Probing the link between citrate and malonyl-CoA in perfused rat hearts

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1Department of Biochemistry and 2Department of Nutrition, University of Montreal, Montreal, Québec, Canada H3C 3J7; 3Department of Nutrition, Case Western Reserve University, Cleveland, Ohio 44106-7139; and 4Department of Physiology, George Washington University, Medical Center, Washington, DC 20037-2337

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Poirier, Myriame, Geneviève Vincent, Aneta E. Reszko, Bertrand Bouchard, Joanne K. Kelleher, Henri Brunengraber, and Christine Des Rosiers. Probing the link between citrate and malonyl-CoA in perfused rat hearts. Am J Physiol Heart Circ Physiol 283: H1379–H1386, 2002—Little is known about the sources of cytosolic acetyl-CoA used for the synthesis of malonyl-CoA, a key regulator of fatty acid oxidation in the heart. We tested the hypothesis that citrate provides acetyl-CoA for malonyl-CoA synthesis after its mitochondrial efflux and cleavage by cytosolic ATP-citrate lyase. We expanded on a previous study where we characterized citrate release from perfused rat hearts (Vincent G, Comte B, Poirier M, and Des Rosiers C. Cytosolic release rates, ranging from 6 to 22 nmol/min, can support a net increase in malonyl-CoA concentrations induced by changes in substrate supply, at most 0.7 nmol/min. In experiments with [U-13C](lactate + pyruvate) and [1-13C]oleate, we show that the acetyl moiety of malonyl-CoA is derived from both pyruvate and long-chain fatty acids. This 13C-labeling of malonyl-CoA occurred without any changes in its concentration. Hydroxycitrate, an inhibitor of ATP-citrate lyase, prevents increases in malonyl-CoA concentrations and decreases its labeling from [U-13C](lactate + pyruvate). Our data support at least a partial role of citrate in the transfer from the mitochondria to cytosol of acetyl units for malonyl-CoA synthesis. In addition, they provide a dynamic picture of malonyl-CoA metabolism: even when the malonyl-CoA concentration remains constant, there appears to be a constant need to supply acetyl-CoA from various carbon sources, both carbohydrates and lipids, for malonyl-CoA synthesis.

THE INTRACELLULAR CONCENTRATION of malonyl-CoA modulates a number of physiological and pathophysiological events. These effects of malonyl-CoA are related to its inhibition of carnitine palmitoyl transferase 1. The latter controls the entry of long-chain acyl-CoA into mitochondria, where they are β-oxidized for energy production. In addition, carnitine palmitoyl transferase 1 activity affects cytosolic concentrations of long-chain acyl-CoAs, which influence signal transduction and binding of nuclear transcription factors (5, 16, 19, 42). In lipogenic tissues such as the liver, malonyl-CoA is both a modulator of fatty acid oxidation and an intermediate of fatty acid synthesis (25). In nonlipogenic tissues such as the heart and skeletal muscle, cytosolic malonyl-CoA modulates fatty acid oxidation and is a component of a fuel-sensing and signaling mechanism that responds to changes in the cell’s substrate supply and energy expenditure (31, 33). Recently, a dysregulated malonyl-CoA metabolism has been implicated in insulin resistance (33), apoptosis (19), and functional recovery of the heart after ischemia (20).

In the heart, the regulatory role of cytosolic malonyl-CoA is supported by the kinetic and regulatory properties of enzymes involved in its metabolism (13, 14, 20, 23, 33, 39). Malonyl-CoA is synthesized by the predominant 280-kDa isofrom of acetyl-CoA carboxylase (ACC) or ACCβ. This enzyme is regulated at the transcriptional and posttranslational levels, the latter via phosphorylation by 5′-AMP-activated protein kinase and protein kinase A. It is also activated by citrate and inhibited by long-chain acyl-CoA. Because tissue acetyl-CoA levels are lower than the Km of ACCβ for acetyl-CoA (117 μM), the ACCβ activity seems to be substrate driven. The only known fate of cardiac malonyl-CoA is recycling to acetyl-CoA via malonyl-CoA decarboxylase (MCD). Much less is known about the regulation of MCD compared with ACCβ. Small variations in the energy demand of the heart could modulate MCD activity and results in rapid changes in malonyl-CoA levels (13, 17). It is unclear as to whether cardiac
MCD is regulated by phosphorylation as in the extensor digitorum longus muscle (14, 35).

Several issues regarding the proposed mechanism of fuel regulation by malonyl-CoA still remain unresolved. First, how can one explain that long-chain fatty acids (LCFA) are \( \beta \)-oxidized at an intracellular malonyl-CoA concentration that should inhibit completely the prevailing muscle isoform of carnitine palmitoyl transferase? Proposed explanations include malonyl-CoA protein binding and/or compartmentation (3, 13, 15, 18, 25). Second, how are cytosolic acetyl-CoA and malonyl-CoA levels correlated with fat and carbohydrate oxidation and the citric acid cycle (CAC) flux in mitochondria? Third, what is the source(s) of cytosolic acetyl-CoA for malonyl-CoA synthesis? One hypothesis is that acetyl-CoA generated by mitochondrial pyruvate dehydrogenase is channeled to carnitine acetyl transferase and translocase (2, 23, 24). A second hypothesis is that mitochondrial acetyl-CoA is transferred to the cytosol via citrate, the citrate transporter and cytosolic ATP-citrate lyase. The activities of enzymes involved in mitochondrial transfer of acetyl units via citrate or acetylcarnitine are small compared with the CAC flux rate (1, 4, 10, 27, 36). However, these activities could be sufficient to maintain the very small pool of malonyl-CoA (between 0.2 and 4 nmol/g wet wt) (4, 17, 20, 23, 37).

We previously demonstrated a modulation of mitochondrial citrate efflux in perfused rat hearts, reflected by rates of citrate release (40). In the present study, we examined the link between mitochondrial citrate synthesis/efflux and malonyl-CoA synthesis. To test the hypothesis that mitochondrial acetyl-CoA is transferred to the cytosol via citrate, we used hydroxycitrate, an inhibitor of ATP-citrate lyase (41). We also investigated the sources of the acetyl moiety of malonyl-CoA with \(^{13}\text{C}\)pyruvate and oleate by mass isotopomer analysis (8) to test the hypothesis of a preferential channeling of pyruvate-derived acetyl-CoA to malonyl-CoA.

**EXPERIMENTAL PROCEDURES**

**Chemicals**

The sources of chemicals, biological products, and \(^{13}\text{C}\)-substrates have been identified previously (11, 12, 40). \(^{[3H]}\)acetyl-CoA (25 \( \mu \)Ci/mol) was obtained from ICN (Montreal, Quebec, Canada). Hydroxycitrate (Citrin K; Garcinia Cambogia Extract Potassium Salt) was a generous gift from Dr. V. Badmaev of Sabinsa (Piscataway, NJ). The dialyzed albumin solution (13.4%, BSA fraction V, fatty acid poor, Bayer; Kankakee, IL) and the stock solution of 20 mM sodium oleate complexed to albumin were prepared and stored as described previously (40).

**Heart Perfusions**

Animal experimentation was approved by the local animal ethics committee in compliance with guidelines of the Canadian Council on Animal Care. Procedures for isolation and perfusion of rat hearts in the Langendorff mode have been described elsewhere (11, 12, 40). Briefly, the hearts of fed male Sprague-Dawley rats (180–210 g, Charles River Breeding Laboratories; St-Constant, Quebec, Canada) were perfused at a constant pressure of 70 mmHg with nonrecirculating modified Krebs-Henseleit buffer (pH 7.4) containing 119 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl\(_2\), 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 25 mM NaHCO\(_3\), 5.5 mM glucose, and 8 mM insulin as well as other additives indicated below. The setup for continuous monitoring of functional parameters, using instruments linked to a microcomputer, has been described earlier (40). At the end of the experiments, the hearts were freeze clamped and stored in liquid nitrogen.

**Perfusion Protocols**

A two 30-min step protocol was designed to document the effect of selected metabolic perturbations that differentially affected the citrate release rate (40) on malonyl-CoA \( I \) net synthesis, as reflected by an increase in its tissue levels over 30 min; and 2) \(^{13}\text{C}\)-labeling. All hearts underwent an initial 30-min perfusion with 5.5 mM glucose, 8 mM insulin, 0.5 mM lactate, and 0.05 mM pyruvate to ascertain that the tissue [13C]-labeling. All hearts underwent an initial 30-min perfusion with 5.5 mM glucose, 8 mM insulin, 0.5 mM lactate, and 0.05 mM pyruvate to ascertain that the tissue level of malonyl-CoA would be the same at the beginning of all metabolic interventions. One group of hearts was freeze clamped after 30 min. Parameters measured in effluent or tissue for this 0- to 30-min period served as baseline values. All other groups of hearts underwent an additional 30 min of perfusion before freeze clamping. In addition to the substrates listed in Table 1, the perfusion buffer contained 5 mM glucose and 8 mM insulin. The buffer composition for protocol \( I \), which served as a control, is that used for the baseline period. Buffer modifications for the 30- to 60-min period included an increase in the concentration of pyruvate from 0.05 to 0.5 mM, the addition of a fatty acid, either 0.4 mM oleate, a LCFA complexed to 4% albumin, or 0.2 mM octanoate and 0.2 mM hydroxycitrate. All hearts underwent initial 30-min perfusion with nonrecirculating modified Krebs-Henseleit buffer containing 5.5 mM glucose, 8 mM insulin, 0.5 mM lactate, and 0.05 mM pyruvate. One group of hearts was quick frozen at 30 min. Parameters measured in effluent or tissue for this group served as baseline values. All other groups of hearts underwent an additional 30 min of perfusion (protocols 1–5A) with modifications of the substrates present in the buffer (except for protocol \( I \), which served as a control). In addition to the substrates listed, the perfusion buffer contained 5 mM glucose and 8 mM insulin.

**Table 1. Protocols of heart perfusion experiments**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Lactate, mM</th>
<th>Pyruvate, mM</th>
<th>Oleate, mM</th>
<th>Octanoate, mM</th>
<th>Other Additions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>0.5 (^{13}\text{C})</td>
<td>0.05 (^{13}\text{C})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>0.2</td>
<td>0.4</td>
<td>4% albumin, 50 ( \mu )M carnitine</td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>1.0 (^{13}\text{C})</td>
<td>0.2 (^{13}\text{C})</td>
<td>0.4 (^{13}\text{C})</td>
<td>4% albumin, 50 ( \mu )M carnitine</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>0.2</td>
<td></td>
<td>1 mM hydroxycitrate</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>0.05</td>
<td></td>
<td>1 mM hydroxycitrate</td>
<td></td>
</tr>
<tr>
<td>5A</td>
<td>0.5 (^{13}\text{C})</td>
<td>0.05 (^{13}\text{C})</td>
<td></td>
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</tr>
</tbody>
</table>

All hearts underwent initial 30-min perfusion with nonrecirculating modified Krebs-Henseleit buffer containing 5.5 mM glucose, 8 mM insulin, 0.5 mM lactate, and 0.05 mM pyruvate. One group of hearts was quick frozen at 30 min. Parameters measured in effluent or tissue for this group served as baseline values. All other groups of hearts underwent an additional 30 min of perfusion (protocols 1–5A) with modifications of the substrates present in the buffer (except for protocol \( I \), which served as a control). In addition to the substrates listed, the perfusion buffer contained 5 mM glucose and 8 mM insulin.

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ate, a medium-chain fatty acid, and the addition of 1 mM hydroxycitrate. Note that albumin was present only in the LCFA group. The ionized calcium and endogenous free fatty acid concentrations of the 4% albumin-containing buffer were determined to be 1.2 and 0.3 mM, respectively. The total free fatty acid concentration for the LCFA group was 0.7 mM. For protocols 1A, 3A, and 5A, the buffer composition was identical to that of protocols 1, 3, and 5, respectively, except that unlabeled lactate, pyruvate, and oleate were replaced with [U,13C3](lactate + pyruvate) and [1-13C]oleate. For protocol 3A, [U,13C3](lactate + pyruvate) was present for the 0- to 60-min period and [1-13C]oleate was present for the 30- to 60-min period. For protocols 1A and 5A, in which hearts were perfused in the absence or in the presence of 1 mM hydroxycitrate, respectively, [U,13C3](lactate + pyruvate) was present for the last 20 min of the 30- to 60-min time period. Thus for protocol 5A, hearts were perfused for 10 min in the presence of 1 mM hydroxycitrate to allow for ATP-citrate lyase inhibition before the addition of 13C-substrates.

The following constraints were linked to the use of hydroxycitrate. First, hydroxycitrate was available as the potassium salt. The total potassium concentration in the perfusion buffer was maintained at 4.8 mM by lowering its potassium chloride content to 2.1 mM. Second, because hydroxycitrate lowers the ionized calcium concentration from 0.94 ± 0.01 to 0.68 ± 0.01 mM (P < 0.05, n = 9, unpaired t-test), although it did not change the total calcium concentration (1.23 ± 0.03 mM, n = 9). Increasing the total calcium concentration of the buffer containing hydroxycitrate to 1.7 mM reestablished its ionized calcium concentration to 0.98 ± 0.02 mM (n = 5). Third, there was a background contamination by citrate in the hydroxycitrate solution (0.5% or 50 μM in a 1 mM solution) that prevented the assessment of citrate release rates.

Analytic Procedures

Gas chromatography-mass spectrometry (GC-MS) methods for the determination of citrate release (40) and 13C mass isotopomer distribution (MID) of tissue malonyl-CoA (32) have been described previously. Tissue malonyl-CoA levels were measured radioenzymatically using a modification of the procedure of McGarry and Brown (26). In brief, 0.05 g wet wt of powdered hearts was homogenized in 1 ml of 10% trichloroacetic acid, followed by centrifugation at 14,000 rpm for 15 min at 4°C. Pellets were kept for protein determination by the Bradford method (6). Supernatants were washed six times with 1 ml of ether, dried in a Speed-Vac, and stored at −80°C. The incubation mixture for malonyl-CoA assay (final volume 1 ml) contained 50 μl lyophilized extract (resuspended in 1 M potassium phosphate buffer-10 mM EDTA (pH 7.1)), 200 μmol potassium phosphate buffer (pH 7.1), 2 μmol EDTA, 2.5 μmol dithiothreitol, 0.2 μmol NADPH, 1 mg BSA, 0.75 mmol [3H]acetyl-CoA (0.9 μCi/nmol), and 5 μM rat liver fatty acid synthase (21). All assays were calibrated by spiking duplicate samples with 0.0625 nmol malonyl-CoA. We verified the linearity of the assay buffer samples were conducted by the Clinical Biochemistry Laboratory of Notre-Dame Hospital.

Calculations, Data Presentation, and Statistical Analyses

13C-labeling of metabolites. Mass isotopomers of malonyl-CoA containing 1 and 2 13C atoms are identified as M, with i = 1 and 2. The absolute molar percent enrichment (MPE) of a given mass isotopomer Mi was calculated as follows

MPE (Mi) = Percent Am/[Am + ∑Am] (1)

where Am and Ai represent the peak areas from GC ion chromatograms corrected for natural abundance, corresponding to unlabeled (M) and 13C-labeled (Mi) mass isotopomers, respectively.

Data are expressed as means ± SE for n heart perfusions. As an index of the contractile activity of the heart, we report only dp/dtmax values, which were constant and hence averaged for a given 30-min period. Statistical analyses were conducted by either paired t-test (dp/dtmax and citrate release), unpaired t-test (13C-labeling of malonyl-CoA), one-way ANOVA followed by a Bonferroni multiple-comparison posttest (tissue citrate levels), or nonparametric Kruskal-Wallis one-way ANOVA followed by Dunn’s multiple-comparison test (tissue malonyl-CoA levels). We tested for differences between values obtained for hearts perfused during the 30- to 60-min period to those obtained during the 0- to 30-min baseline period and for the effect of hydroxycitrate. A probability of P < 0.05 was considered to be significant.

RESULTS

Cardiac Contractile Parameters

In the baseline period, perfused hearts maintained a constant spontaneous beating at 309 ± 5 beats/min, a coronary flow rate of 11 ± 1 ml/min, a left ventricular developed pressure of 113 ± 2 mmHg, a rate-pressure product of (34.9 ± 0.8) × 10−3 mmHg·beats·min−1 (not shown), and a dp/dtmax of 3,267 ± 167 mmHg/s (Fig. 1, left). Compared with the baseline period (0–30 min), the contractile activity (dp/dtmax) decreased in the 30- to 60-min period in all groups except in hearts perfused
with 0.5 mM pyruvate (Fig. 1, middle). Hydroxycitrate addition to control hearts resulted in a 20% decline of dP/dt\text{\textsubscript{max}} values (Fig. 1, right). This effect of hydroxycitrate was not due to calcium chelation (9), because it was also observed after normalization of the buffer concentration of ionized calcium (not shown). Changes in dP/dt\text{\textsubscript{max}} values paralleled those of the left ventricular developed pressure and of the rate-pressure product, whereas values of coronary flow rate and heart rate remained similar to the baseline values (not shown).

**Citrate Efflux and Malonyl-CoA Concentrations**

Under all conditions, citrate release rates were constant for a given 30-min period, ranging from 7 to 22 nmol/min. In control hearts, citrate release rates (Fig. 2A) and tissue levels (Fig. 2B) were the same in the 30- to 60-min period than in the baseline period. Increasing pyruvate concentration from 0.05 to 0.5 mM, or adding octanoate or oleate to the perfusion buffer during the 30- to 60-min period, increased citrate release rates and tissue citrate levels in parallel. A similar correlation between citrate release rates and tissue levels was observed in our previous study in normoxic Langendorff-perfused rat hearts (40), although not in the swine heart in vivo (28).

In hearts perfused under control conditions and in those perfused with 0.5 mM pyruvate, malonyl-CoA levels were similarly increased by 30% at 60 min compared with 30 min (Fig. 2C), reflecting net malonyl-CoA synthesis. Malonyl-CoA levels were not increased in the presence of oleate but markedly increased in the presence of octanoate, in agreement with recent data from Longnus et al. (22).

Addition of hydroxycitrate did not modify tissue citrate levels (Fig. 3A). However, it prevented the increase in tissue malonyl-CoA levels during the 30- to 60-min period (Fig. 3B). This effect was not observed in perfusions with 1 mM citrate (not shown).

**Sources of Acetyl Moiety for Malonyl-CoA Synthesis**

We recently developed a technique to assess the 13C MID of malonyl-CoA (32). With the use of this technique, we probed the origin of the acetyl moiety of malonyl-CoA using [U-13C3](lactate + pyruvate) and [1-13C]oleate in the same perfusion experiments (protocol 3A). Decarboxylation of [U-13C3]pyruvate forms M2 isotopomers of acetyl-CoA and malonyl-CoA, whereas [1-13C]oleate oxidation forms M1 isotopomers. Note that in these nonrecirculating perfusions with 25 mM bicarbonate buffer, the incorporation of 13CO\text{\textsubscript{2}} derived from the oxidation of the 13C-substrates is negligible. This is demonstrated by the absence of the M3 isotopomer of malonyl-CoA.

In hearts perfused with [U-13C3](lactate + pyruvate) and [1-13C]oleate, malonyl-CoA was enriched in both M1 and M2 isotopomers (Fig. 4A). From these M2 and M1 enrichment values, one can estimate the percentage of malonyl-CoA molecules that arose from acetyl-CoA generated by pyruvate decarboxylation and oleate oxidation, respectively, following a reasoning similar to that used previously for estimating the relative contribution of these pathways to the acetyl moiety of citrate (cf. Eqs. 5 and 6 of Ref. 11, respectively). Note that this calculation does not require any corrections for recycling of [13C]citrate isotopomers in the TCA cycle, but it is subject to some uncertainties because it assumes the existence of only one pool of malonyl-CoA (3, 13, 15, 18).

![Fig. 2. Citrate release rates (A) and tissue levels of citrate (B) and of malonyl-CoA (C) of hearts perfused with various substrate mixtures. Data shown are means ± SE for 5–11 experiments. Hearts were perfused for 30 (left) and 60 min with 5 mM glucose, 8 nM insulin, and other substrates (right), as indicated below. Citrate release rates were averaged for the 15- to 30- and 45- to 60-min periods. *P \leq 0.05, all data at 30–60 min versus those at 0–30 min. gww, Grams wet weight.](http://ajpheart.physiology.org/)
DISCUSSION

The goal of the present study was to shed some light on the sources of the acetyl moiety of cytosolic malonyl-CoA in the heart. There are two possible pathways for transferring acetyl units from mitochondria to the cytosol: 1) citrate transporter and ATP-citrate lyase and 2) carnitine acetyl translocase and transferase (Fig. 5). The activities of enzymes involved in the two putative processes, albeit low (1, 4, 10, 27, 36), seem sufficient to maintain the very small pool of malonyl-CoA in the heart. We decided to investigate first the citrate pathway because our previous study (40) had identified a sizeable citrate efflux from the perfused rat heart (5–25 nmol/min). This efflux of citrate amounts to 0.2–1% of CAC flux and is modulated by the nature of the substrates offered to the heart and by energy demand.

In the present study, we confirmed the relationship between citrate release rates and tissue content (40) (Fig. 2, A and B). In addition, we compared changes in citrate release and in malonyl-CoA tissue levels induced by modifications of substrate supply (Fig. 2, B and C). No correlation was found, but citrate release rates were far in excess of increases in malonyl-CoA content based on the following. Malonyl-CoA concentrations were measured 30 min after changing the substrate profile in the perfusate. Most likely, the various substrate mixtures affected differentially the supply of substrate (acyt-CoA) and/or the levels of allosteric regulators of ACC (citrate and long-chain acyl-CoA). Malonyl-CoA accumulation over time could have resulted from an increased substrate flux through ACC with no change or a decrease in flux through MCD. We do not know the time course of these changes, but it is likely to have been fairly rapid (4, 13, 17, 23, 37). Because the half-life of malonyl-CoA in similarly perfused rat hearts is ~1.5 min (32), let us assume that the changes in malonyl-CoA concentrations occurred over 5 min. The net rate of malonyl-CoA accumulation would then be 0.2–0.7 nmol·min⁻¹·g⁻¹.

Fig. 3. Effect of HC on tissue levels of citrate (A) and malonyl-CoA (B) in perfused hearts. Data shown are means ± SE of 4 and 7 experiments with 5.5 mM glucose, 0.5 mM [U-13C₃]lactate, and 0.05 mM [U-13C₃]pyruvate in the absence (control conditions; –HC) or in the presence of 1 mM HC, respectively. The data, obtained during the 30- to 60-min period, are expressed as percentages of corresponding baseline data obtained for hearts perfused under control conditions during the 0- to 30-min period. *P < 0.05, effect of HC; values compared with control for the 30- to 60-min period.

From the M2 enrichment of malonyl-CoA (9.9%), one can calculate a 10% contribution of exogenous pyruvate decarboxylation to the acetyl moiety of malonyl-CoA. To calculate the contribution of exogenous fatty acid oxidation, one has to consider two factors: 1) only one of nine acetyl units of the oleate molecule was labeled with ¹³C, and 2) the albumin preparation contained 0.3 mM unlabelled free fatty acids, which must be added to the 0.4 mM concentration of [1-¹³C]oleate. Therefore, the contribution of exogenous fatty acids to the acetyl moiety of malonyl-CoA was obtained by multiplying the M1 enrichment of malonyl-CoA (5.8%) by [9 × (0.4 + 0.3/0.4), yielding 91%. Note that this value of 91% probably overestimates slightly the true contribution of exogenous fatty acids to malonyl-CoA because some unlabeled free fatty acids in the albumin preparation could have less than nine acetyl units (e.g., palmitate).

We also tested the effect of hydroxycitrate on malonyl-CoA labeling from [U-¹³C₃]lactate + pyruvate (protocols 1A and 5A). Hydroxycitrate decreased slightly (22%), but significantly, the M2 enrichment of malonyl-CoA (Fig. 4B).
wet wt \(^{-1}\). This compares to citrate release rates ranging from 7 to 22 nmol/min. Note that citrate release rates represent the difference between citrate efflux from mitochondria and citrate disposal via cytosolic ATP-citrate lyase. These observations are compatible with citrate being involved in the transfer of acetyl units.

We used hydroxycitrate, an inhibitor of ATP-citrate lyase (41), to further test the participation of the citrate pathway in the heart. Saha et al. (34) have shown a decrease in malonyl-CoA concentrations in rat muscle incubated in the presence of hydroxycitrate. In our study, the addition of 1 mM hydroxycitrate to the perfusate prevented the increase in malonyl-CoA concentration over baseline levels (Fig. 3B). This effect was observed despite 1) a lowering of the contractile activity of the heart, a condition that could potentially increase the activity of ACC and/or decrease that of MCD (17, 33); and 2) the known capacity of hydroxycitrate to act as an allosteric activator of ACC (34). In fact, each of these two factors should have favored an increase, rather than a decrease, in tissue malonyl-CoA levels. These observations are also compatible with the participation of cytosolic ATP-citrate lyase in the generation of acetyl-CoA for malonyl-CoA synthesis.

Additional arguments for the citrate pathway were obtained from experiments with \(^{13}\)C-labeled substrates. First, in perfusions with \([U-^{13}\text{C}_3]\text{lactate + pyruvate}\) and \([1-^{13}\text{C}]\text{ooleate}\), we showed that acetyl units used for malonyl-CoA synthesis are generated from both decarboxylation of exogenous pyruvate/lactate and \(\beta\)-oxidation of exogenous LCFA. The relative contributions of these two processes to malonyl-CoA formation (10% and 90%) are similar to those reported for the acetyl moiety of citrate in rat hearts perfused under similar conditions (40). Despite some uncertainties in the calculation of these estimates, these data argue against a preferential channeling of pyruvate-derived acetyl-CoA to malonyl-CoA via acetylcarnitine transferase/translocase (2, 23, 24). In studies conducted by Lysiak et al. (24) in isolated rat heart mitochondria, the formation of acetylcarnitine from pyruvate was favored by low concentrations of catalytic intermediates of the CAC. Thus, in the presence of high carnitine concentration, the formation of acetylcarnitine had to be the major fate of acetyl-CoA derived from pyruvate dehydroxylation. Furthermore, the decrease in acetylcarnitine formation upon addition of malate is probably explained by the incorporation of acetyl groups into citrate. In the present study, the mix of substrates provided (some of them anaplerotic) presumably insured adequate constant concentrations of CAC intermediates as reflected by a sizeable citrate efflux.

Hydroxycitrate prevented the increase in tissue malonyl-CoA levels and lowered the extent of \(^{13}\)C-labeling of malonyl-CoA from \([U-^{13}\text{C}_3]\text{lactate + pyruvate}\). Thus, despite the presence of 1 mM hydroxycitrate, some acetyl units derived from \([U-^{13}\text{C}_3]\text{lactate + pyruvate}\) were converted to malonyl-CoA. These data can be interpreted in two ways. First, the concentration of hydroxycitrate (1 mM) might not have been sufficient to fully inhibit ATP-citrate lyase. Hydroxycitrate, a tri-anion at physiological pH, does not permeate easily through cell membranes. This is why the concentration of malonyl-CoA in incubating muscle (34) decreased as hydroxycitrate concentrations were raised from 1 to 4 mM. This also explains why the \(K_i\) for hydroxycitrate of isolated ATP-citrate lyase (0.05–
0.6 μM (38, 41) is three to four orders of magnitude lower than the concentration of hydroxycitrate required to inhibit fatty acid synthesis by 50% in the intact liver (2 mM) (7). Second, it is possible that the citrate-cleavage pathway and the acetylcarntine transferase/translocase pathway both contribute to the acetyl moiety of malonyl-CoA, at least under some circumstances.

In conclusion, our data support the role of citrate in the transfer of acetyl units from the mitochondria to cytosol in the heart. Results from this and our previous study (32) show that measurements of malonyl-CoA concentrations and related enzyme activities provide only a partial picture of malonyl-CoA metabolism. Cycling between acetyl-CoA and malonyl-CoA does not change the malonyl-CoA concentration in the heart, where the only known fate of malonyl-CoA is degradation to acetyl-CoA. However, cytosolic acetyl-CoA might be hydrolyzed to acetate as occurs in numerous organs (30) or converted to acetylcarntinate before export. Thus, although the low level of malonyl-CoA in cytosol remains fairly constant, there is probably a constant need to supply acetyl-CoA to ACCβ. This study with 13C-substrates provides a dynamic picture of the carbon sources and fluxes leading to malonyl-CoA even when its concentration does not change. Results from this study do not exclude, however, a contribution of the acetylcarntinate pathway in the transfer of acetyl units from the mitochondria to cytosol. The citrate and the acetylcarntinate pathways might coexist and ensure a constant supply of acetyl-CoA to ACCβ under various conditions. One factor that could determine the contributions of the two pathways could be the relative availability of anaplerotic substrates versus acetyl-CoA and carntinate in mitochondria. Another potential factor is the regulation of ATP:citrate lyase activity by covalent modification (29). Further studies will investigate the respective roles of citrate and acetylcarntinate pathways to the supply of acetyl moiety of malonyl-CoA.

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