Malonyl-CoA metabolism in cardiac myocytes and its relevance to the control of fatty acid oxidation

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1. Viable myocytes were obtained from rat hearts. Oxidation of [$1\text{-}^{14}\text{C}$]palmitate by these cells could be decreased by the addition of glucose (5 mM) or lactate (2 mM). In the presence of glucose, insulin decreased and adrenaline increased palmitate oxidation. 2. The myocytes contained activities of ATP citrate-lyase, acetyl-CoA carboxylase and the condensing enzyme of the fatty acid elongation system. No fatty acid synthase activity was demonstrable in myocytes. 3. In rat hearts perfused with 5 mM

glucose, malonyl-CoA content was acutely raised by insulin. In the presence of glucose + insulin, perfusion with palmitate or adrenaline decreased the malonyl-CoA content. 4. It is concluded that malonyl-CoA can be synthesized within cardiac myocytes and that the level of this metabolite can be acutely regulated. This is likely to have consequences for the regulation of carnitine palmitoyltransferase in the heart.

INTRODUCTION

It is generally accepted that long-chain fatty acids are an important metabolic fuel for the aerobic heart. Their availability in the circulation is increased in conditions such as fasting, diabetes or in the early hours following acute myocardial infarction [1–3]. Metabolic mechanisms whereby use of fatty acids can predominate over that of carbohydrate fuels (the ‘glucose fatty acid cycle’ [4]) have been well documented and involve inhibition of glucose entry, inhibition of hexokinase and phosphofructokinase and inactivation of pyruvate dehydrogenase (see [5] for a review). That β -oxidation of fatty acids is necessary for this suppression of carbohydrate utilization is suggested by observations that inhibitors of the overt form of carnitine palmitoyltransferase (CPT₁) such as tetradecylglycidate or etomoxir can overcome this suppression of glucose oxidation and pyruvate dehydrogenase [6,7]. Although key regulation site(s) have not been clearly defined, there also is evidence that this interplay between fatty acids and carbohydrate fuels is not entirely one-sided; i.e. under appropriate circumstances carbohydrate fuels can decrease fatty acid utilization by heart cells. Elevation *in vivo* of blood lactate decreases fatty acid extraction and oxidation by the heart [8–11], with similar effects being seen in the perfused heart [12–14] or in isolated myocytes [15,16]. Glucose suppresses removal of oleate by the working perfused heart [17], and glucose, or particularly glucose + lactate or lactate + dichloroacetate, decrease oxidation of ^{14}C -labelled long-chain fatty acids by perfused heart or incubated myocytes [14,18]. Two lines of evidence support a possible role of the CPT enzyme system in such ‘reverse glucose fatty acid cycle’ effects in heart muscle. First, carbohydrate fuels inhibit the oxidation of oleate, but not that of octanoate (the oxidation of which is mainly independent of the CPT system) [14]. Second, when decreasing production of $^{14}\text{CO}_2$ from [^{14}C]palmitate, carbohydrate fuels also decrease tissue long-chain acylcarnitine content [13]. It was inferred from ref. [13] that there was also a concomitant rise in the cytosolic content of long-chain fatty acyl-CoA, suggesting a metabolic ‘cross-over’ at heart CPT₁ when carbohydrate fuel was provided. Taken together, these various findings suggest a significant role for the CPT system, in particular CPT₁, in the

regulation of cardiac fuel selection. Possible importance of CPT₁ has also been highlighted by the observations that several drugs that inhibit CPT₁ {e.g. 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate (‘POCA’) [19], oxfenicine [20–22], tetradecylglycidate [23] and etomoxir [6,24]} protect the ischaemic heart.

There has been ample documentation that CPT₁, which is associated with the outer membrane of mitochondria, plays a major role in regulating the entry of long-chain fatty acids into mitochondrial oxidative metabolism in the liver (for reviews see [25–27]). In particular malonyl-CoA, the product of acetyl-CoA carboxylase, which is a key regulatory step in hepatic fatty acid synthesis, is a potent inhibitor of CPT₁ in liver mitochondria, thereby providing an inverse regulatory link between hepatic fatty acid synthesis and β -oxidation. Heart mitochondria contain an isoform of CPT₁ which is slightly smaller than and immunologically distinct from liver CPT₁ [28,29]. However this heart (and skeletal-muscle) form of CPT₁ can also be inhibited by malonyl-CoA, moreover with a sensitivity that is as much as 50 times that of the liver enzyme [30–32]. Heart muscle is not regarded as a ‘lipogenic’ tissue. However, an isoform of acetyl-CoA carboxylase that is distinct from the liver enzyme has been isolated from rat heart [33,34], and malonyl-CoA has been detected in rat heart tissue [35]. It was not clear to what extent these measurements of acetyl-CoA carboxylase and malonyl-CoA were representative of cardiac myocytes, rather than the non-myocyte cells of the heart, nor have there been any reports of acute regulation of malonyl-CoA concentration in the heart.

In this study we have examined the capability of extracts from viable isolated myocytes to synthesize and dispose of malonyl-CoA. In addition we show that insulin, adrenaline and non-esterified fatty acid supply can each acutely regulate malonyl-CoA concentration in the perfused rat heart. The findings are consistent with malonyl-CoA playing a regulatory role in fuel selection by the heart.

MATERIALS AND METHODS

Materials

Fine chemicals, hormones and enzymes were from Sigma Chemical Co. (Poole, Dorset, U.K.) or Boehringer Mannheim (Lewes,

Abbreviation used: CPT₁, the overt form of carnitine palmitoyltransferase (EC 2.3.1.21).

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East Sussex, U.K.). In addition, collagenase (Type 1 from *Clostridium histolyticum*) was from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.), and $\text{NaH}^{14}\text{CO}_3$, $[1\text{-}^{14}\text{C}]\text{acetyl-CoA}$, $[^3\text{H}]\text{acetyl-CoA}$, $[2\text{-}^{14}\text{C}]\text{malonyl-CoA}$ and $[1\text{-}^{14}\text{C}]\text{palmitate}$ were from Amersham International (Amersham, Bucks., U.K.). Fatty acid synthase (EC 2.3.1.85) was purified from rat livers. These were homogenized in 10 mM Tris/HCl buffer (pH 7.4) containing 225 mM mannitol, 75 mM sucrose, 0.5 mM EDTA and 0.5 mM dithiothreitol. The homogenate was centrifuged for 10 min at 9200 g_{av} and the resulting supernatant was then re-centrifuged at 100000 g_{av} for 1 h. The supernatant was fractionated with $(\text{NH}_4)_2\text{SO}_4$, the fraction between 25 and 40% saturation being saved. This was dissolved in 20 mM Bis-Tris-propane buffer (pH 7.0) containing 30 mM NaCl, 3 mM EDTA and 1 mM dithiothreitol and then dialysed for 12–15 h against two changes each of 100 volumes of the same buffer. Finally the extract was chromatographed on a Pharmacia f.p.l.c. HiLoad 16/10 Q Sepharose column previously equilibrated with 20 mM Bis-Tris-propane buffer (pH 7.0) containing 30 mM NaCl, 3 mM EDTA and 1 mM dithiothreitol. With a linear gradient of 0–0.6 M NaCl dissolved in the same buffer over 2 column vol., fatty acid synthase activity was eluted between 0.2 and 0.25 M NaCl with a specific activity of 0.17 $\mu\text{mol}/\text{min}$ per mg of protein. Sodium palmitate was associated with fatty-acid-poor BSA [36] and standardized by a colorimetric assay [37].

Animals

These were male rats of the Sprague–Dawley strain bred at University College London. These were maintained at 20–22 °C and had constant access to drinking water and to Rat & Mouse No. 3 Breeding Diet (Special Diet Services, Witham, Essex, U.K.). This contained (by wt.) 21% protein, 4% fat and 39% starches and sugars. The light/dark cycle was 13 h/11 h, with light from 06:00 to 19:00 h. Rats used for isolation of myocytes were 10–12 weeks old (300–350 g body wt.); all other rats were 6–8 weeks old (200–250 g body wt.).

Isolation of cardiac myocytes

This was basically by the method of ref. [38], with some modifications. Hearts were perfused retrogradely through the aorta for 45 min with Krebs–Henseleit bicarbonate medium containing 5.5 mM glucose, streptomycin (0.1 mg/ml) and collagenase (0.5 mg/ml). For the first 35 min the perfusate contained 25 μM CaCl_2 . Then over the ensuing 5 min the $[\text{Ca}^{2+}]$ in the perfusate was very gradually raised to 1.3 mM. This procedure appeared to lessen cell damage attributable to the ‘calcium paradox’ phenomenon, which occurs on returning the $[\text{Ca}^{2+}]$ in the medium to physiological levels. As described in [38], the myocyte isolation procedure was concluded by centrifugation of the cells through Krebs–Henseleit bicarbonate medium containing fatty-acid-poor BSA (40 mg/ml). This procedure significantly decreased the final yield of myocytes, but appreciably increased the proportion of viable cells recovered. All myocyte preparations that were subsequently used for experimentation had a high (> 70%) proportion of rod-shaped cells, and more than 95% of the cells excluded Trypan Blue. Hormone-responsiveness and metabolic features of these myocyte preparations are described in the Results and discussion section.

Palmitate oxidation by isolated cardiac myocytes

Immediately after isolation, portions of cells were incubated in 50 ml silicone-treated Erlenmeyer flasks fitted with glass centre

wells. The incubation medium was 1.0 ml of Krebs–Henseleit bicarbonate buffer containing BSA (40 mg/ml) and 0.5 mM sodium $[1\text{-}^{14}\text{C}]\text{palmitate}$ (2 $\mu\text{Ci}/\mu\text{mol}$). Other additions are indicated in Tables 1 and 2. The flasks were stoppered with rubber Suba-seals and gassed through the stoppers with a stream of O_2/CO_2 (19:1) for 2–3 min. The gas supply was then removed, and the flasks were incubated with shaking (70 cycles/min) at 37 °C for 1 h. Incubations were terminated by injection through the stoppers of 1 ml of 1 M HCl into each flask outer well, followed by injection of 0.5 ml of 1 M Hyamine hydroxide (in methanol) into each centre well. The flasks were returned to the bath and incubated with shaking for a further 1 h, after which the contents of the centre wells were taken for scintillation counting. Blank incubations containing $[^{14}\text{C}]\text{palmitate}$ but no cells were always performed in parallel.

Perfusion and freeze-clamping of hearts

Before perfusion, hearts were trimmed to remove as far as possible any adhering fat. Recirculating Langendorff perfusion was performed at 37 °C as described in [39] at a pressure of $80 \pm 5 \text{ cmH}_2\text{O}$. The perfusate consisted of 100 ml of Krebs–Henseleit bicarbonate medium gassed with O_2/CO_2 (19:1) containing 5 mM glucose and fatty-acid-poor BSA (20 mg/ml). Other additions are indicated in Table 4. After 45 min of perfusion, hearts were removed from cannulae and immediately crushed between stainless steel plates precooled in liquid nitrogen [40]. Hearts were stored in liquid nitrogen until extracted for measurement of metabolites.

Preparation of tissue extracts for measurement of metabolites

Individual freeze-clamped hearts were ground to a powder under liquid nitrogen and then homogenized in 6% (v/v) HClO_4 , followed by centrifugation at 2000 g for 10 min at 4 °C. The supernatant was made to 0.1 M in triethanolamine hydrochloride and the pH was adjusted to 6.0 with KOH (10 M and then 1 M). After removal of the KClO_4 precipitate by centrifugation, the resulting supernatants were used immediately for assay of malonyl-CoA. The 2000 g pellets from the HClO_4 treatment were homogenized in 6 ml of 0.2 M KOH containing 10 mM dithiothreitol and then incubated at 55 °C for 2 h. After transfer to ice and addition of 0.3 ml of 60% HClO_4 , followed by centrifugation at 2000 g for 10 min, 0.1 ml of near-saturated KH_2PO_4 was added to the resulting supernatant and the pH adjusted to 7.0 with KOH. Precipitated KClO_4 was removed by centrifugation and the extracts were stored at –70 °C before assay of carnitine and CoASH (representing long-chain fatty-CoA and carnitine derivatives).

Preparation of tissue extracts for assay of enzyme activities

Samples of whole heart muscle or freshly isolated myocytes were homogenized in 10 mM Tris/HCl buffer (pH 7.4) containing 225 mM mannitol, 75 mM sucrose, 0.5 mM EDTA and 0.5 mM dithiothreitol by using a motor-driven Teflon pestle in a glass Potter/Elvehjem homogenizer (radial clearance 0.19 mm). Centrifugation was carried out at 9200 g_{av} for 10 min, followed by centrifugation of the resulting supernatant at 100000 g for 60 min. The 9200 g and 100000 g pellets were resuspended in the same medium and all fractions were frozen at –70 °C. Mean values for 14 separate preparations of myocytes indicated that the 9200 g pellet contained 86% of the recovered cell protein, whereas the 100000 g pellet and supernatant contained respectively 2% and 12% of the cell protein.

Measurements of metabolite contents

Malonyl-CoA was measured by the method of ref. [41], in which [^3H]acetyl-CoA is converted by fatty acid synthase into light-petroleum-soluble material in proportion to malonyl-CoA. All assays were calibrated by malonyl-CoA internal standards. CoASH was measured by the recycling assay described in [42]. All assays were calibrated by CoASH internal standards. L-Carnitine was measured by the method of ref. [43], in which [^{14}C]acetyl-CoA is converted by carnitine acetyltransferase into [^{14}C]acetylcarnitine in proportion to carnitine. The [^{14}C]acetylcarnitine was separated from [^{14}C]acetyl-CoA on columns of Dowex 2-X8 (200–400 mesh, Cl form).

Measurements of enzyme activities

Acetyl-CoA carboxylase (EC 6.4.1.2) in 100000 g supernatants was measured radiochemically at 37 °C [44] as incorporation of [^{14}C]bicarbonate into acid-stable material. Assays were carried out with or without 10 mM trisodium citrate with an equimolar amount of MgCl_2 to compensate for chelation of Mg^{2+} by citrate [44]. Fatty acid synthase (EC 2.3.1.85) in 100000 g supernatants was assayed spectrophotometrically at 25 °C as described in [45] or radiochemically at 37 °C [46] as incorporation of [$2\text{-}^{14}\text{C}$]malonyl-CoA into fatty acids. In the radiochemical assay the co-substrates NADPH and acetyl-CoA were both omitted from blanks. Any remaining incorporation of [^{14}C]malonyl-CoA in these blank incubations was believed to be attributable to fatty acid elongation activity. Traces of this were detected in 100000 g supernatants (see the Results and discussion section), and this activity would be expressed in the absence of NADPH and acetyl-CoA provided that the tissue extract contained traces of endogenous long-chain fatty acyl-CoA. Fatty acid elongation activity was measured radiochemically at 37 °C as the incorporation of [$2\text{-}^{14}\text{C}$]malonyl-CoA into light-petroleum-soluble material in the presence of palmitoyl-CoA. This was done essentially as described in [47]. The reaction mixtures were 1.0 ml and contained 100 mM Tris/HCl buffer (pH 7.4), fatty-acid-poor BSA (4 mg/ml), 30 μM palmitoyl-CoA and 100 μM [$2\text{-}^{14}\text{C}$]malonyl-CoA. The reactions were started by addition of 0.1 ml of tissue extract and stopped after 10 or 20 min for extracts from whole heart and myocytes respectively. ATP citrate-lyase (EC 4.1.3.8), lactate dehydrogenase (EC 1.1.1.27), NADPH-cytochrome *c* reductase (EC 1.6.2.4) and succinate-dependent cytochrome *c* reductase (EC 1.3.99.1) were assayed spectrophotometrically at 25 °C as described in [48], [49], [50] and [51] respectively.

Measurement of DNA and protein

These were as described in [52] and [53] respectively.

Statistical methods

Statistical significance was assessed by Student's *t* test for paired or unpaired samples as appropriate. Values are expressed as means $\pm$ S.E.M.

RESULTS AND DISCUSSION

Metabolic performance as an index of myocyte viability

It was essential that the myocyte preparation used for estimation of enzyme activities (see below) should be intact and retain normal metabolic characteristics. Therefore, in addition to the visual indices of viability mentioned in the Materials and methods section, we also assessed the capability of the cells to oxidize [^{14}C]palmitate, together with the effects of carbohydrate fuels

Table 1 Effects of carbohydrate substrates and insulin on oxidation of [$1\text{-}^{14}\text{C}$]palmitate by cardiac myocytes

Myocytes were incubated as described in the Materials and methods section for 1 h with 0.5 mM [$1\text{-}^{14}\text{C}$]palmitate and fatty-acid-poor BSA (40 mg/ml). Concentrations of other indicated additions were: glucose, 5 mM; sodium lactate, 2 mM; insulin, 8 nM. In all cases the rates of $^{14}\text{CO}_2$ production are expressed relative to those in the presence of glucose + insulin. Abbreviation: n.s., not statistically significant ($P > 0.05$).

Expt. no.	<i>n</i>	Additions	Relative rate of palmitate oxidation	Statistical significance (paired <i>t</i> test)	
				Effect of insulin	Effect of glucose/lactate
1	8	None	283 $\pm$ 58	$P < 0.01$	$P < 0.025$
		Glucose	150 $\pm$ 16		
		Glucose + insulin	100		
2	4	None	271 $\pm$ 96	n.s.	$P < 0.025$
		Insulin	169 $\pm$ 19		
		Glucose + insulin	100		
3	6	None	188 $\pm$ 8	n.s.	$P < 0.005$
		Lactate	107 $\pm$ 9		
		Lactate + insulin	137 $\pm$ 16		
		Glucose + insulin	100		

and hormones on this process. Oxidation of [^{14}C]palmitate to $^{14}\text{CO}_2$ was linear with time for at least 2 h (results not shown). Supplementation of the incubation medium with L-carnitine did not enhance the rate of oxidation of palmitate to CO_2 , suggesting that the procedure for myocyte isolation did not cause any serious depletion of this important metabolite.

Table 1 shows that both glucose and lactate at physiological concentrations significantly decreased oxidation of palmitate to CO_2 by approx. 40%. In these particular experiments insulin caused a further significant decrease in palmitate oxidation, provided that glucose was present. No significant effects of insulin were seen in the absence of carbohydrate substrates or in the presence of lactate (Table 1). This effect of insulin to suppress fatty acid oxidation further was not always observed, however (see Exp. 1 of Table 2). It is suggested that this rather variable effect of insulin may be secondary to insulin's ability to stimulate glucose transport in the isolated cells and, in turn, this may depend on the activity and intracellular disposition of glucose transporters at the time of cell isolation. To facilitate comparison of different experimental sets, we expressed all these measurements of oxidation of palmitate to CO_2 relative to those seen with glucose + insulin present. This particular condition was chosen because it represented the least variable absolute rate of [^{14}C]palmitate oxidation. This was found to be $0.79 \pm 0.16 \mu\text{mol}$ of $^{14}\text{CO}_2$ /hour per mg of DNA ($n = 11$). Table 2 demonstrates that adrenaline increased oxidation to CO_2 by myocytes in a statistically significant 2-fold effect, which essentially overcame the suppressive effect of glucose on fatty acid oxidation. In the absence of glucose the increase in [^{14}C]palmitate oxidation was only approx. 30% and was not statistically significant ($P > 0.25$). The α_1 -adrenergic agonist phenylephrine significantly inhibited oxidation of palmitate to CO_2 when glucose + insulin were present. It is possible that this is due to enhancement of glucose oxidation resulting from an elevation of mitochondrial $[\text{Ca}^{2+}]$ leading to activation of pyruvate dehydrogenase phosphatase [54]. The β -adrenergic agonist isoprenaline, at least in part, mimicked the effect of adrenaline to increase palmitate oxidation and also overcame the inhibitory effect of phenylephrine.

The effects of insulin and adrenaline seen with these myocyte

Table 2 Effects of adrenergic agonists on oxidation of [$1\text{-}^{14}\text{C}$]palmitate by cardiac myocytes

Myocytes were incubated as described in the Materials and methods section for 1 h with 0.5 mM [$1\text{-}^{14}\text{C}$]palmitate and fatty-acid-poor BSA (40 mg/ml). Concentrations of other indicated additions were: glucose, 5 mM; insulin, 8 nM; adrenergic agonists, all at 5 μM . In all cases the rates of $^{14}\text{CO}_2$ production are expressed relative to those in the presence of glucose + insulin. Abbreviation: n.s., not statistically significant ($P > 0.05$).

Expt. no.	<i>n</i>	Additions	Relative rate of palmitate oxidation	Statistical significance of effects of adrenergic agonists (paired <i>t</i> test)
1	5	None	188 $\pm$ 29	n.s.
		Adrenaline	246 $\pm$ 45	
		Glucose	112 $\pm$ 12	
		Glucose + adrenaline	208 $\pm$ 31	$P < 0.025$
		Glucose + insulin	100	$P < 0.005$
2	7	Glucose + insulin + adrenaline	200 $\pm$ 16	
		Glucose + insulin	100	$P < 0.05$
		Glucose + insulin + phenylephrine	74 $\pm$ 12	
		Glucose + insulin + isoprenaline	140 $\pm$ 11	$P < 0.01$
		Glucose + insulin + isoprenaline + phenylephrine	130 $\pm$ 23	$P < 0.005$ (versus phenylephrine alone)

preparations can be qualitatively related to other effects noted in the perfused heart (see below).

Cytosolic enzymes involved in malonyl-CoA synthesis and disposal

Activities measured in 100000 *g* supernatants were compared between myocytes and whole heart and, in some instances, epididymal adipose tissue of the same rats (Table 3). The activity of ATP citrate-lyase was 4-fold higher ($P < 0.05$) in myocyte extracts than in those from whole heart. Acetyl-CoA-dependent incorporation of [^{14}C]bicarbonate into acid-stable products was seen in extracts from both whole heart and myocytes, with the myocyte extracts showing a 3-fold higher ($P < 0.05$) citrate-dependent carboxylase activity. There are two likely origins of the citrate-independent carboxylase activity. First, the mitochondrial enzyme propionyl-CoA carboxylase appears to be abundant in heart, has some activity with acetyl-CoA as substrate and is likely to appear in 100000 *g* supernatant fractions if mitochondria are damaged during tissue homogenization. For obvious reasons the tissue-homogenization procedure with isolated myocytes is performed in a more gentle manner than with the relatively tough pieces of whole heart tissue. Second, dephosphorylation of acetyl-CoA carboxylase (from rat liver) increases its citrate-independent activity (although the citrate-dependent activity is not greatly different for the dephospho- and the various phospho- forms of the enzyme) [55]. It is not known to what extent the isolation of myocytes followed by their homogenization and subcellular fractionation influences the phosphorylation state of the myocyte acetyl-CoA carboxylase. Fatty acid synthase activity could not be detected in 100000 *g* supernatants from whole heart by the spectrophotometric assay. As a precaution we checked whether heart extracts might contain an inhibitor of fatty acid synthase. However, this was discounted, since addition of heart 100000 *g* supernatant material to assays of fatty acid synthase in adipose tissue extracts caused no

Table 3 Enzyme activities in 100000 *g* supernatants

Values are means $\pm$ S.E.M. of the number of separate preparations indicated in parentheses and are expressed in pmol/min per mg of soluble protein; n.d., not detectable by the assay.

Enzyme assay	Tissue source		
	Whole heart	Isolated myocytes	Epididymal adipose tissue
ATP citrate-lyase	710 $\pm$ 110 (4)	2990 $\pm$ 820 (4)	9800 $\pm$ 890 (4)
Acetyl-CoA carboxylase			
(a) without Mg-citrate	95 $\pm$ 15 (8)	75 $\pm$ 30 (4)	—
(b) with Mg-citrate	170 $\pm$ 15 (8)	295 $\pm$ 70 (4)	—
(c) citrate-dependent activity [(b) — (a)]	75 $\pm$ 15	225 $\pm$ 55	—
Fatty acid synthase			
(a) spectrophotometric	n.d. (6)	—	6900 $\pm$ 400 (6)
(b) radiochemical			
(i) with acetyl-CoA + NADPH	186 $\pm$ 30 (6)	72 (2)	10900 (2)
(ii) without acetyl-CoA + NADPH	30 $\pm$ 10 (6)	93 (2)	100 (2)

inhibition (results not shown). In a more sensitive radiochemical assay some fatty acid synthase activity was detected as incorporation of [^{14}C]malonyl-CoA into lipids by 100000 *g* supernatants from whole heart tissue. Only approx. 15% of this incorporation was observable in blanks from which acetyl-CoA + NADPH had been omitted (likewise, omission of these co-substrates of fatty acid synthase decreased incorporation of malonyl-CoA by adipose-tissue extracts by 99%). By contrast, with 100000 *g* supernatants from isolated myocytes there was no 'true' fatty acid synthase activity detectable above the blank value. It was therefore concluded that the small amount of fatty acid synthase activity detected in extracts from whole heart must have originated from cells other than myocytes (e.g. endothelial cells, adipocytes etc.).

Fatty acid elongation activity

Some 86% of the total elongation activity as measured by incorporation of [$2\text{-}^{14}\text{C}$]malonyl-CoA into light-petroleum-soluble material was detected in the 9200 *g* pellet from myocyte homogenates. This pellet also contained 97% of the total succinate dehydrogenase activity and 92% of the total NADPH-cytochrome *c* reductase activity. Since 80% of the total lactate dehydrogenase activity was measured in the 100000 *g* supernatant, it was concluded that the relatively gentle homogenization conditions were sufficient to break the cells, but did not greatly disrupt the sarcoplasmic reticulum. Fatty acid elongation activity in the 9200 *g* pellet was 1.05 ± 0.08 nmol/min per mg of protein ($n = 14$). These assays contained 60 μM fatty-acid-poor BSA, and under these conditions incorporation of [^{14}C]malonyl-CoA was maximal with 30–60 μM palmitoyl-CoA. Higher concentrations of palmitoyl-CoA were inhibitory (results not shown). When assayed at 30 μM palmitoyl-CoA the K_m of the elongation system for malonyl-CoA was found to be 50 μM (Figure 1). Addition of NADPH or NADH to the assays had no effect on the incorporation of [^{14}C]malonyl-CoA: i.e. the rate of the condensation reaction of the elongation system did not appear to

Figure 1 Eadie-Hofstee plot to determine the K_m for malonyl-CoA of the fatty acid elongation system

The concentration of malonyl-CoA was varied from 10 to 600 μM ([S] given in mM). Palmitoyl-CoA was present at 30 μM . The values are from a representative experiment. Linear regression analysis ($r = -0.983$) indicated a K_m value of 50 μM .

be dependent on the subsequent activities of 3-oxoacyl-CoA reductase or *trans*-2-enoyl-CoA reductase. Some 17% of the total fatty acid elongation activity in myocyte homogenates was found in the 100 000 *g* supernatant. It is suggested, provided that this fraction contains some endogenous long-chain fatty acyl-CoA, that these traces of elongation activity may be responsible for the apparent fatty acid synthase activity seen in the absence of acetyl-CoA + NADPH (Table 3). It was concluded from these experiments that cardiac myocytes contain a particulate system that condenses malonyl-CoA into lipids. It is likely that this represents the fatty acid elongation system.

Acute regulation of malonyl-CoA content in the perfused heart

The values are shown in Table 4. As for the experiments with isolated myocytes, we have made statistical comparisons against perfusions that contained glucose+insulin. The hearts were from fed rats, and when perfused with glucose+insulin their malonyl-CoA content per g wet wt. was not significantly different from that measured *in vivo* in hearts from fed rats by McGarry et al. [35]. Omission of insulin from the perfusate significantly decreased the malonyl-CoA content by 41%. Even with insulin present, both palmitate and adrenaline decreased the malonyl-CoA content by 53% and 47% respectively, to values which were not significantly different from those measured *in vivo* in

hearts from 24 h- or 48 h-starved rats [35]. We also measured the contents of acid-precipitated alkali-labile (i.e. long-chain) esters of CoA and carnitine. Omission of insulin significantly increased the content of acylcarnitine. Perfusion with palmitate significantly increased the content of both long-chain esters. Perfusion with adrenaline also significantly increased the content of both long-chain esters, in particular acylcarnitine. It was also noteworthy that adrenaline substantially increased the content of the total long-chain ester pool. Presumably this is because adrenaline had increased lipolysis within myocytes and/or other cells within the heart.

General discussion

Scholte et al. [56] made use of the 'calcium paradox' to damage perfused hearts so that cytosolic contents leaked into the perfusate. A CO_2 -fixing activity that was dependent on citrate was detected in such perfusates. This activity was suggestive of acetyl-CoA carboxylase, but its cellular source was not determined. Before that, it had been claimed that acetyl-CoA carboxylase was absent from heart [57]. More recently, two groups [33,34] identified an acetyl-CoA carboxylase in rat heart. This has a subunit size ($M_r = 280\,000$) distinct from the isoform that is found predominantly in lipogenic tissues such as liver and adipose tissue ($M_r = 265\,000$). In these latter studies the cell type from which the cardiac carboxylase came was still not totally certain. Cardiac adipocytes can probably be discounted as a possible source, because adipose tissue appears to contain only the M_r -265 000 protein [34]; however, other non-myocytes such as endothelial cells could not be totally excluded. The present study shows unambiguously that cardiac myocytes contain a significant acetyl-CoA carboxylase activity.

The intracellular water space in rat heart is 0.5 ml/g wet wt. [58]. Assuming that malonyl-CoA contents as measured *in vivo* [35] or in perfused heart (Table 4) are reasonably representative of those in myocytes, crude 'global' concentrations of malonyl-CoA in myocytes may range from approx. 3 to 9 μM . These are at least one order of magnitude in excess of those that inhibit heart mitochondrial CPT₁ [30–32,59] and, of necessity, one must therefore expect most of the malonyl-CoA within myocytes to be bound to cell components other than CPT₁. The molecular abundance in heart cells of the condensing enzyme of the fatty acid elongation system is not known, but with a K_m for malonyl-CoA of 50 μM (Figure 1) it is possible that this enzyme could be a significant 'sink' for the binding of malonyl-CoA. In this respect it is noteworthy that heart crude mitochondrial fractions contain extensive overt low-affinity binding sites for malonyl-

Table 4 Metabolite contents of perfused hearts

Hearts were perfused for 45 min as described in the Materials and methods section with 5 mM glucose and fatty-acid-poor BSA (20 mg/ml). Concentrations of other indicated additions were: insulin, 8 nM; adrenaline, 5 μM ; sodium palmitate, 0.5 mM. ^A $P < 0.05$, ^B $P < 0.02$, ^C $P < 0.01$, ^D $P < 0.001$ for comparisons against perfusions with glucose + insulin. The values are means $\pm$ S.E.M. of 5–6 measurements in each case.

Expt. no.	Additions to perfusate	Heart wet wt. (g)	Metabolite content (nmol/g wet wt.)			
			Malonyl-CoA	Acyl-CoA (A)	Acylcarnitine (B)	[(A) + (B)]
1	Insulin	2.2 $\pm$ 0.1	3.9 $\pm$ 0.4	11.0 $\pm$ 2.1	35.1 $\pm$ 2.6	46.1 $\pm$ 3.5
	None	2.4 $\pm$ 0.1	2.3 $\pm$ 0.1 ^C	10.4 $\pm$ 1.3	45.9 $\pm$ 2.5 ^B	56.3 $\pm$ 2.0 ^A
2	Insulin	2.3 $\pm$ 0.3	3.0 $\pm$ 0.3	13.9 $\pm$ 2.3	36.1 $\pm$ 2.0	53.8 $\pm$ 2.3
	Insulin + palmitate	1.9 $\pm$ 0.1	1.4 $\pm$ 0.3 ^C	22.5 $\pm$ 2.8 ^A	48.0 $\pm$ 4.7 ^A	68.9 $\pm$ 7.6
3	Insulin	2.1 $\pm$ 0.1	3.6 $\pm$ 0.3	11.9 $\pm$ 1.5	39.9 $\pm$ 1.4	49.8 $\pm$ 2.0
	Insulin + adrenaline	2.1 $\pm$ 0.1	1.9 $\pm$ 0.5 ^B	18.6 $\pm$ 1.0 ^C	66.2 $\pm$ 5.9 ^C	84.7 $\pm$ 6.5 ^D

CoA (K_a determined as 30 μ M [31]). The possibility that these binding sites are related to the fatty acid elongation system merits further investigation.

Liver acetyl-CoA carboxylase is subject to complex acute regulation based on polymerization/depolymerization and protein phosphorylation/dephosphorylation. Conditions such as carbohydrate feeding or insulin administration lead to a more active polymerized form, whereas glucagon and adrenaline have the opposite effect [60–66]. In the longer term, expression of the liver carboxylase protein is also under nutritional and hormonal regulation (reviewed in [67]). Thampy [33] has shown that the heart isoform of acetyl-CoA carboxylase is activated by citrate, inhibited by palmitoyl-CoA and activated by incubation with a phosphoprotein phosphatase preparation. However, there have so far been no reports of whether the heart carboxylase can be acutely regulated by hormones such as insulin or adrenaline. Our findings (Table 4) suggest that this may be so, in that insulin increased the malonyl-CoA content and this effect was antagonized by adrenaline. The effect of adrenaline could, however, be indirect. Adrenaline increased the long-chain fatty acyl-CoA content (as also did palmitate), and it is possible that this is the signal which (presumably) inhibits the carboxylase, leading to a decrease in malonyl-CoA rather than a change in protein phosphorylation. Bianchi et al. [34] showed that, unlike the liver enzyme, the abundance of acetyl-CoA carboxylase in heart is not affected by fasting and refeeding. It is therefore possible that the decrease *in vivo* in heart malonyl-CoA content after fasting [35] was brought about by acute effects, as reported here with the isolated perfused heart.

Finally, although experimental conditions differ, one can make some qualitative comparisons between the effects of insulin to raise malonyl-CoA content in the perfused heart (Table 4) and to decrease oxidation of fatty acid to CO_2 by incubated myocytes (Table 1). Likewise the effects of adrenaline to decrease malonyl-CoA (Table 4) and to increase oxidation of fatty acid to CO_2 (Table 2) are likely to be related. In conclusion, this study provides evidence in support of the conjecture that malonyl-CoA plays a regulatory role in the myocyte, linking the control of CPT_1 to the activity of acetyl-CoA carboxylase.

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