Citrate release by perfused rat hearts: a window on mitochondrial cataplerosis

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Vincent, Genevieve, Blandine Comte, Myriame Poirier, and Christine Des Rosiers. Citrate release by perfused rat hearts: a window on mitochondrial cataplerosis. Am J Physiol Endocrinol Metab 278: E846–E856, 2000.—Cytoplasmic citrate is proposed to play a crucial role in substrate fuel selection in the heart. However, little is known about factors regulating the transfer of citrate from the mitochondria, where it is synthesized, to the cytosol. Further to our observation that rat hearts perfused under normoxia release citrate whose 13C labeling pattern reflects that of mitochondrial citrate (B. Comte, G. Vincent, B. Bouchard, and C. Des Rosiers, J. Biol. Chem. 272: 26117–26124, 1997), we report here data indicating that this citrate release is a specific process reflecting the mitochondrial efflux of citrate, a process referred to as cataplerosis. Indeed, measured rates of citrate release, which vary between 2 and 21 nmol/min, are modulated by the nature and concentration of exogenous substrates feeding acetyl-CoA (fatty acid) and oxaloacetate (lactate plus pyruvate) for the mitochondrial citrate synthase reaction. Such release rates that represent at most 2% of the citric acid cycle flux are in agreement with the activity of the mitochondrial tricarboxylic acid transporter whose participation is also substantiated by 1) parallel variations in citrate release rates and tissue levels of citrate plus malate, the antiporter, and 2) a lowering of the citrate release rate by 1,2,3-benzenetricarboxylic acid, a specific inhibitor of the transporter. Taken together, the results from the present study indicate that citrate cataplerosis is modulated by substrate supply, in agreement with the role of cytosolic citrate in fuel partitioning, and occurs, at least in part, through the mitochondrial tricarboxylic acid transporter.

energy metabolism; citric acid cycle; 13C mass isotopomer analysis; mitochondrial tricarboxylic acid transporter

As the first intermediate committed to the citric acid cycle (CAC), citrate plays a crucial role in cardiac energy metabolism. However, its metabolic role may not be limited to the mitochondria. Indeed, cytosolic citrate is proposed to also play a central role in substrate fuel partitioning (for a recent review see Ref. 28). An increase in cytosolic citrate, after its efflux from the mitochondria where it is synthesized, could restrict 1) glucose utilization by inhibiting glycolysis at the level of phosphofructokinase (PFK; see Refs. 11 and 8 for review) and/or 2) long-chain fatty acid (LCFA) β-oxidation after its conversion to malonyl-CoA, an inhibitor of carnitine palmitoyltransferase I (CPT-I; see Refs. 1, 19, 20, 21, 31). The regulatory role of cytosolic citrate has long been recognized in lipogenic tissues such as the liver and adipose tissues, where it also supplies acetyl units for de novo fatty acid synthesis. In nonlipogenic tissues, a generalized theory of fuel sensing was formulated recently on the basis of experimental evidence obtained in β-cells (26) and skeletal muscle (28, 30, 31) whereby cytosolic citrate would act as a signal to cells that they have an excess of fuel for their immediate needs. Further investigations are, however, required to support such a mechanistic scheme in the heart.

In cardiac cells, a regulatory role for cytosolic citrate as an inhibitor of glycolysis was first proposed in 1963 by Garland et al. (11). However, the significance of mitochondrial citrate efflux, or cataplerosis, was questioned because of the low activity of the mitochondrial tricarboxylate transporter (5, 32). Nevertheless, a mitochondrial citrate efflux was demonstrated in isolated heart mitochondria (5, 14, 27). This efflux, which occurred in response to added malate, was favored at a high NADH-to-NAD+ concentration ratio or state 4 respiration (14) and was restricted by 1,2,3-benzenetricarboxylic acid (BTC), a specific inhibitor of the mitochondrial tricarboxylate transporter (5, 23, 27). To the best of our knowledge, mitochondrial citrate cataplerosis has not been examined in the intact heart. However, cardiac citrate release has been documented in vivo in humans (33, 34) and ex vivo in the perfused rat heart (6, 7). Of potential clinical relevance, myocardial citrate release was increased in patients with coronary artery disease in a proportion that correlated with disease severity (33, 34). Furthermore, a relationship between cardiac citrate release and mitochondrial citrate cataplerosis was suggested by our finding (6, 7) that rat hearts perfused with 13C-labeled substrates release citrate whose 13C labeling pattern reflects that of mitochondrial citrate. This finding, and the possibility that a dysregulated myocardial citrate release represents a specific chronic alteration of energy metabolism in patients with ischemic disease, prompted us to further examine this process.

The purpose of the present study was to elucidate factors modulating citrate release by the perfused rat heart. Citrate release rates were quantitated by isotope...
dilution gas chromatography-mass spectrometry (GC-MS) and flow rate measurements in hearts perfused under normoxia with various substrate mixtures, including physiological LCFA. In view of the crucial role of mitochondrial citrate in the CAC, we hypothesized that, if citrate release reflects mitochondrial citrate cataplerosis, it should be specifically modulated by 1) the energy demand, which is a determinant of citrate utilization by the CAC and 2) the supply of substrates for citrate synthesis, namely acetyl-CoA and anaplerotic oxaloacetate (OAA). Furthermore, the release of citrate should not be correlated with that of 1) lactate dehydrogenase (LDH), an index of membrane damage, or 2) the CAC intermediate succinate, an index of oxygen deprivation (16). Finally, provided that citrate release involves the mitochondrial tricarboxylate transporter, it should be inhibited by 1,2,3-BTC but not by its inactive chemical analog 1,2,4-BTC.

**EXPERIMENTAL PROCEDURES**

**Chemicals**

Chemicals, organic solvents, enzymes, coenzymes, insulin, and BSA (fraction V) were purchased from Boehringer Mannheim (Laval, Quebec), Fisher Scientific (Montreal, Quebec), Sigma-Aldrich Chemicals (Milwaukee, WI), Anachemia (Dorval, Quebec), and ICN Canada (Montreal, Quebec). [2,2,3,3-2H4]succinate, [2,2,4,4-2H4]citrate, [U-13C3]lactate (99%), Sigma-Aldrich Chemicals (Milwaukee, WI), Anachemia (Dorval, Quebec), and Fisher Scientific (Montreal, Quebec), and BSA (fraction V) were purchased from Boehringer Mannheim Chemicals, organic solvents, enzymes, coenzymes, insulin, and BSA (fraction V) were purchased from Boehringer Mannheim (Laval, Quebec), Fisher Scientific (Montreal, Quebec), Sigma-Aldrich Chemicals (Milwaukee, WI), Anachemia (Dorval, Quebec), and ICN Canada (Montreal, Quebec). [2,2,3,3-2H4]succinate, [2,2,4,4-2H4]citrate, [U-13C3]lactate (99%), [U-13C]pyruvate (99%), [1-13C]oleic acid, and [1,2-13C2]octanoic acid (99%) were obtained from Isotec (Miamisburg, OH), Cambridge Isotopes Laboratories (Woburn, MA), and CDN Isotopes (Pointe-Claire, Quebec). RS-3-hydroxy-[2,2,3,4,4-2H4]butyrate (RS-[2H4]-BHB) and [1,2,3,6-13C4]citrate were synthesized as described previously (9, 15). The derivatization agent N-methyl-N-(t-butyldimethylsilyl))trifluoroacetamide was supplied by Regis Chemical (Morton Grove, IL). All aqueous solutions were made with water purified by a “Milli-Q” system (Millipore, St-Laurent, Quebec). The albumin solution [BSA, fraction V, fatty acid poor (Bayer, Kankakee, IL): 1.2 kg in 8 liters of Krebs buffer without glucose] was dialyzed at 4°C against 25 liters of the same buffer for 8 h. The buffer was changed six times (for a total of 150 liters of Krebs buffer in 48 h) to reduce the background citrate concentration to the low micromolar range (1.2 ± 0.1 µM). The dialyzed albumin was stored at −80°C at a final concentration of 13.4%. A 20 mM solution of sodium oleate, unlabeled or labeled, complexed to albumin was prepared by first dissolving oleic acid (99%) in a 10% molar excess of 1 N NaOH and then mixing with the dialyzed solution. All of these13C-labeled substrates are metabolized to ketone bodies (KB), an index of cellular necrosis, and tissue levels of citrate and lactate, an indicator for citrate efflux from the mitochondria (5, 14, 27).

For the addition of 13C substrate(s), the unlabeled substrate(s) was replaced by the corresponding labeled substrate(s), either [1,2-13C2]octanoate or [1-13C]oleate ([U-13C3]lactate + pyruvate). We reported previously that plateau enrichment of effluent citrate is reached within 20 min (7). All of these 13C-labeled substrates are metabolized to mitochondrial citrate by different pathways: [1-13C]oleate and [1,2-13C2]octanoate are β-oxidized to M1 and M2 isopomers of acetyl-CoA, respectively, and [U-13C3]lactate + pyruvate are decarboxylated to M2 acetyl-CoA and carboxylated to M3 OA.

Heart Perfusion

Animal experiments were approved by the local animal care committee in compliance with the guidelines of the Canadian Council on Animal Care. After opening the chest and inserting a cannula in the aorta, hearts were excised and transferred to a Langendorff perfusion setup, as described previously (6, 7, 16). Briefly, the hearts of fed male Sprague-Dawley rats (180–240 g; Charles-River, St-Constant, Quebec) were anesthetized by intraperitoneal injection of pentobarbital sodium (65 mg/kg) and then were perfused retrogradely through the aorta at a constant pressure of 70 mmHg with a nonrecirculating modified Krebs-Ringer bicarbonate buffer containing (in mM) 119 NaCl, 4.8 KCl, 1.3 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3 (pH 7.4) and various additives (substrates, hormones, or drugs), as indicated. The perfusate was gassed directly with 5% CO2-95% O2 at 37°C. For heart perfusions in the presence of albumin, the buffer contained in a stirred reservoir was pumped through a filter at a rate of 80 ml/min at the top of a jacketed glass oxygenator with a large inner surface area exposed to 5% CO2-95% O2. The oxygenated buffer was delivered to the heart through the aortic cannula by a pump whose rate was adjusted to keep the perfusion pressure at 70 mmHg (between 10 and 15 ml/min). Excess oxygenated buffer was returned to the buffer reservoir through an overflow outlet.

The following functional parameters were monitored continuously through instruments linked to a microcomputer: 1) coronary flow, using an electromagnetic flow probe (model FM501; Carolina Medical Electronics, King, NC), 2) temperature, using a thermocouple (Yellow Springs Instrument, Yellow Springs, OH), and 3) contractile function (heart rate, systolic and diastolic pressures, dp/dtmax), using a saline-filled latex balloon inserted in the left ventricular cavity, inflated to achieve an initial end-diastolic pressure of 5 mmHg, and connected to a pressure transducer (Digit-Med Heart Performance Analyzer, Micro-Med, Louisville, KY). The hearts were allowed to beat spontaneously throughout the experiments.

Experimental Design

All heart perfusion experiments included a 20-min stabilization period followed by a 30- to 90-min perfusion period (Fig. 1). Three groups of perfusion experiments were conducted. Under these conditions, we quantitated citrate release rates and documented the following parameters: 1) contractile activity (from continuous monitoring of dp/dtmax), an index of the energy demand, 2) 13C labeling of citrate isolated from the effluent perfusate and tissue, to assess the origin of the effluent citrate, 3) 13C enrichment of the acetyl (C4-a5) and OAA (C1-2,3,4,5) moieties of effluent citrate, to probe the origin and supply of substrates for the citrate synthase reaction, 4) the release rate of ketone bodies (KB), an index of acetyl-CoA spillover from the CAC, and 5) the release rate of succinate, a CAC intermediate whose release rate is increased by oxygen deprivation (16), 6) the release of LDH, an index of cellular necrosis, and 7) tissue levels of citrate and lactate, an indicator for citrate efflux from the mitochondria (5, 14, 27).

For the addition of 13C substrate(s), the unlabeled substrate(s) was replaced by the corresponding labeled substrate(s), either [1,2-13C2]octanoate or [1-13C]oleate ([U-13C3]lactate + pyruvate). We reported previously that plateau enrichment of effluent citrate is reached within 20 min (7). All of these 13C-labeled substrates are metabolized to mitochondrial citrate by different pathways: [1-13C]oleate and [1,2-13C2]octanoate are β-oxidized to M1 and M2 isopomers of acetyl-CoA, respectively, and [U-13C3]lactate + pyruvate are decarboxylated to M2 acetyl-CoA and carboxylated to M3 OAA.

Group 1: Perfusion in the presence of the medium-chain fatty acid octanoate. As a follow-up to our previous work (6, 7), citrate release rates were assessed initially in isolated rat hearts perfused with 11 mM glucose, 1 mM lactate, 0.2 mM pyruvate, and 0.2 mM octanoate. Compared with this substrate mixture, referred to as the control condition, other interventions were designed to 1) accelerate the rate of citrate utilization by the CAC, by increasing energy demand with the β-adrenergic agonist isoproterenol or 2) limit its rate of synthesis by lowering the availability of substrates for the citrate synthase reaction, either acetyl-CoA or OAA. Isoproterenol was infused at a final concentration of 1 µM. Acetyl-CoA
Sample Processing

Samples of effluent perfusate were collected on ice and processed as follows: 1) 10 ml remained untreated, and 2) 10 ml were treated with 1 M sodium borohydride (NaBH₄; 500 µl/10 ml) to reduce OAA to malate and acetoacetate to BHB. For the analysis of effluent citrate, samples (10 ml) were concentrated to 4 ml under a stream of air at 50°C. For the analysis of effluent citrate in albumin-containing buffer, samples of perfusate were first treated with saturated sulfosalicylic acid (250 µl) and centrifuged for 10 min at 10,000 rpm. Next, after pH adjustment (between 4 and 7), the supernatants were concentrated to 4 ml and centrifuged through an Ultrafree-4 Millipore filter at 6,000 rpm for 30 min. All samples were stored at −20°C until further analysis. At the end of the perfusion experiments, the hearts were quickly freeze-clamped and stored in liquid nitrogen.

Analytical Procedures

GC-MS. Methods to determine ¹³C mass isotopomer distribution and concentrations of the following metabolites isolated from effluent perfusates or tissues have been previously described in detail (6, 7, 16): citrate (C₄₋₆) as well as its acetyl (C₄₋₅) and OAA (C₅₋₇) moieties, BHB, succinate, fumarate, and pyruvate. The internal standards used for quantification of succinate, BHB, and citrate releases were [2,2,3-²H₃]-succinate, RS-[2,2,3,4,4,4-²H₆]BHB, and [1,2,3,6-¹³C₄]- or [2,2,4,4-²H₄]citrate, respectively.

All metabolites were analyzed as their tert-butyldimethylsilyl derivatives on a Hewlett Packard 5890 Series II plus gas chromatograph coupled to a 5972 mass selective detector equipped with an HP-5 fused silica capillary column (50 m, 0.2-mm inner diameter, 0.33-µm film thickness). The mass spectrometer was operated in the electron impact mode (70 eV) after automatic and manual calibration (to improve sensitivity in the high mass range). The split injection ratio was ~10:1, the carrier gas used was helium (0.6–0.7 ml/min), and the injection port was at 280°C. The transfer line to the mass spectrometer was at 300°C. Appropriate ion sets were monitored with a dwell time of 45–75 ms/ion. The GC-MS was operated in the electron impact mode (70 eV) after automatic and manual calibration (to improve sensitivity in the high mass range). The split injection ratio was ~10:1, the carrier gas used was helium (0.6–0.7 ml/min), and the injection port was at 280°C. The transfer line to the mass spectrometer was at 300°C. Appropriate ion sets were monitored with a dwell time of 45–75 ms/ion. The GC temperature program, elution times, and ions monitored for...
analysis of the 13C mass isotopomer distribution of CAC metabolites have been reported previously (6, 7, 16). Although the [4H4]citrate internal standard is an M4 citrate isotopomer, its major ion is at mass-to-charge ratio 461, corresponding to a M3 isotopomer due to the loss of water in the fragmentation process.

Other assays. The concentrations of tissue citrate and malate were determined using standard enzymatic assays (2) on a Roche Cobas Fara spectrophotometer. LDH release was assessed as described previously (3). Analyses of total and ionized calcium levels in perfusion buffer samples were conducted by the Clinical Biochemistry Laboratory of Notre-Dame Hospital.

Calculations

13C labeling of metabolites. The absolute molar percent enrichment (MPE) of individual 13C-labeled mass isotopomers (Mi) of a given metabolite was calculated as follows

\[ \text{MPE (Mi)} = \frac{\% A_{Mi}}{A_{Mi} + \sum A_{Mi}} \]  

where \( A_{Mi} \) and \( A_{Mi} \) represent the peak areas, corrected for natural abundance, corresponding to the unlabeled (M) and 13C-labeled (M_i) mass isotopomers, respectively.

Release rates of metabolites. Calculations of the concentration of a metabolite, using the corresponding heavy labeled internal standard, have been described previously (16). Total release rates of the metabolites were obtained by multiplying their perfusate concentrations (nmol/ml) by the coronary flow rate (ml/min). For all metabolites (citrate, KB, or succinate), a solution of Krebs buffer containing the internal standards of glutamate, lactate, and pyruvate was used as a background signal.

Flux parameters. The development of equations to calculate the relative contributions of octanoate and pyruvate to citrate formation, from the 13C labeling pattern of citrate released by hearts perfused with [1-13C]octanoate or [U-13C2]pyruvate + lactate, have been described in detail previously (7). In the present study, we report the following flux ratios: 1) the contribution of octanoate or oleate to the acetyl moiety of citrate; 2) the contribution of pyruvate to the acetyl and OAA moieties of citrate, and 3) the contribution of the unlabeled pyruvate arising from glucose or glycogen to tissue pyruvate. With [1,2-13C2]octanoate as the only labeled substrate, we measured the mole fraction (MF) in M2 of the acetyl moiety of citrate (instead of the MF in M1 for [1,13C]octanoate, as in our previous study (6)). The MF in a given mass isotopomer (Mi) of a metabolite is equivalent to MPE/100. Then, the equation to calculate the flux ratio (octanoate oxidation/citrate synthesis) becomes

\[ FC_{\text{OCT-AC(CIT)}} = 4 \times \frac{AC_{\text{CIT}}^{\text{M1}}}{OCT_{M2}} \]  

where \( FC_{\text{OCT-AC(CIT)}} \) is the fractional contribution (FC) of OCT to the acetyl moiety of citrate (AC(CIT)) through \( \beta \)-oxidation, and OCT(M2) is the MF in M2 of intracellular octanoate, which we assumed is equal to extracellular octanoate, since octanoate is not a physiological fatty acid. Factor 4 takes into account that octanoate is oxidized to four acetyl-CoA, only one of which is labeled. Using the same principle, with [1-13C]oleate as the labeled fatty acid, the FC of oleate (OLE) to the acetyl moiety of citrate (AC(CIT)) through \( \beta \)-oxidation may be calculated using this similar equation

\[ FC_{\text{OLE-AC(CIT)}} = 9 \times \frac{AC_{\text{CIT}}^{\text{M1}}}{OLE_{M1}} \]  

The MF in M1 of intracellular oleate (OLE(M1)) was assumed to be equal to extracellular oleate. Because oleate is a physiological fatty acid, its enrichment could be diluted in the cell. Thus the calculated flux ratio represents a minimal estimate of the actual flux ratio.

Statistical Analysis

Origin and Magnitude of Citrate Release by Perfused Rat Hearts

Isolated rat hearts perfused under normoxia released citrate in the effluent perfusate under all conditions examined. The measured citrate release rates varied between 2 and 21 nmol/min, depending on the nature of the substrate mixture supplied to the heart, but remained constant under a given perfusion condition for up to 90 min. Such release rates represent at most 2% of the total CAC flux rate (estimated at 2.0 ± 0.3 µmol/min in the Langendorff-perfused rat heart; see Ref. 7). Citrate release rates were highest in hearts perfused with a mixture of substrates feeding both acetyl-CoA (fatty acid) and anaplerotic OAA (lactate and pyruvate) to the citrate synthase reaction (see sections on Modulation of Citrate Release for details). They were lowest, but still measurable with precision, when the hearts were perfused with 11 mM glucose alone (2.4 ± 0.5 nmol/min, n = 4). As shown in Fig. 2,

![Fig. 2. Correlation between the mass isotopomer distribution of tissue and effluent citrate labeled from various 13C precursors of acetyl-CoA and oxaloacetate (OAA). Hearts were perfused for 40–70 min under normoxia with various substrate mixtures as follows: either 1) 11 mM glucose, 1 mM lactate, 0.2 mM pyruvate, 0.2 mM [1,13C2]octanoate, and 1 µM isoproterenol (α, n = 5), 2) 11 mM glucose, 1 mM lactate, 0.2 mM pyruvate, and 0.02 mM [1,13C2]octanoate (α, n = 8), 3) 11 mM glucose, and 0.2 mM [1,13C2]octanoate (α, n = 8), or 4) 5.5 mM glucose, 1 mM [U-13C]lactate, 0.2 mM [U-13C]pyruvate, and 0.4 mM [1-13C]oleate (α, n = 6). MPE of the individual 13C-labeled M1, M2, and M3 isotopomers of citrate, isolated from the effluent perfusate and freeze-damped tissue, was determined by gas chromatograph-mass spectrometry (GC-MS). Data are means ± SE of n heart perfusion experiments. Linear regression analysis: slope = 0.95 ± 0.05; r = 0.99, P < 0.05. Error bars are included in the symbols.](image)
the $^{13}$C mass isotopomer distribution of citrate isolated from the effluent reflected that of tissue citrate. The $^{13}$C substrates used in this study are known to label citrate through the mitochondrial citrate synthase reaction, after their conversion to acetyl-CoA (M1 or M2 isotopomers) and/or anaplerotic OAA (M3 isotopomers). Therefore, we concluded that effluent citrate is likely to be of mitochondrial origin. The results presented below demonstrate the modulation of citrate release by substrate supply and provide evidence for the specificity of the process.

Modulation of Citrate Release: Experiments with Octanoate (Group 1)

Functional status. Hearts perfused under normoxia for up to 70 min with 11 mM glucose, 1 mM lactate, 0.2 mM pyruvate, and 0.2 mM octanoate (control condition) maintained constant spontaneous beating at 280 ± 4 beats/min, a coronary flow rate of 10.4 ± 0.3 ml/min, a rate pressure product of $(23.6 ± 0.7) \times 10^3$ mmHg x beats/min (not shown), and a contractile activity (dP/dt max) of $2,379 ± 121$ mmHg/s (data not shown). The contractile activity of the heart was not modified significantly by lowering the exogenous octanoate concentration from 0.2 to 0.02 mM or by removing lactate and pyruvate. However, it was increased significantly by the infusion of 1 µM isoproterenol to 146 ± 13% of the control value (P < 0.05, n = 5; paired t-test). Thus, from the contractile activity of the heart, which is the main determinant of its energy demand, we concluded that the rate of citrate utilization by the CAC was similar under all conditions, except with isoproterenol with which it was augmented.

Metabolic status: Availability and source of substrates for citrate synthesis. Let us examine how the availability of the two substrates for the citrate synthase reaction varied under the various perfusion conditions of group 1. Although the availability of anaplerotic OAA for citrate synthesis was not assessed directly in this group of perfusions, we predicted from previous studies (6, 7) that removal of lactate plus pyruvate from the buffer would lower the supply of anaplerotic OAA. The availability and sources of acetyl-CoA for citrate synthesis are inferred from 1) the flux ratio (octanoate oxidation/citrate synthesis) evaluated from the M2 enrichment values of the C$_{4-i}$ of effluent citrate using Eq. 2 (Fig. 3A) and 2) the KB release rates (Fig. 3B). The observed variations in these two parameters are compatible with the known metabolism of octanoate. Indeed, because octanoate $\beta$-oxidation is not regulated at CPT-I, its concentration in the perfusion buffer sets the rate of acetyl-CoA generation. Accordingly, whenever octanoate was supplied at 0.2 mM, a concentration leading to maximal $\beta$-oxidative rates (17), the acetyl-CoA of citrate was predominantly supplied by octanoate $\beta$-oxidation [~80% as indicated by the flux ratios (octanoate oxidation/citrate synthesis); Fig. 3A] at a rate exceeding citrate synthesis (as indicated by KB release; Fig. 3B). Note that, under control conditions, the KB release rate represents as much as 25% of the rate of 1) octanoate uptake or 2) acetyl-CoA oxidation in the CAC (6). When octanoate was supplied at 0.02 mM, only 30% of the acetyl-CoA of citrate arose from $\beta$-oxidation, and the KB release rate was close to the detection level, in agreement with a decreased supply of acetyl-CoA.

Citraterelase and tissue levels of citrate and malate. Hearts perfused under control conditions for 70 min released citrate at a constant rate of 21 ± 1 nmol/min (Fig. 3C). This rate was decreased significantly by 1) accelerating citrate utilization by the CAC through the addition of 1 µM isoproterenol or by 2) limiting the supply of substrates for citrate synthesis, either acetyl-CoA, by the lowering of exogenous octanoate concentration from 0.2 to 0.02 mM, or anaplerotic OAA, by the removal of exogenous lactate and pyruvate. The similar decrease in citrate release rates under conditions where the delivery of either acetyl-CoA or OAA was limited emphasizes the requirement of both substrates for the synthesis of excess citrate relative to the CAC flux. It is noteworthy that, although the citrate release rate was decreased under limited OAA supply, the KB release rate was increased, suggesting a greater acetyl-CoA spillover from the CAC.

Comparison of Fig. 3, C and D, reveals that citrate release rates varied in parallel with tissue levels of citrate plus malate, the antiporter for the mitochondrial tricarboxylate transporter. Note the closer relationship between citrate release rates and tissue levels of citrate plus malate than with tissue citrate alone.

Modulation of Citrate Release Experiments with Physiological Substrate Mixtures (Group 2)

In addition to the results from the first group of perfusion experiments, we documented citrate release in hearts perfused with substrate mixtures mimicking the physiological state, either the fed (5.5 mM glucose, 0.5 or 1 mM lactate, 0.05 or 0.2 mM pyruvate, and 8 nM insulin) or the fasted state (5.5 mM glucose, 1 mM lactate, 0.2 mM pyruvate, 8 nM insulin and 0.4 mM LCFA oleate).

Functional status. Hearts perfused under normoxia with 5.5 mM glucose, 0.5 or 1 mM lactate, 0.05 or 0.2 mM pyruvate, and 8 nM insulin maintained constant spontaneous beating at a rate exceeding citrate synthesis (as indicated by KB release; Fig. 3B). Note that, under control conditions, the KB release rate represents as much as 25% of the rate of 1) octanoate uptake or 2) acetyl-CoA oxidation in the CAC (6). When octanoate was supplied at 0.02 mM, only 30% of the acetyl-CoA of citrate arose from $\beta$-oxidation, and the KB release rate was close to the detection level, in agreement with a decreased supply of acetyl-CoA.

Citraterelase and tissue levels of citrate and malate. Hearts perfused under control conditions for 70 min released citrate at a constant rate of 21 ± 1 nmol/min (Fig. 3C). This rate was decreased significantly by 1) accelerating citrate utilization by the CAC through the addition of 1 µM isoproterenol or by 2) limiting the supply of substrates for citrate synthesis, either acetyl-CoA, by the lowering of exogenous octanoate concentration from 0.2 to 0.02 mM, or anaplerotic OAA, by the removal of exogenous lactate and pyruvate. The similar decrease in citrate release rates under conditions where the delivery of either acetyl-CoA or OAA was limited emphasizes the requirement of both substrates for the synthesis of excess citrate relative to the CAC flux. It is noteworthy that, although the citrate release rate was decreased under limited OAA supply, the KB release rate was increased, suggesting a greater acetyl-CoA spillover from the CAC.

Comparison of Fig. 3, C and D, reveals that citrate release rates varied in parallel with tissue levels of citrate plus malate, the antiporter for the mitochondrial tricarboxylate transporter. Note the closer relationship between citrate release rates and tissue levels of citrate plus malate than with tissue citrate alone.

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1 The acetyl moiety of effluent citrate (C$_{4-i}$) labeled from [1,2-$^{13}$C]octanoate was enriched solely in M2 isotopomers (data not shown). M1 enrichment of the acetyl moiety was low and imprecise (not significant, when tested against the null hypothesis, not shown), indicating negligible recycling of $^{13}$C label through malate $\rightarrow$ pyruvate $\rightarrow$ acetyl-CoA reactions.
Metabolic status: availability and source of substrates for citrate synthesis. In an attempt to explain the similar citrate release rates in hearts perfused with octanoate and oleate, we determined the relative contribution of pyruvate and oleate to the formation of acetyl-CoA and anaplerotic OAA. This was done by perfusing hearts with a mixture of 13C-labeled substrates (0.4 mM [1-13C]oleate, 1 mM [U-13C3]lactate, and 0.2 mM [U-13C3]pyruvate) and determining the 13C labeling of effluent citrate and of tissue pyruvate, succinate, and fumarate, as described previously (6, 7). Note that KB release by hearts perfused with oleate was low (15.5 ± 1.7 nmol/min, n = 6, not shown), in agreement with a regulated β-oxidation rate (Ref. 22 and Tables 1 and 2).

As shown in Table 1, tissue pyruvate was only enriched in M3 isotopomers, suggesting a negligible decarboxylation of malate to pyruvate through the malic enzyme reaction. Tissue citrate was enriched in M1, M2, and M3 isotopomers. Enrichment in isotopomers of higher masses was <1.5%, indicating that the proportion of citrate molecules labeled in both the acetyl and OAA moieties was small. The labeling pattern of citrate is compatible with the formation of M1 and M2 acetyl-CoA from oleate β-oxidation and pyruvate decarboxylation, respectively, and of M3 OAA from pyruvate carboxylation. Isotope dilution between tissue citrate and succinate was evaluated at 1.1 ± 0.6 (Eq. 10 of Ref. 7). Tissue succinate and fumarate showed similar enrichment in M1 and M2 isotopomers, whereas tissue fumarate was more enriched in M3 isotopomers. This can be explained by the entry of M3 isotopomers at the level of malate and/or OAA by anaplerotic carboxylation of [U-13C3]pyruvate.

The isotopic data in Table 1 allow the calculation of several flux ratios. From the MPE of tissue pyruvate in M3 isotopomers, we conclude that pyruvate converted to acetyl-CoA or OAA arose predominantly from exogenously supplied pyruvate and/or lactate (0.76 ± 0.06 from FCPYRe-PYRI; Eq. 2 of Ref. 7); the remaining (1 - FCPYRe-PYRI = 0.24 ± 0.06) arose from glucose and/or glycogen. These data are in agreement with other studies conducted in vivo (35) and ex vivo in the perfused rat heart (4) showing a preferential oxidation of exogenous lactate and/or pyruvate relative to glucose. Other flux ratios are shown in Table 2. The acetyl moiety of citrate was predominantly supplied by oleate β-oxidation [80 ± 8% from the flux ratio (oleate oxidation/citrate synthesis); Eq. 3]. The contribution of pyruvate...
vate decarboxylation to the acetyl moiety of citrate was substantial (11 \pm 3%; Eq. 5 of Ref. 7) and was greater than that of pyruvate carboxylation to the OAA moiety of citrate (2.4 \pm 0.2%; Eq. 4 of Ref. 7). Note that, because the formation of M3 OAA through the metabolism of citrate isotopomers in the CAC was low (31.9 \pm 2.2%; Eqs. 8 and 9 of Ref. 7), we obtained good precision on flux measurements (as indicated by the SE) of anaplerotic pyruvate carboxylation despite substantial pyruvate decarboxylation.

Specificity of Citrate Release (Groups 1–3)

Effect of 1,2,3- and 1,2,4-BTC (group 3). FUNCTIONAL STATUS. The addition of 10 mM 1,2,3-BTC or 1,2,4-BTC to hearts perfused with 5.5 mM glucose, 1 mM lactate, and other substrates at mM concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated. Samples of effluent perfusate were collected on ice and processed for the determination of citrate concentrations as indicated.


<table>
<thead>
<tr>
<th>Metabolites</th>
<th>MPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>ND</td>
</tr>
<tr>
<td>OAA (citrate)</td>
<td>9.35 \pm 0.80</td>
</tr>
<tr>
<td>Acetyl-CoA (citrate)</td>
<td>8.88 \pm 0.85</td>
</tr>
<tr>
<td>Citrate</td>
<td>14.5 \pm 0.5</td>
</tr>
<tr>
<td>Succinate</td>
<td>15.2 \pm 1.0</td>
</tr>
<tr>
<td>Fumarate</td>
<td>15.3 \pm 0.6</td>
</tr>
</tbody>
</table>

Data, expressed as molar percent enrichment (MPE), are the means \pm SE of 6 heart perfusion experiments. For all metabolites, the MPE in M4 to M6 isotopomers was below 1.5% (data not shown). Data are from heart perfusion experiments with 5.5 mM glucose, 1 mM [U-13C3]lactate, 0.2 mM [U-13C3]pyruvate, 0.4 mM [1-13C]oleate-4% albumin, 50 \mu M carnitine, and 8 nM insulin (group 2 in Fig. 1). Effluent perfusates (citrate) and freeze-dried hearts (pyruvate, succinate, and fumarate) were processed for analysis of the 13C labeling of metabolites by GC-MS. ND, not detected; NA, not applicable. OAA, oxaloacetate.


<table>
<thead>
<tr>
<th>Flux Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FColeate–AC(CIT)</td>
<td>Olate oxidation/CS</td>
</tr>
<tr>
<td>FCpyruv–AC(CIT)</td>
<td>PDC/CS</td>
</tr>
<tr>
<td>FCpyruv–OAA(CIT)</td>
<td>PC/CS</td>
</tr>
<tr>
<td>PC/PDC</td>
<td>0.25 \pm 0.03</td>
</tr>
</tbody>
</table>

Data are means \pm SE of 6 heart perfusion experiments. Data are from heart perfusion experiments with 5.5 mM glucose, 1 mM [U-13C3]lactate, 0.2 mM [U-13C3]pyruvate, 0.4 mM [1-13C]oleate-4% albumin, 50 \mu M carnitine, and 8 nM insulin (group 2 in Fig. 1). Flux ratio for oleate oxidation/citrate synthesis (FColeate–AC(CIT)) was calculated from the M1 enrichment value of the C4,5 of effluent citrate (AC(CIT)) determined at plateau (50–60 min). Flux ratios pyruvate decarboxylation/citrate synthesis (FCpyruv–AC(CIT); PDC/CS) and pyruvate carboxylation/citrate synthesis (FCpyruv–OAA(CIT); PC/CS) were calculated from the M3 MPE of tissue pyruvate, and the M2 MPE of the C3,4 or the corrected M3 MPE of the C3,4 of OAAC(CIT) of effluent citrate (50–60 min), as described previously (6, 7).

LDH release (groups 1–3). Under all conditions examined, LDH release rates were 20- to 50-fold lower than those measured in hearts reperfused after 90 min of low-flow ischemia or 40 min of hypoxia (3, 16). Furthermore, the LDH release rates did not correlate with the citrate release rates (r = -0.1, P = 0.95, not significant). A significant increase in LDH release was observed during isoproterenol infusion (from 18 to 2 to 62 \pm 14 \mu M/min, P < 0.05, n = 4; paired t-test). Similarly, LDH release was increased upon removal of 1,2,3-BTC (from 19 \pm 7 to 74 \pm 20 \mu M/min, P < 0.05, n = 4; one-way ANOVA for repeated measures followed by a Dunnett multiple comparison test) but not of 1,2,4-BTC, thus raising the possibility of a detrimental effect associated with the inhibition of citrate transport from the mitochondria to the cytosol.
Succinate release (groups 1–3). Under all conditions, succinate release remained between 2 and 20 nmol/min, which is three- to sixfold lower than rates reported for oxygen-deprived hearts (16). There was no correlation between citrate and succinate release rates \( (r = 0.48, P = 0.2) \), indicating that these two cataplerotic reactions are regulated independently (data not shown).

**DISCUSSION**

Results from this study demonstrate that citrate release by rat hearts perfused ex vivo under normoxia is specifically modulated by various perfusion interventions. The specificity of this process is also supported by the lack of correlation between the release rates of citrate and those of LDH, an index of cellular membrane damage, or of succinate, another CAC intermediate whose release increases with oxygen deprivation (16). Furthermore, 1) the identical \(^{13}\)C labeling of tissue and effluent citrate resulting from \(^{13}\)C-labeled substrates feeding acetyl-CoA and OAA to the mitochondrial citrate synthase reaction and 2) a modulation of citrate release rates by the nature and concentrations of these substrates indicate that citrate release reflects mitochondrial citrate cataplerosis. Altogether, the results from the present study provide a basis for discussing the following aspects relevant to mitochondrial cataplerosis: 1) the participation of the tricarboxylate transporter relative to other transporters, 2) its modulation by metabolic conditions, and 3) its link to the regulatory role of cytosolic citrate. A schematized overview of the metabolic processes relevant to this discussion is presented in Fig. 6.

In the heart, the existence of mitochondrial citrate cataplerosis has often been questioned because of the low activity of the mitochondrial tricarboxylate transporter. The activity of this transporter may be low when compared with lipogenic tissue such as the liver. Still this low activity is compatible with the 1) citrate release rates reported in the present study and 2) a role of cytosolic citrate as a “signaling molecule” in nonlipogenic tissues (28). The participation of this transporter in mitochondrial citrate cataplerosis in the intact heart is substantiated by the following results from our investigation. First, citrate release by the heart was inhibited by 1,2,3-BTC, the only known specific inhibitor of the mitochondrial tricarboxylate transporter, but not by 1,2,4-BTC, the inactive chemical analog. Second, citrate release rates varied in parallel with the tissue levels of citrate plus malate, the antiporter. A closer relationship between citrate release rates and tissue levels of citrate plus malate than with tissue citrate supports the suggestion of Ruderman et al. (28) that cytosolic citrate would be reflected more directly in the sum of whole cell concentration of citrate plus malate than citrate alone. Our data indicate that the tissue ratio citrate/malate varies with the availability and/or source of acetyl-CoA for citrate synthesis. Indeed, this ratio was close to 1 for hearts perfused with 0.2 mM octanoate but was only 0.5 for hearts perfused with 0.02 mM octanoate.

Regarding the relatively low inhibition of citrate release (25%) by 1,2,3-BTC, there is a possibility that, due to steric hindrance, 1,2,3-BTC does not easily reach the extramitochondrial space. Thus a greater concentration of exogenous 1,2,3-BTC would be necessary for maximal inhibition of the mitochondrial transporter. In guinea pig heart mitochondria, 50 mM 1,2,3-BTC achieved maximal inhibition of the activity of the transporter (27), whereas, in rat heart mitochondria, the same concentration resulted in only 25% inhibition (5). We did not attempt to use such a high concentration of 1,2,3-BTC or 1,2,4-BTC in the intact beating heart.

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**Fig. 5.** Effect of 10 mM 1,2,3-benzenetricarboxylic acid (BTC; hatched bars) and 1,2,4-BTC (filled bars) on citrate release rates. Data are from heart perfusion experiments depicted in Fig. 1 as group 3. Hearts were perfused under normoxia with 5.5 mM glucose, 8 nM insulin, 1 mM lactate, and 0.2 mM pyruvate. Samples of coronary effluent perfusate were collected on ice and processed for the determination of citrate concentrations by GC-MS. Citrate release rate values, assessed during the last 5 min of each 30-min time period, are expressed as percentage \((\%\) of those measured before the addition of 1,2,3- or 1,2,4-BTC. Data are means \(\pm\) SE of 4 heart perfusion experiments. *\(P < 0.05\), one-way ANOVA for repeated measures followed by the Dunnett multiple comparison test.

**Fig. 6.** Schematic overview of metabolic processes and factors relevant to mitochondrial citrate cataplerosis in the heart (see discussion). ACL, ATP citrate lyase; CS, citrate synthase; PDH, pyruvate dehydrogenase; Tmt, mitochondrial tricarboxylate transporter; Tc, cell membrane citrate transporter; αKG, α-ketoglutarate; PFK, phosphofructokinase; ACC, acetyl-CoA carboxylase; CPT, carnitine palmitoyltransferase +, activation; −, inhibition.
since a decrease in contractile function, possibly due to calcium chelation, was already observed at a concentration of 10 mM. An alternative explanation for the low inhibition by 1,2,3-BTC would be the participation of the following additional mechanism for the transfer of citrate from the mitochondria to the cytosol (13): the conversion of citrate to α-ketoglutarate in the mitochondria through normal operation of the CAC, followed by the transport of α-ketoglutarate from the mitochondria to the cytosol by the dicarboxylate transporter, and the conversion of α-ketoglutarate to cytosolic citrate by reversal of the cytosolic NADP-linked isocitrate dehydrogenase (ICDH) reaction. In the heart, the activity of the mitochondrial dicarboxylate transporter is severalfold higher than that of the tricarboxylate transporter (12). Although the occurrence of this alternative pathway was not tested specifically in the present study, the following data from previous heart perfusion experiments with [U-13C5]glutamate suggest that it is unlikely to be involved (6). Under normoxia, the maximal contribution of reversal of the NADP-ICDH to citrate formation was evaluated at most at 5%, with the remaining 95% being formed through the citrate synthase reaction. Because the activity of cytosolic NADP-ICDH is at most 2% of total ICDH activity (25), this sets its maximal contribution at 0.1% of the CAC, which is 20-fold lower than the citrate release rate, i.e., 2% of the CAC.

Therefore, based on the above considerations, it is likely that the mitochondrial tricarboxylate transporter participates in the transfer of citrate from the mitochondria to the cytosol under our conditions. Besides, our observation of an increased LDH release associated with the addition of 1,2,3-BTC, but not 1,2,4-BTC, suggests that the activity of this transporter, and thus mitochondrial citrate cataplerosis, could be important for the integrity of myocardial cells. At present, we do not have an explanation for this unexpected finding. Mitochondrial transmembrane carrier deficiencies have recently been considered as potential causes of mitochondriopathies (12). It is proposed that defects in these carriers may induce imperfect energy metabolism, probably as a result of osmotic disturbances within the mitochondria. So far, little is known about the heart mitochondrial citrate transporter aside from its mRNA expression in the heart (12). This subject is, however, beyond the scope of the present study.

What then triggers mitochondrial citrate cataplerosis in the heart? How does cardiac mitochondrial citrate cataplerosis integrate itself with a role of cytosolic citrate as a signaling molecule? Before attempting to answer these questions, let us first summarize the proposed role of cytosolic citrate in fuel signaling in nonlipogenic tissues, such as the heart (28). Cytosolic citrate would act as a signal to the cell that it has an excess of fuel for its immediate needs. Thus an increase of cytosolic citrate could restrain the use of both fatty acid (via malonyl-CoA CPT-I inhibition) and glucose (via PFK inhibition) for energy production. In the heart, the regulatory role of cytosolic citrate on glycolysis was first proposed in 1963 by Garland et al. (11) who reported a correlation between citrate tissue levels and PFK activity. As for a link between cytosolic citrate and malonyl-CoA synthesis, it remains to be clarified. Malonyl-CoA is synthesized from acetyl-CoA by acetyl-CoA carboxylase (ACC). In skeletal muscle, experimental evidence supports a role for cytosolic citrate both as 1) a precursor of acetyl-CoA, after citrate cleavage by the ATP citrate lyase, and 2) an allosteric activator of ACC (28). However, the kinetic and regulatory properties of the cardiac ACC, predominantly the ACCβ isoform, are such that its activity appears to depend more on the acetyl-CoA supply than on citrate activation (19, 29). Therefore, could cytosolic citrate be a precursor of malonyl-CoA in the heart? To the best of our knowledge, this possibility has not been examined. The activity of the cytosolic ATP citrate lyase in the heart is low [0.2 ± 0.03 vs. 2.8 ± 0.03 μmol/min × g wet wt (1), but probably sufficient to maintain the very small pool of malonyl-CoA [nmol/g wet wt (1, 19, 21)].

In light of the above considerations, let us now examine how our data on citrate release rates, reflecting mitochondrial citrate cataplerosis, are in agreement with the proposed regulatory role of cytosolic citrate. Our series of heart perfusions with the MCFA octanoate indicate that citrate release rates are modulated by 1) the energy demand of the heart and 2) the availability of substrates for the citrate synthase reaction. Although the effect of energy demand should be substantiated by studies in the working rat heart perfused at different workloads, these data support a role for cytosolic citrate as a signal to the cell that the fuels available are in excess for its immediate needs. For hearts perfused with octanoate and physiological concentrations of lactate and pyruvate, we proposed previously that the high production of mitochondrial acetyl-CoA and NADH from octanoate β-oxidation, which results in state 4 respiration (18), inhibits pyruvate decarboxylation and favors anaplerotic pyruvate carboxylation (6, 7). This sets up conditions for citrate accumulation and efflux from the mitochondria to the cytosol. We emphasized that a surge of anaplerotic pyruvate carboxylation was required to balance the citaplerotic efflux of citrate. In view of the proposed regulatory role of cytosolic citrate, such a link between pyruvate anaplerosis and citrate cataplerosis would support a role for these reactions in metabolic signaling in the heart.

However, other factors appear to contribute to the high rate of citrate release by hearts perfused with glucose, lactate, pyruvate, and the LCFA oleate. Indeed, under these conditions, the relative substrate flux through pyruvate carboxylation and decarboxylation differs markedly from that measured in hearts perfused with glucose, lactate, pyruvate, and the MCFA octanoate (from the flux ratio pyruvate carboxylation-to-pyruvate decarboxylation reported in Table 2 and Ref. 6: 0.25 ± 0.03 vs. 2.8 ± 0.7, respectively). We can only speculate on the nature of the other factor(s) involved. We exclude 1) a difference in the rate of citrate utilization by the CAC, since hearts perfused with octanoate
and oleate showed similar dP/dt\textsubscript{max} values and 2) the participation of another anaplerotic reaction aside from pyruvate carboxylation, for example, at the level of α-ketoglutarate or succinate, since there is little tracer dilution between tissue citrate and succinate. However, there is a possibility that citrate release in the effluent underestimates mitochondrial citrate cataplerosis because of 1) citrate metabolism in the cytosol by the ATP citrate lyase and 2) the presence of a regulated transport system for citrate in the plasma membrane. Little is known about these two processes in the heart, although a sodium-dependent transporter was characterized in tissues such as the kidney and liver (24). As for the ATP citrate lyase, a role for this enzyme in the removal of citrate from the cytosol was suggested (5) where its activity would be limited by the low level of cytosolic CoASH resulting from LCFA activation. In contrast, the activation of MCFA such as octanoate occurs in the mitochondria. Thus, in hearts perfused in the absence of LCFA, citrate release may represent a minimal estimate of mitochondrial citrate cataplerosis due to higher activity of the ATP citrate lyase.

Despite these uncertainties regarding the magnitude of mitochondrial citrate cataplerosis, as reflected by citrate release, the observed variations in citrate release rates in hearts perfused with the various substrate mixtures appear nevertheless compatible with a regulatory role of cytosolic citrate in glycolysis and fatty acid oxidation. Indeed, this dual role of citrate requires its presence in the cytosol under all metabolic conditions, although its concentration should be higher when glycolysis is inhibited. Accordingly, citrate release was observed under all conditions examined, even in the presence of glucose as sole substrate. Furthermore, citrate release rates were highest in hearts perfused with physiological concentrations of glucose, lactate, pyruvate, and a fatty acid, either the LCFA oleate or the MCFA octanoate. Under these conditions, we documented active β-oxidation and a low contribution of glycolysis to tissue pyruvate formation (data from Table 2 and Ref. 6). Similarly, in vivo studies with normal human subjects showed that myocardial citrate release correlated positively with LCFA uptake but negatively with glucose uptake (33, 34). Interestingly, these correlations were not observed in patients with coronary artery disease, suggesting dysregulation. As for a possible link between citrate cataplerosis and malonyl-CoA, although only circumstantial, the results from heart perfusion with glucose, insulin, lactate, and pyruvate appear more compatible with a role of citrate as a precursor, rather than an activator, of malonyl-CoA formation. Indeed, under these conditions, where tissue malonyl-CoA and the glycolytic rate were expected to be greater than in hearts perfused in the additional presence of LCFA (1, 19), citrate release rates were lower. Still, they were modulated by physiological concentrations of exogenous lactate and pyruvate.

In conclusion, the results from this study demonstrate that citrate release by rat hearts perfused ex vivo under normoxia is a specific process, reflecting citrate efflux from the mitochondria to the cytosol or cataplerosis. They indicate that citrate cataplerosis is modulated by energy demand and by the nature and concentrations of substrates feeding the citrate synthase reaction, either acetyl-CoA or anaplerotic OAA. These changes are in agreement with the crucial roles of citrate 1) in the mitochondria for energy production and 2) in the cytosol as a signal molecule regulating substrate fuel selection. However, under some conditions, such as in the absence of LCFA, the measured citrate release rates may underestimate the magnitude of mitochondrial citrate cataplerosis due to citrate metabolism in the cytosol by the ATP citrate lyase. The participation of this enzyme, which is essential to the role of citrate as a precursor of malonyl-CoA, remains to be demonstrated. Finally, our results indicate that mitochondrial citrate cataplerosis occurs at least in part through the tricarboxylate transporter and suggest a potential physiological significance of this transporter for the integrity of myocardial cells.

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REFERENCES


E856 CARDIAC CITRATE CATAPLEROSIS