips and hydrolyzed in 10 ml 1 N KOH/30% MeOH with reference β-hydroxy fatty acid for subsequent GC/MS analysis of free fatty acid and acylcarnitine β-hydroxy fatty acid content.

RESULTS

Effect of duration of ischemia on β-hydroxy fatty acid production. After aerobic perfusion with 0.75 mM palmitate, ischemia for 0–10 min effected progressive accumulation of β-hydroxypalmitate and β-hydroxystearate (Fig. 1). Half-maximal accumulation was evident by 2 min. β-Hydroxypalmitate accumulation was about twice that of β-hydroxystearate. As previously reported, the significant levels of β-hydroxymyristate present in the aerobic heart did not change appreciably during ischemia (5).

Intracellular distribution of β-hydroxy fatty acids. The intracellular distribution of β-hydroxy fatty acids between mitochondria and cytosol of ischemic heart was determined by prompt chilling, homogenization, and differential centrifugation. All three tissue fractions (mitochondria, cytosol, and low-speed pellet) were assayed for β-hydroxy fatty acid content; when compared with direct assay of frozen tissue, total recovery ranged from 95 to 105%. By use of marker enzyme distribution (Methods) total mitochondrial and cytosolic β-hydroxy fatty acid contents were calculated from the values obtained for the isolated mitochondrial and cytosolic fractions. Results for β-hydroxypalmitate are shown in Fig. 2b. 44% of total β-hydroxypalmitate was in the cytosol by 2 min of ischemia and this increased to 54% by 10 min of ischemia. Similar results were obtained for β-hydroxy-

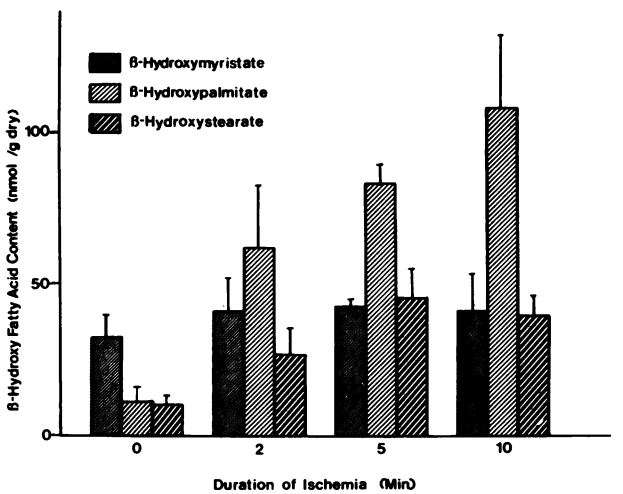

FIGURE 1 Heart β-hydroxy fatty acid production as function of duration of ischemia. Excised rabbit hearts were flushed and perfused by Langendorff method with oxygenated Krebs-Ringer-bicarbonate solution containing 10 mM glucose. After 15 min of nonrecirculating unrestricted preflow at 37°C, the heart was perfused aerobically with perfusate supplemented with 0.75 mM palmitate/1% albumin for 20 min. Coronary effluent was continuously collected to monitor lactate-to-pyruvate ratios to ensure aerobic function (lactate-to-pyruvate ratios ≤ 12). After prompt coronary flow restriction for various lengths of time, empty heart was quickly frozen for β-hydroxy fatty acid analysis. During ischemia, mean coronary effluent lactate-to-pyruvate ratios were >30. Shown are the means and standard deviations of at least five experiments for each time.

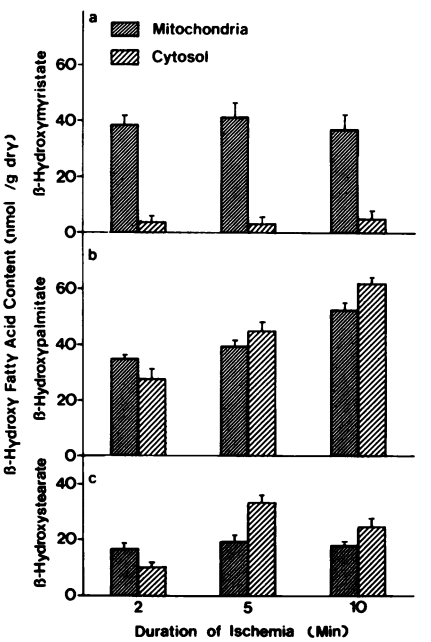

FIGURE 2 Calculated intracellular distribution of β-hydroxy fatty acid as a function of duration of ischemia. Perfusions were performed as in Fig. 1. At end of ischemic perfusion the heart was flushed with ice-cold saline containing 10 mM KCN, a 1–2-g apical section was removed, and the remainder was freeze-clamped for total β-hydroxy fatty acid analysis. The apical piece was weighed, minced, sonicated, homogenized, and fractionated by differential centrifugation for analysis for: β-hydroxymyristate (a), β-hydroxypalmitate (b), and β-hydroxystearate (c). Mitochondrial and cytosolic marker enzyme distributions were used to calculate fatty acid distributions (Methods). Shown are the means of three to six experiments for each time and standard deviations. Zero time whole tissue content of each fatty acid is shown in Fig. 1.

stearate (Fig. 2c). In contrast the distribution of β-hydroxymyristate (Fig. 2a) was relatively invariant in both tissue spaces. The data suggest prompt and continuous efflux of β-hydroxypalmitate and β-hydroxystearate from the mitochondria into the cytosol during ischemia. On the other hand, most β-hydroxymyristate appears to be in a metabolically inactive pool with little change in distribution during ischemia.

Chemical state(s) of β-hydroxy fatty acid. The distribution of β-hydroxy fatty acid among free fatty acid, acylcarnitine, and acyl-CoA in heart was determined after varying lengths of ischemic perfusion with palmitate. Portions of heart were extracted for acyl-CoA by method of Mancha et al. (17), which yielded quantitative recovery of standard β-hydroxypalmitoyl-CoA (93%). A separate portion of heart was extracted for total lipids (exclusive of acyl-CoA) by Lopes-Cardoza's modification of the Bligh and Dyer extraction (19), which was quantitative for standard β-hydroxypalmitate (99%) and standard palmitoylcarnitine (98%). Tissue content of β-hydroxypalmitoyl-CoA was relatively invariant after 2 min ischemia, whereas β-hydroxypalmitoyl-carnitine progressively increased through 10 min (Fig. 3a) at which point it was threefold greater than β-hydroxypalmitoyl-CoA. Tissue-free β-hydroxy-palmitate content was comparable to CoA ester content and was relatively invariant. Similar distribution was obtained for β-hydroxystearate (Fig. 3b). Essentially all tissue β-hydroxypalmitate and β-hydroxystearate was accounted for in these three lipid classes. In contrast only 25% of tissue β-hydroxymyristate existed as free acid or esters of CoA or carnitine (data not shown). The relatively stable pool of β-hydroxymyristate must exist primarily as some other class of lipid, possibly phospholipid.

Analysis of β-hydroxypalmitate in lipid subfractions was also performed on mitochondria and cytosolic fractions. The data represent an estimate because the cytosolic fraction is contaminated by 7.8% total tissue mitochondria and the mitochondrial fraction is contaminated by 1.8% of total tissue cytosol, according to marker enzyme distributions (Methods). The analytic accuracy for β-hydroxypalmitoyl-CoA was limited by the relatively small quantity of this ester in both cell fractions. Table I shows the results of two experiments with hearts perfused ischemically for 10 min. In the cytosol fraction 59% of the β-hydroxypalmitate was found as the carnitine ester, 15% as the CoA ester, and 26% as the free acid. In the mitochondrial fraction, 41% of the β-hydroxyplamitate existed as the carnitine ester, 8% as the CoA ester, and 51% as the free acid. β-Hydroxy-palmitate recovered as free fatty acid in these experiments was significantly greater than that found when frozen whole heart was promptly extracted and assayed (Fig. 3); this probably reflects hydrolysis of β-hydroxypalmitoyl esters during cell fractionation prior to lipid extraction.

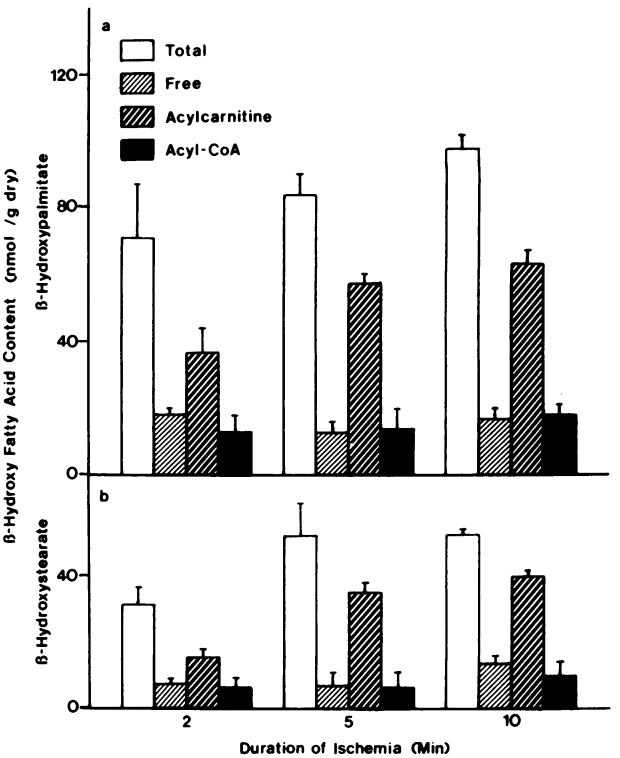

FIGURE 3 Chemical states of tissue β-hydroxy fatty acid as a function of duration of ischemia. Perfusions were performed as in Fig. 1. Portions of frozen heart were directly assayed for total β-hydroxy fatty acid content. Additional portions were extracted with chloroform and methanol for total lipids, which were then separated by sequential thin-layer chromatography; the free fatty acid and acylcarnitine fractions were identified and assayed for β-hydroxy fatty acid content. A third portion of frozen heart was extracted for acyl-CoA and subsequently analyzed for β-hydroxy fatty acid content. Results are shown for: β-hydroxypalmitate (a) and β-hydroxystearate (b). The mean and standard deviations are shown for three to six experiments for each time. Zero time total tissue content of each fatty acid is shown in Fig. 1.

These results are consistent with prompt and continuous conversion of intramitochondrial β-hydroxy acyl-CoA to β-hydroxyacylcarnitine, which is then transferred to the cytosol.

Changes in tissue CoA and carnitine levels during ischemia. Tissue levels of CoA and carnitine (free and esters) were determined before and after varying periods of ischemia (Figs. 4 and 5). After the initial aerobic perfusion with 0.75 mM palmitate, levels of acyl and acetyl esters of carnitine and CoA were relatively high, as previously found in rat heart by Oram et al. (20). By 2 min ischemia acetyl-CoA decreased by ~50% and like acetylcarnitine, continued to drop

TABLE I
Intracellular Distribution and Chemical States of β-Hydroxypalmitate

	β-Hydroxypalmitate content, nmol/g dry			
	Cytosol		Mitochondria	
Lipid subfractions*	Expt. A	Expt. B	Expt. A	Expt. B
Carnitine ester	30.1 (51%)	48.3 (66%)	8.8 (39%)	19.5 (44%)
Free fatty acid	18.8 (32%)	15.1 (21%)	12.1 (54%)	21.3 (48%)
CoA ester	10.3 (17%)	10.0 (13%)	1.4 (7%)	3.9 (8%)
Total subfractions	59.2 (100%)	73.4 (100%)	22.3 (100%)	44.7 (100%)
Total by direct assay	50.5	62.1	17.3	41.9

* Two hearts (A & B) were perfused ischemically for 10 min as described in Fig. 1. Cytosolic and mitochondrial fractions were separated by differential centrifugation of homogenate (Methods). Carnitine esters, free fatty acids, and CoA esters were extracted from portions of the subcellular fractions and assayed for β-hydroxypalmitate as previously described. Total by direct assay refers to direct hydrolysis of aliquots of subcellular fractions. The percent distribution of β-hydroxypalmitate among the three lipid subfractions are shown in parentheses.

through 10 min. Levels of free CoA and carnitine were relatively constant during the ischemic period.

During 10 min of ischemia the mean long-chain acyl-CoA content rose from 145±11 to 190±15 nmol/g dry for a mean increase of 45 nmol/g dry. Total β-hydroxypalmitoyl- and β-hydroxystearoyl-CoA content after 10 min ischemia was 28.3 nmol/g dry (Fig. 3). If one assumes that all the β-hydroxypalmitate and -stearate found prior to ischemia existed as CoA esters [e.g., 21.0 nmol/g dry (Fig. 1)], the calculated increment of β-hydroxypalmitoyl- and -stearoyl-CoA during ischemia would be 7.2 nmol/g dry, which constitutes at least 16% of the incremental long-chain acyl-CoA. This calculated percentage approximates 63% if none of these β-hydroxy fatty acids existed as CoA esters prior to ischemia.

Over 10 min of ischemia the average total long-chain acylcarnitine content rose from 956±50 to 2,179±224 nmol/g dry for a mean increase of 1,223 nmol/g dry. The average β-hydroxypalmitoyl- and β-hydroxy-stearoylcarnitine content after 10 min ischemia was 98.7 nmol/g dry, which could represent as much as 8% of incremental long-chain acylcarnitine.

DISCUSSION

These studies show that β-hydroxy fatty acids produced by the isolated ischemic heart oxidizing palmitate egress rapidly from the mitochondria and accumulate in the cytosol primarily as carnitine esters.

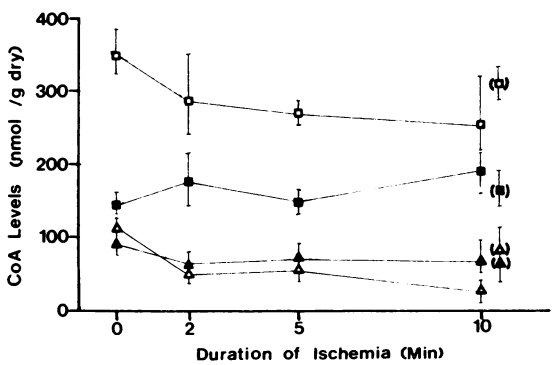

FIGURE 4 Heart CoA levels as a function of duration of ischemia. Perfusions were performed as in Fig. 1. Portions of frozen heart were extracted and enzymatically assayed for: total CoA, □; acetyl-CoA, △; acyl-CoA, ■; and free CoA, ▲, content. Values in ( ) are the corresponding aerobic CoA content. Shown are the averages and ranges for four to five experiments for each time.

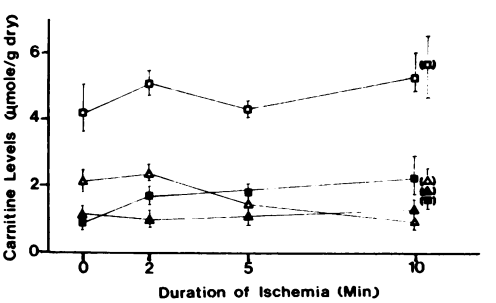

FIGURE 5 Heart carnitine levels as a function of duration of ischemia. Perfusions were performed as in Fig. 1. Portions of frozen heart were extracted and assayed for: total carnitine, □; acetylcarnitine, △; acylcarnitine, ■; and free carnitine, ▲, content. Values in ( ) are the corresponding aerobic carnitine content. Shown are the averages and ranges for four to five experiments for each time.

These data suggest that not all the CoA esters of these β-oxidation intermediates are bound by β-hydroxyacyl-CoA dehydrogenase; therefore, some are available for transfer from the mitochondria by the carnitine transferase system (Fig. 6). According to the Stanley and Tubbs "leaky hosepipe" theory, under conditions of slow β-oxidation flux CoA esters of β-hydroxy fatty acid and α,β-unsaturated fatty acids may diffuse from the β-oxidation locus (21). Because β-hydroxy-palmitoylcarnitine has been shown to be an acceptable substrate for the carnitine-acyl transferase (22), the β-hydroxy fatty acids are most likely transferred to the cytosol as carnitine esters by this system.

Our mitochondrial preparation method yielded mitochondria is quantities similar to that of Palmer et al. (23) for isolation of subsarcolemmal mitochondria from rat heart, although interfibrillar mitochondria are not quantitatively recovered. The method chosen allowed for rapid, one-step separation of mitochondria. Our data, therefore, applies primarily to subsarcolemmal mitochondria. However, as demonstrated by Matlib et al. (24) these two mitochondrial populations are probably not metabolically different. No current evidence exists to suggest that the two populations of mitochondria have differential response(s) to ischemia. Finally, our use of marker enzymes corrected for any cross-contamination between mitochondria and cytosol due to the isolation procedure (15).

This is the first reported attempt to identify acyl moieties of the increased long-chain acyl CoA and acylcarnitine pools found in ischemic myocardium. Our estimated cytosol/mitochondria distribution (63:37) of β-hydroxypalmitoylcarnitine approaches the cytosol/mitochondria distribution (75:25) of total long chain fatty acylcarnitine in the ischemic rat heart as reported by Idell-Wenger et al.(15), although the contribution of β-hydroxy fatty acylcarnitines to the ischemia-induced increase in acyl-carnitine was relatively small. However, the assay accuracy for β-hydroxy fatty acyl-CoA was limited by the very small amount of these esters and the probable propensity for hydrolysis during tissue fractionation. Nevertheless, β-hydroxy fatty acyl-CoA may contribute significantly to the increased tissue long-chain fatty acyl-CoA levels found with ischemia, because the β-hydroxypalmitate accumulated during ischemia is proportional to perfusate palmitate concentration (5). Because long-chain fatty acyl-CoA and/or-carnitine may affect such reactions as the adenine nucleotide translocase system (7) Na+, K+-ATPase (8), and carnitine-acyl transferase (25), accumulation of CoA and carnitine esters of β-hydroxy fatty acid during ischemia may have important pathophysiological consequences.

The significant amount of free β-hydroxy fatty acid indicates hydrolysis of the β-hydroxy fatty acid-CoA and/or carnitine esters, possibly by tissue hydrolase(s). Other investigators have detected acyl-CoA and acylcarnitine hydrolase activities in a variety of organs, including heart (26, 27). Although the effect of ischemia on these enzymes is unknown, Sobel et al. (28) have demonstrated the accumulation of lysophosphatides due to increased phospholipase activity in the ischemic heart. Crass and Pieper (29) have observed that hypoxic perfusion of isolated rat heart prompts accumulation of free fatty acid derived from ^{14}C-labeled endogenous lipids. Recently, Lochner et al. (30) have reported that rat heart free fatty acid levels increase during low-flow hypoxia and correlate with depression of mitochondrial function. Because free

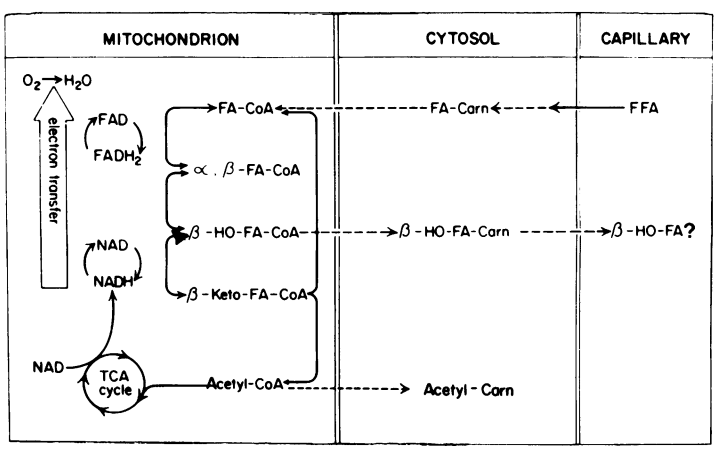

FIGURE 6 Schema of myocardial cell compartmentation, fatty acid oxidation and β-hydroxy fatty acid (β-HO-FA) production. The carnitine-palmitoyl-CoA transferases and palmitoyl-CoA synthetase are not depicted. FA-Carn, fatty acylcarnitine; α,β-FA-CoA, α,β-mono-unsaturated fatty acyl-CoA; β-keto-FA-CoA, β-keto-fatty acyl-CoA.

fatty acids are permeable to the sarcolemma, these β-hydroxy fatty acids may escape into the coronary effluent. We have previously detected small quantities of β-hydroxy fatty acids in coronary effluent from ischemic rabbit heart (5).

Although in these studies we assayed only for β-hydroxy fatty acid (14 to 18 carbons long) it is likely that during ischemia α,β-unsaturated intermediates also accumulate. Lopes-Cardoza et al. (19) showed that during state 4 oxidation of palmitate by rat liver mitochondria the accumulation of mono-unsaturated β-oxidation intermediates is about half of the β-hydroxy intermediates.

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