FEBS Letters 581 (2007) 4491–4494

Fatty acid chain-elongation in perfused rat heart: Synthesis of stearoylcarnitine from perfused palmitate

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Received 4 August 2007; accepted 14 August 2007

Available online 22 August 2007

Edited by Sandro Sonnino

Abstract **Rat hearts perfused for up to 60 min in the working mode with palmitate, but not with glucose, resulted in substantial formation of palmitoylcarnitine and stearoylcarnitine. To test whether lipolysis of endogenous lipids was responsible for the increased stearoylcarnitine content or whether some of the perfused palmitate underwent chain elongation, hearts were perfused with hexadecanoic-16,16,16-d_3 acid (M+3). The pentafluorophenacyl ester of deuterium labeled stearoylcarnitine had an M+3 (639.4 *m/z*) compared to the unlabeled M+0 (636.3 *m/z*) consistent with a direct chain elongation of the perfused palmitate. Furthermore, the near equal isotope enrichment of palmitoyl- (90.2 ± 5.8%) and stearoylcarnitine (78.0 ± 7.1%) suggest that both palmitoyl- and stearoyl-CoA have ready access to mitochondrial carnitine palmitoyltransferase and that most of the stearoylcarnitine is derived from the perfused palmitate.**

Keywords: Fatty acid elongation; Mitochondria; Heart; Palmitoylcarnitine; Stearoylcarnitine

1. Introduction

The fatty acid demand of non-lipogenic tissues, such as skeletal muscle and heart, is met by uptake from the circulation. In these tissues the major metabolic fate of fatty acids is their β-oxidation primarily in the mitochondrial matrix for production of ATP. However, fatty acids also are needed for the synthesis of phospholipids which represent a major constituent of membranes. The fatty acid composition of these phospholipids often shows tissue as well as organelle specificity which requires remodeling of the free fatty acid derived from the circulation. While the reactions required for tissue specific remodeling (chain elongation, desaturation) are relatively well known for lipogenic tissues, little is known for non-lipogenic tissues such as the heart and skeletal muscle. In studies with isolated rat cardiac myocytes Hagve and Sprecher [1] could find no evidence for chain elongation of ^{14}C-labeled 18:2, 18:3, 20:4, and 20:5 fatty acids. Likewise, no chain elongation activity could be detected by Hamilton and Saggerson [2] in any heart subcellular fraction either by measuring directly the condensing enzyme with palmitoyl-CoA and malonyl-CoA as the substrates or by determining the incorporation of radioactivity from malonyl-CoA into lipid-soluble products in the presence of palmitoyl-CoA. In contrast, Jimenez et al. [3] reported the ability of cardiac myocytes to metabolize radiolabeled linoleic acid to higher and more unsaturated metabolites and that this activity significantly decreased with age. In the Langendorf perfused rabbit heart, Ford et al. [4] documented a significant conversion of [9,10-^{3}H] octadec-9′-enoic acid to [^{3}H] eicosenoylcarnitine in ischemic, but not in control, myocardium.

Recently, we observed that rat hearts perfused in the working mode with unlabeled palmitate plus glucose, but not with glucose alone, also contain increasing amounts of stearoylcarnitine with time. This finding lead us to postulate that some of the perfused palmitic acid was used to synthesize stearic acid. In the present work we directly tested this hypothesis by perfusing isolated rat hearts with stable isotope labeled palmitic acid (hexadecanoic-16,16,16-d_3 acid) and analyzing the long-chain acylcarnitines by HPLC/MS.

2. Materials and methods

2.1. Chemicals

Unlabeled palmitic acid (⩾99.0% GC) was from Fluka and hexadecanoic-16,16,16-d_3 acid (min 99 at.% D) from Isotech. All other chemicals were of highest quality commercially available.

2.2. Heart perfusion

Six month Fisher 344 rats were obtained from a colony maintained by the National Institute of Aging (Harlan Sprague Dawley, Inc., IN). The animals, housed in our animal care facility in a temperature and humidity controlled room and 12 h light/dark cycle, had free access to food and water until the experiments. On the day of an experiments the animals were weighed, injected with 500 U heparin (IP), killed with Na-pentobarbital (100 mg/kg body weight) and the hearts canulated for perfusion. The perfusion protocol consisted of a 15 min non-recirculating perfusion in the Langendorf mode with Krebs–Henseleit buffer containing 5.5 mM glucose and 0.1 U/L insulin, followed by 15 and 60 min perfusion in the working mode (left atrial flow 35–50 ml/min at a preload of 10–15 mmHg) with 5.5 mM glucose/0.1 U/L insulin/3% BSA alone (glucose perfused) or with 1.2 mM unlabeled palmitate (M+0) or hexadecanoic-16,16,16-d_3 acid (M+3) complexed to 3% BSA in the presence of glucose/insulin (palmitate perfused) [5]. At the end of perfusion the hearts were freeze-clamped, powdered under liquid nitrogen and the powdered tissue stored at −60 °C.

2.3. Fatty acid and acylcarnitine analysis

Unlabeled and stable isotope labeled palmitic acid was methylated and the methylesters subjected to gas chromatography (inj. temp.: 240 °C; temp. gradient 60–320 °C) with mass spectrometric detection

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0014-5793/$32.00
doi:10.1016/j.febslet.2007.08.026

[6]. Acylcarnitines were isolated by silica gel solid phase extraction, derivatized with pentafluorophenacyl trifluoromethanesulfonate and analyzed by HPLC/MS [7].

3. Results and discussion

In rat hearts perfused with unlabeled palmitic acid plus glucose, but not with glucose alone, there is an increase in myocardial palmitoylcarnitine and myristoylcarnitine, as well as of stearoylcarnitine (Table 1). While the increase in palmitoylcarnitine (and myristoylcarnitine) in palmitate perfused hearts is expected, the increase in stearoylcarnitine is not expected and raises questions about the source of stearate present in stearoylcarnitine. Is it a contaminant present in commercial palmitic acid, is it released from endogenous lipids during perfusion with palmitate or is it derived from perfused palmitate by chain elongation?

To address the purity of unlabeled palmitic acid used in perfusion experiments we first analyzed the palmitic acid by gas chromatography/mass spectrometry (GC/MS). Out of four commercially available palmitic acid preparations only one was >99% pure (Fluka) even though all were labeled and sold as >99% pure. Since stearate was below the limit of detection in the commercial palmitate obtained from Fluka and used in the current perfusion experiments, stearate contamination can not be the source for stearoylcarnitine formation (data not shown). Although the lack of long-chain acylcarnitine accumulation in hearts perfused with glucose alone made lipolysis as the source of the stearoyl moiety unlikely, increased generation of endogenous stearate due to increased lipid remodeling during palmitate perfusion could not be ruled out. Thus, to determine if the stearoyl moiety of stearoylcarnitine is derived from lipolysis of endogenous lipids or by chain elongation of perfused palmitate, rat hearts were perfused with hexadecanoic-16,16,16-d_3 acid (M+3) (by GC/MS free of stearate contamination, data not shown) and the myocardial acylcarnitines analyzed. Fig. 1 shows the chromatographic separation and mass spectrometric detection of acylcarnitine pentafluorophenacyl esters formed by the heart during a 60 min perfusion with stable isotope labeled palmitate (hexadecanoic-16,16,16-d_3 acid) (Fig.1A). For comparison, a chromatogram of long-chain acylcarnitine pentafluorophenacyl esters obtained by perfusing hearts with unlabeled palmitic acid under otherwise identical conditions as in Fig.1A is presented in Fig. 1B. The insets in Fig. 1A and B show the mass isotopomer distribution of palmitoylcarnitine and stearoylcarnitine as well as of myristoylcarnitine pentafluorophenacyl esters. As shown in inserts in Fig. 1A when hearts are perfused with hexadecanoic-16,16,16-d_3 acid most of the palmitoylcarnitine formed is of M+3 species (611.3 *m/z*) with 9.8 ± 5.8% ($n = 4$, mean ± S.D.) representing M+0 species (608.3 *m/z*). Since the perfused deuterated palmitic acid was greater than 99 at.% deuterium, the presence of M+0 species indicates some dilution of the perfused palmitic acid by unlabeled endogenous palmitic acid. As with palmitoylcarnitine, most of the formed stearoylcarnitine was of M+3 species (639.4 *m/z*) with 22.0 ± 7.2% ($n = 4$, mean ± S.D.) of unlabeled M+0 (636.3 *m/z*). Thus, the formation of deuterated stearoylcarnitine (M+3) during palmitate perfusion provides unequivocal evidence for fatty acid chain elongation in the heart. In addition, the presence of M+3 myristoylcarnitine indicates β-oxidation of labeled palmitate (compare Fig. 1A vs 1B). The near identical enrichment of stable isotope in palmitoyl- and stearoylcarnitine as judged from the 611.3/608.3 and 639.4/636.3 *m/z* ratios, i.e., 90.2 ± 5.8% and 78.0 ± 7.1%, respectively suggests that a fraction of the perfused palmitic acid is directly chain elongated to stearate at a site, probably mitochondria, where both, palmitoyl-CoA (substrate) and stearoyl-CoA (product) have direct access to carnitine palmitoyltransferase. Consistent with our interpretation are data published by Ford et al. [4] who perfused rabbit hearts with radiolabeled oleic acid and found that the chain elongated product, eicosenoic acid, was confined to the carnitine fraction with no accumulation in other lipid classes, such as triglycerides, phospholipids, and fatty acids. Our data clearly show that rat heart contains all the enzymes necessary for fatty acid chain elongation and also explain the finding reported by Cinti et al. [8] that the content of stearic acid relative to palmitic acid in the heart is nearly twice that found in plasma.

In further support of fatty acid chain elongation in the heart it has been shown recently that ELOVL6 is expressed in the heart [9–12]. This enzyme catalyzes the malonyl-CoA dependent chain elongation of myristic, lauric and palmitic acids to stearic acid with a substrate preference for palmitic acid [9–11]. In addition to ELOVL6, rat heart also expresses ELOVL1 and ELOVL5 [12], with ELOVL1 having a substrate preference for saturated and monounsaturated fatty acids and ELOVL5 for polyunsaturated fatty acids [13].

In summary, using the fatty acid (hexadecanoic-16,16,16-d_3 acid) perfused working rat heart model we provide experimental evidence for an active fatty acid chain elongation pathway in the heart. While the data do not allow conclusion about the subcellular localization of the pathway, the near identical isotope enrichment in palmitoylcarnitine and stearoylcarnitine suggests that at least some of the substrate palmitoyl-CoA and product stearoyl-CoA of the chain elongation have ready access to carnitine palmitoyltransferase and is indicative of a mitochondrial fatty acid chain elongation pathway. Further studies are required to elucidate the mechanism and substrate

Table 1
Palmitoyl-, stearoyl-, and myristoylcarnitine contents of rat hearts at baseline (no perfusion), after 15 and 60 min perfusion in the working mode with unlabeled palmitic acid plus glucose and perfusion for 60 min with glucose only

Substrate/perfusion (min)	Myristoylcarnitine (C14)	Palmitoylcarnitine (C16)	Stearoylcarnitine (C18)
Baseline (no perfusion)	1.6 ± 0.6	2.5 ± 1.0	1.3 ± 0.3
Palmitate + glucose/15 min	15.0 ± 0.8	166.6 ± 31.7	12.7 ± 3.6
Palmitate + glucose/60 min	28.3 ± 5.9	119.2 ± 39.0	32.9 ± 9.9
Glucose only/60 min	3.7 ± 0.9	3.8 ± 1.2	2.8 ± 0.6

Values represent the means ± S.E.M. of four separate experiments and are expressed as nmol/g tissue wet weight.