Assessing the Reversibility of the Anaplerotic Reactions of the Propionyl-CoA Pathway in Heart and Liver*  

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While a number of studies underline the importance of anaplerotic pathways for hepatic biosynthetic functions and cardiac contractile activity, much remains to be learned about the sites and regulation of anaplerosis in these tissues. As part of a study on the regulation of anaplerosis from propionyl-CoA precursors in rat livers and hearts, we investigated the degree of reversibility of the reactions of the propionyl-CoA pathway. Label was introduced into the pathway via NaH13CO3, [U-13C3]propionate, or [U-13C3]lactate and isotopomers in isotopic equilibrium under all conditions tested. The mass isotopomer distributions of propionyl-CoA, methylmalonyl-CoA, and succinyl-CoA revealed that, in intact livers and hearts, (i) the propionyl-CoA carboxylase reaction is slightly reversible only at low propionyl-CoA flux, (ii) the methylmalonyl-CoA racemase reaction keeps the methylmalonyl-CoA enantiomers in isotopic equilibrium under all conditions tested, and (iii) the methylmalonyl-CoA mutase reaction is reversible, but its reversibility decreases as the flow of propionyl-CoA increases. The thermodynamic disequilibrium of the combined reactions of the propionyl-CoA pathway explains the effectiveness of anaplerosis from propionyl-CoA precursors such as heptanoate.

Adequate energy production via the citric acid cycle (CAC) requires not only a constant supply of acetyl-CoA, but also a fairly constant pool of the catalytic intermediates which carry the acetyl groups as they are oxidized. Although the process of anaplerosis in microorganisms was discovered in the 1960s (1), its importance for the homeostasis of mammalian cells (particularly cardiomyocytes) was recognized only in the 1980s (2, 3) (for a recent review, see Ref. 4). While the crucial role of anaplerosis for hepatic gluconeogenesis is self-evident, investigations in the heart suggested that stimulating anaplerosis from exogenous substrates could become part of the treatment of myocardial reperfusion injury and other cardiomyopathies (5–10).

In a recent clinical study (11), the hypoglycemia as well as the mechanical performance of the heart and muscle of patients suffering from long chain fatty acid oxidation defects was greatly improved by replacing the fat component of their therapeutic diet, i.e. trioctanoin (a medium-even-chain triglyceride) by triheptanoin (a medium-odd-chain triglyceride). The only difference between the metabolisms of octanoate and heptanoate is the production of propionyl-CoA from the latter. In a follow-up investigation in pig hearts, propionate infusion was found to be a very effective anaplerotic substrate. Anaplerosis from 0.25 mM [U-13C3]propionate amounted to 9% of the rate of the CAC (12, 13). This led us to investigate the regulation of the propionyl-CoA pathway in livers and hearts of animals.

Propionyl-CoA is formed from the activation of propionate and from the catabolism of isoleucine, valine, threonine, methionine, cholesterol, odd-chain fatty acids, and C5-ketone bodies (14). In mammalian tissues, the metabolism of propionyl-CoA (Fig. 1) involves (i) the formation of (S)-methylmalonyl-CoA by biotin-dependent propionyl-CoA carboxylase (15), (ii) the conversion of (S)-methylmalonyl-CoA to succinyl-CoA by methylmalonyl-CoA racemase (16), and (iii) the conversion of (R)-methylmalonyl-CoA to succinyl-CoA by cobalamin-dependent methylmalonyl-CoA mutase (17–19) (for a recent review, see Ref. 20).

Early in vitro work with purified enzymes revealed different degrees of reversibility of the reactions catalyzed by propionyl-CoA carboxylase (21, 22), methylmalonyl-CoA racemase (23), and methylmalonyl-CoA mutase (24). In the presence of a mixture of purified propionyl-CoA carboxylase, methylmalonyl-CoA racemase, and methylmalonyl-CoA mutase, only a few percent of the radioactivity of [1-14C]succinyl-CoA (prepared from [1-14C]succinate) was recovered in CO2 released by the reversal of propionyl-CoA carboxylase (16). From these studies on purified enzymes, one cannot draw conclusions on the degree of reversibility of the reactions of the propionyl-CoA pathway in intact organs.

To the best of our review of the literature, there have been only few attempts at testing the reversibility of the propionyl-CoA pathway in intact mammalian tissues, in the absence of biotin or cobalamin deficiency (25). A characterization of the reversibility and of the degree of thermodynamic equilibrium of the pathway would allow one to predict the efficiency of anaplerotic interventions with propionyl-CoA precursors. We took advantage of recent developments in mass isotopomer analysis2 (26) to evaluate the reversibility of propionyl-CoA carboxylase and methylmalonyl-CoA mutase in intact rat livers and hearts.

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‡ The abbreviations used are: CAC, citric acid cycle; HPLC, high performance liquid chromatography.

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2 Mass isotopomers are designated as Mn, where n is the number of atomic mass units above the molecular weight of the unlabeled isotopomer M.
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EXPERIMENTAL PROCEDURES

Materials

Chemicals and biochemicals were obtained from Sigma. NaH\(^{13}\)CO\(_3\), \([U-^{13}\)C\(_3\)]propionate, \([U-^{13}\)C\(_2\)]lactate, and \([U-^{13}\)C\(_2\)]pyruvate (99%) were purchased from Isotopic (Miamisburg, OH). A standard of \([U-^{13}\)C\(_3\)]propionyl-CoA was prepared from \([U-^{13}\)C\(_3\)]propionate and purified by HPLC (27).

Organ Perfusion Experiments

Hearts from overnight-fasted Sprague-Dawley rats (180–220 g) were perfused in the Langendorff mode with non-recirculating Krebs-Henseleit bicarbonate buffer (12 ml/min) containing 5.5 mM glucose, 11.1 mM lactate, 6.2 mM pyruvate, 1 mM carmine, and 100 milliliters/ml insulin. Three protocols were conducted.

Protocol 1—Unlabeled propionate (0–1 mM) was added to the perfusate from zero time. At \(t = 15\) min, unlabeled bicarbonate was replaced by 40% enriched NaH\(^{13}\)CO\(_3\) (28), and the heart was quick-frozen at up to 35 min. The enrichment of HCO\(_3^-\)/CO\(_2\) was measured in samples of effluent perfusate taken just before clamping the heart.

Protocol 2—Unlabeled propionate (0.1–1 mM) was added to the perfusate from zero time. At \(t = 15\) min, unlabeled propionate was replaced by \([U-^{13}\)C\(_2\)]lactate, and the heart was quick-frozen at 35 min.

Protocol 3—Unlabeled propionate (0–1 mM) was added to the perfusate from zero time. At \(t = 15\) min, unlabeled lactate and pyruvate were replaced by 99% enriched \([U-^{13}\)C\(_2\)]lactate + \([U-^{13}\)C\(_2\)]pyruvate, and the heart was quick-frozen at 35 min.

Almost identical protocols were conducted on perfused rat livers (29) from overnight-fasted rats except that (i) no insulin or carmine was added to the perfusate, (ii) the concentrations of unlabeled propionate ranged from 0 to 3 mM, and Protocol 2 did not include lactate and pyruvate in the perfusate.

In Vivo Pig Experiments

Four anesthetized pigs were thoracotomized and fitted with a pump-controlled bypass between the femoral artery and a branch of the left anterior descending coronary artery (10). NaH\(^{13}\)CO\(_3\) was infused into the bypass at a rate calculated to achieve a 15% enrichment of HCO\(_3^-\)/CO\(_2\) in the vascular myocardium. The enrichment of HCO\(_3^-\)/CO\(_2\) was measured in samples of venous blood from the infused territory just before taking punch biopsies of the myocardium at 10 and 60 min (two pigs at each time point).

Sample Preparation

Frozen tissue (0.2–1 g) is extracted with 4 volumes of ice-cold 6% perchloric acid using a Polytom homogenizer. After centrifugation, the acid extract is mixed with 3 volumes of 75 mM potassium phosphate buffer pH 4. The solution is transferred to a glass syringe from which it is slowly pushed through an oligonucleotide purification cartridge (ABI Masterpiece, Applied Biosystems) as described by Deutsch et al. (30) for the isolation of long chain acyl-CoAs from tissue extracts. The cartridge is then rinsed with 5 ml of water. The short chain acyl-CoAs are eluted from the cartridge with 1 ml of 50% acetonitrile in water. The acetonitrile/water fraction is evaporated in a Savant vacuum centrifuge at room temperature. The residue is dissolved in 1 ml of water which is divided into two aliquots for the assays of the mass isomer distribution of (i) propionyl-CoA via propionsarcosine pentafluorobenzyl ester and (ii) methylmalonyl-CoA\(^3\) and succinyl-CoA via the tert-butyldimethylsilyl derivatives of the corresponding acids (27, 28). The measured mass isomer distributions were corrected for the contributions of naturally occurring heavy isotopes (31). In the experiments with NaH\(^{13}\)CO\(_3\) tracer, the enrichment of HCO\(_3^-\)/CO\(_2\) in perfusate or blood was measured by gas chromatography-mass spectrometry (32).

Calculations

The fractional contribution of succinyl-CoA to methylmalonyl-CoA via the reversal of the methylmalonyl-CoA mutase reaction (FC\(_{\text{Succ-CoA} \rightarrow \text{MMA-CoA}}\)) can be calculated from the M2 enrichments of methylmalonyl-CoA and succinyl-CoA labeled from \([U-^{13}\)C\(_3\)]propionate or from \([U-^{13}\)C\(_2\)]lactate + \([U-^{13}\)C\(_2\)]pyruvate. The calculation assumes that the two methylmalonyl-CoA enantiomers are in isotopic equilibrium. This assumption is validated under “Discussion.” Since, with the above tracers, M2 succinyl-CoA is the only source of M2 methylmalonyl-CoA, the balance of M2 isotomers of methylmalonyl-CoA and succinyl-CoA yields Equation 1,

\[
\text{MFM}_2 \text{MMA-CoA} = \frac{\text{FC}_{\text{Succ-CoA} \rightarrow \text{MMA-CoA}} \times \text{MFM}_2 \text{Succ-CoA}}{\text{MFM}_2 \text{MMA-CoA}} \quad (\text{Eq. 1})
\]

where \(\text{MFM}_2 \text{MMA-CoA}\), and \(\text{MFM}_2 \text{Succ-CoA}\), are the mole fractions of M2 methylmalonyl-CoA and M2 succinyl-CoA, respectively. Similar equations can be written for the balances of the M1 and M3 isotomers of methylmalonyl-CoA and succinyl-CoA. We chose not to use the other equations for the following reasons. The M1 enrichments of the CoA esters are often very low and are measured over high M1 natural abundances of the tert-butyldimethylsilyl derivatives of methylmalonyl-CoA and succinate (28% both for M1 versus 10.9% for M2). Second, the equation with the M3 isotomers has an additional term that takes into account that M3 propionyl-CoA is also a source of M3 methylmalonyl-CoA, thus compounding the effect of biological and analytical variability of the measured parameters.

A similar rationale is the basis of Equation 2, which allows calculating the fractional contribution of methylmalonyl-CoA to propionyl-CoA via the reversal of the propionyl-CoA carboxylase reaction.

\[
\text{FC}_{\text{Pr-CoA} \rightarrow \text{Succ-CoA}} = \frac{\text{FC}_{\text{Pr-CoA} \rightarrow \text{Succ-CoA}} \times \text{MFM}_2 \text{Pr-CoA}}{\text{MFM}_2 \text{MMA-CoA}} \quad (\text{Eq. 2})
\]

Data Presentation

Data are expressed as means of the parameters calculated from triplicate gas chromatography-mass spectrometry injections in the analysis of a given tissue or effluent perfusate sample for a given perfusion. We present data from about 50 organ perfusion experiments. For each of the experimental conditions chosen, we ran all perfusions with one parameter (time or concentration of one substrate) being allowed to vary.

RESULTS

We tested the reversibility of the reactions of the propionyl-CoA pathway in perfused rat livers and hearts using three types of isotopic tracers, i.e. NaH\(^{13}\)CO\(_3\), \([U-^{13}\)C\(_2\)]propionate, or \([U-^{13}\)C\(_2\)]lactate + \([U-^{13}\)C\(_2\)]pyruvate, in the presence of increasing concentrations of propionate that are within physiological/therapeutic range.

Perfusions with NaH\(^{13}\)CO\(_3\)—First, we followed the time course of the labeling of liver methylmalonyl-CoA and succinyl-CoA from bicarbonate. Fig. 2A shows that the labeling of methylmalonyl-CoA plateaued at about one-third that of bicarbonate. The labeling of succinyl-CoA was only about \(1/5\) of that of methylmalonyl-CoA. Similar data were obtained in perfused rat hearts, except that the labeling of methylmalonyl-CoA was only \(1/6\) that of bicarbonate (Fig. 2B).

We interpreted data of Fig. 2 as suggesting that the enrichment of methylmalonyl-CoA was diluted via the reversal of the methylmalonyl-CoA mutase reaction. We reasoned that the reversal of the mutase reaction would be decreased by increasing the rate of flow through the propionyl-CoA pathway. We perfused livers and hearts with buffer containing

\(^3\) The mass spectrometric assay of methylmalonyl-CoA does not differentiate between the S and R enantiomers.
40% enriched NaH$^{13}$CO$_3$ and increasing concentrations of unlabeled propionate. The M1 enrichment of methylmalonyl-CoA in livers (Fig. 3A) and hearts (Fig. 3B) increased with the concentration of unlabeled propionate and plateaued at a level very close to the enrichment of bicarbonate. In contrast, the M1 enrichment of succinyl-CoA in livers (Fig. 3A) and hearts (Fig. 3B) plateaued at about one-half and one-fifth, respectively, of that of bicarbonate. Note that the data of Fig. 3, A and B, for 0 mM propionate are similar to those of Fig. 2, A and B (experiments without propionate).

**Perfusions with [U-$^{13}$C$_3$]Propionate—**In livers and hearts perfused with increasing concentrations of [U-$^{13}$C$_3$]propionate, the M3 enrichment of methylmalonyl-CoA increased with the [U-$^{13}$C$_3$]propionate concentration (Fig. 4, A and B). The M3 enrichment of propionyl-CoA was very close to that of methylmalonyl-CoA (not shown). A trace of M2 enrichment of propionyl-CoA was detected in the presence of 0.1 mM [U-$^{13}$C$_3$]propionate, but its value could not be determined with precision. In addition, small amounts of M2 and M1 isotopomers of methylmalonyl-CoA were formed (Fig. 4, A and B). The mass isotopomer distribution of succinyl-CoA (Fig. 4, C and D) showed substantial proportions of M1 to M3 isotopomers.

**Perfusions with [U-$^{13}$C$_3$]Lactate + [U-$^{13}$C$_3$]Pyruvate—**In livers and hearts perfused with 1 mM [U-$^{13}$C$_3$]lactate + 0.2 mM [U-$^{13}$C$_3$]pyruvate and 0 mM unlabeled propionate, all three labeled mass isotopomers of methylmalonyl-CoA were present in variable proportions (see left side of Fig. 5, A and B). In addition, a very small amount of M2 propionyl-CoA was detected (2.8% in liver, 3.9% in heart). When the concentration of unlabeled propionate was increased, (i) the proportions of M1 to M3 isotopomers of methylmalonyl-CoA decreased to almost zero (Fig. 5, A and B), and (ii) no label was detected in propionyl-CoA.

The same livers and hearts contained substantial proportions of M1, M2, and M3 isotopomers of succinyl-CoA (Fig. 5, C and D). In the hearts, the distribution of the succinyl-CoA isotopomers was not affected much by increasing the concentration of unlabeled propionate (Fig. 5D). In contrast, in livers...
perfused under identical conditions, the proportions of the M1 to M3 isotopomers decreased with the concentration of unlabeled propionate (Fig. 5C).

Reversibility of the Methylmalonyl-CoA Mutase Reaction—The M2 enrichments of methylmalonyl-CoA and succinyl-CoA labeled from [U-13C3]propionate (Fig. 4) were introduced into Equation 1 to compute the fractional contribution of succinyl-CoA to methylmalonyl-CoA, an index of the reversibility of the methylmalonyl-CoA mutase reaction. Fig. 6 (solid symbols) shows this index as a function of the propionate concentration. To compute this index for 0 mM propionate (Fig. 6, open symbols), we used the M2 enrichments of methylmalonyl-CoA and succinyl-CoA labeled from [U-13C3]lactate + [U-13C3]pyruvate (left ends of the four panels of Fig. 5). Fig. 6 shows that the contribution of succinyl-CoA to methylmalonyl-CoA is very high (>90%) in liver and heart in the absence of extracellular propionate. As the concentration of propionate increases, this contribution decreases to low levels (about 10%).

To estimate the level of reversibility of the methylmalonyl-CoA mutase reaction in vivo, we infused NaH13CO3 in a branch of the coronary artery of four anesthetized pigs. In the infused myocardial area, the M1 labeling ratios [methylmalonyl-CoA]/[bicarbonate] and [succinyl-CoA]/[bicarbonate] ranged from 36 to 53% and from 1.0 to 2.3%, respectively. These relative percentages are similar to those observed in rat hearts perfused in the absence of propionate (Fig. 2B). The arterial concentration of propionate in the pigs was about 0.03 mM.

DISCUSSION

The present study was designed to characterize the reactions of the propionyl-CoA pathway in liver and heart, emphasizing the reversibility of the reactions and their impact on the labeling pattern of the CoA intermediates. For this purpose, we perfused livers and hearts with NaH13CO3, [U-13C3]propionate, or [U-13C3]lactate + [U-13C3]pyruvate at various propionate concentrations.

The rapid labeling of methylmalonyl-CoA and succinyl-CoA from 40% enriched bicarbonate reflected a rapid turnover of the intermediates (Fig. 2, A and B). The enrichment of succinyl-CoA was low because of dilution of label in the CAC. However, the enrichment of methylmalonyl-CoA, formed by propionyl-CoA carboxylation, was much lower than that of bicarbonate in both livers and hearts, suggesting that the enrichment of methylmalonyl-CoA was diluted via the reversal of the methylmalonyl-CoA mutase reaction. As the concentration of unlabeled propionate in the perfusate was increased, the labeling of methylmalonyl-CoA increased until it equaled that of extracellular bicarbonate (Fig. 3, A and B). This confirmed that, in the
absence of extracellular propionate, the labeling of methylmalonyl-CoA from bicarbonate was diluted by the reversal of the methylmalonyl-CoA mutase reaction. Also, the identical enrichment of methylmalonyl-CoA and bicarbonate at high propionate concentration demonstrated, albeit indirectly, the isotopic equilibration of the methylmalonyl-CoA enantiomers.

We then set out to explore the reversibility of methylmalonyl-CoA mutase by channeling label either via propionyl-CoA (using [U-13C3]propionate) or via the CAC and succinyl-CoA (using [U-13C3]lactate/[U-13C3]pyruvate). The use of uniformly labeled substrates and mass isotopomer distribution analysis provides a greater wealth of information than singly labeled substrates, especially when label passes through the CAC (33, 34). In livers and hearts perfused with increasing concentrations of [U-13C3]propionate, the mass isotopomer distribution of methylmalonyl-CoA was characterized mostly by the M3 isotopomer, the abundance of which was greater than 90% (Fig. 4, A and B). This also reflects the isotopic equilibration of the methylmalonyl-CoA enantiomers. In addition, we observed small but clearly identifiable amounts of M2 and M1 isotopomers of methylmalonyl-CoA, which could not be derived directly from M3 propionate. The origin of the M2 and M1 isotopomers of methylmalonyl-CoA becomes evident when one examines the mass isotopomer distributions of succinyl-CoA, which include M3, M2, and M1 isotopomers (Fig. 4, C and D). In the heart, the proportions of the three enriched mass isotopomers of succinyl-CoA are similar. The markedly different isotopomer enrichment patterns of liver and heart succinyl-CoA at the various propionate concentrations reflects these organs' differential capacity for propionate metabolism as well as for anaplerosis. In the livers, the proportions of the three enriched mass isotopomers of succinyl-CoA are different (M3 > M2 > M1), presumably because of additional isotopic exchanges in the pyruvate → oxaloacetate → phosphoenolpyruvate → pyruvate cycle. In both livers and hearts perfused with [U-13C3]propionate, the M2 and M1 mass isotopomers of succinyl-CoA are clearly the precursors of the M2 and M1 isotopomers of methylmalonyl-CoA. This provides direct evidence for the reversibility of the methylmalonyl-CoA mutase reaction.

Probing the propionyl-CoA pathway with NaH13CO3 or [U-13C3]propionate could not yield information on the possible reversibility of the propionyl-CoA carboxylase reaction. This is why we labeled the pathway via the CAC and succinyl-CoA using [U-13C3]lactate + [U-13C3]pyruvate in the presence of increasing concentrations of unlabeled propionate. With 0 mM propionate, the mass isotopomer distribution of heart succinyl-CoA showed similar proportions of the M1 to M3 isotopomers (left side of Fig. 5D). This again reflects and is compatible with
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A quantitative index of the reversibility of methylmalonyl-CoA mutase, i.e. the fractional contribution of succinyl-CoA to the methylmalonyl-CoA pool was calculated from the M2 enrichments of methylmalonyl-CoA and succinyl-CoA (Fig. 6). In isolated livers and hearts perfused without propionate, more than 90% of methylmalonyl-CoA was derived from the reversal of methylmalonyl-CoA mutase. This high fractional contribution does not represent a net supply of methylmalonyl-CoA, but a rapid interconversion of the two CoA esters at no energy cost. Thus, in the absence of exogenous propionate, the net supply of methylmalonyl-CoA (less than 10%) results from the small flow of propionyl-CoA presumably derived from the catabolism of endogenous aminoisocids. However, the rapid interconversion of methylmalonyl-CoA and succinyl-CoA has a major effect on the former’s labeling pattern. This is because the labeling patterns of methylmalonyl-CoA is strongly influenced by the CAC and related pathways. This explains the major dilution of the enrichment of methylmalonyl-CoA labeled from NaH13CO3 (Fig. 5, A and B, and left sides of Fig. 3, A and B). As the concentration of propionate is increased in the perfusate, the reversibility of methylmalonyl-CoA mutase decreases (Fig. 6), and the labeling of methylmalonyl-CoA from NaH13CO3 becomes very close to that of the latter (Fig. 3, A and B).

To what extent is the methylmalonyl-CoA mutase reaction reversible in vivo? In non-ruminant mammals, the arterial concentration of propionate is very low (5 μM in dogs (35), 30 μM in pigs (13)). From Fig. 6, one would expect marked reversibility of the reaction in the heart. This conclusion is supported by our data obtained in the pig heart perfused in situ with 15% 13C-labeled bicarbonate. In the liver of non-ruminant mammals, the portal vein propionate concentration is about 0.2 mM (35), which should somewhat slow down the reversibility of the mutase reaction. In both heart and liver, the reversibility of the mutase should be markedly decreased after ingestion of a diet rich in triheptanoin, a precursor of propionyl-CoA used to boost anaplerosis in patients with long chain fatty acid oxidation defects (11). Last, in ruminant mammals with high propionate concentrations in the portal and arterial blood, there is probably little reversibility of the mutase.

Propionyl-CoA carboxylase is generally referred to as catalyzing a reversible reaction (20). Kaziro et al. (15) commented that, based on the equilibrium constant of 5.7 and the ΔF of −1028 calories/mol, the reaction is readily reversible. Our data are at variance with this conclusion for two reasons. First, in perfusions with [U-13C3]propionate, propionyl-CoA was only M3 labeled, while methylmalonyl-CoA was labeled in M3, M2, and M1 (Fig. 4, A and B). Second, in perfusions with [U-13C3]lactate + [U-13C3]pyruvate and 0 mM propionate, the labeling of propionyl-CoA was very low (less than 4% M2, not shown). When unlabeled propionate was added to the perfusate, no label was detectable in propionyl-CoA. In contrast, the labeling of methylmalonyl-CoA from [U-13C3]lactate + [U-13C3]pyruvate was substantial in M3, M2, and M1 (Fig. 5, A and B).

We propose that the very low reversibility of the propionyl-CoA carboxylase reaction in intact livers and hearts results from the fact that the mass action ratio of this reaction is far from equilibrium. The equilibrium constant of the propionyl-CoA carboxylase reaction allows one to calculate that, at thermodynamic equilibrium, the mass action ratio ([S]-methylmalonyl-CoA/[propionyl-CoA] should be about 10. This is based on reported values for the mitochondrial [ATP]/[ADP] ratio of 0.5 in liver (36) mitochondrial [Pi] of 7.5 mM (36) and an assumed [HCO3−] of 25 mM. Since the equilibrium constant of methylmalonyl-CoA racemase is 1.0 (23), and since we have evidence that the pool of tissue methylmalonyl-CoA is racemic,
we would expect the mass action ratio $[\text{[S,R]}]/[\text{propionyl-CoA}]$ to be about 20 if the combined propionyl-CoA carboxylase + methylmalonyl-CoA racemase reaction was at thermodynamic equilibrium. In fact, we found that the ratio $[\text{[S,R]}]/[\text{propionyl-CoA}]$ is about 0.6 in rat livers perfused without or with 1 mM propionate (27). We can, therefore, conclude that the propionyl-CoA carboxylase reaction is quite far from thermodynamic equilibrium in the intact liver.

This rationale can be extended to the combined equilibrium constant of the reactions catalyzed by propionyl-CoA carboxylase, methylmalonyl-CoA racemase, and methylmalonyl-CoA mutase ($K \approx 18.6$ (24)). Then, the $[\text{succinyl-CoA}]/[\text{propionyl-CoA}]$ ratio should be about 20 if the combined propionyl-CoA carboxylase + methylmalonyl-CoA racemase reaction was at thermodynamic equilibrium. In fact, we found that the ratio is (i) 3.7 and 0.14 in rat livers perfused without or with 1 mM propionate, respectively, and (ii) 18 and 0.3 in hearts perfused without or with 1 mM propionate, respectively. The measured $[\text{succinyl-CoA}]/[\text{propionyl-CoA}]$ ratio is one to three orders of magnitude smaller than its thermodynamic equilibrium value (12, 27). The dis-equilibrium of the propionyl-CoA to succinyl-CoA reaction sequence reflects the drawing of succinyl-CoA derived from propionyl-CoA into the CAC. This explains the efficiency of anaplerosis from low concentration (0.25 mM) of [U-13C3]propionate in pig heart (9% of the flux through the CAC (13)).

In conclusion, our data demonstrate that in intact normal rat livers and hearts, (i) the methylmalonyl-CoA mutase reaction is partly reversible and that this reversibility is modulated by the propionyl-CoA flux, (ii) the methylmalonyl-CoA enantiomers are maintained in isotopic equilibrium via the reversible methylmalonyl-CoA racemase reaction, and (iii) the reversibility of the propionyl-CoA carboxylase reaction is minor. The dis-equilibrium of the propionyl-CoA to succinyl-CoA reaction sequence explains the effectiveness of anaplerosis from low concentrations of propionyl-CoA precursors such as propionate or heptanoate (11, 13).

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