Radiolabeled Acetate as a Tracer of Myocardial Tricarboxylic Acid Cycle Flux

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The kinetics of [1-14C]acetate oxidation in isolated perfused rat hearts have been determined over a range of perfusion conditions. Effluent measurements demonstrated that 14CO2 cleared biexponentially over 50 minutes after bolus injection of [1-14C]acetate into normoxic hearts perfused with 5 mM glucose and 10 mU/ml insulin. The clearance half-time (t1/2) for the predominant initial clearance phase was 3.1 ± 0.5 minutes (n = 4). MVO2 was varied over a fourfold range by hypoxia and phenylephrine stimulation (t1/2, 7.2 ± 1.2 and 2.2 ± 0.2 minutes, respectively) and in the presence of alternate substrates (lactate, 2 mM; DL-3-hydroxybutyrate, 20 mM; and palmitate, 0.1 mM), which did not modify either tricarboxylic acid (TCA) cycle flux or acetate kinetics. A good correlation (r = 0.93) was observed between k, the rate constant for the initial phase of 14CO2 clearance, and TCA cycle flux, estimated from oxygen consumption. In contrast to results with [1-14C]acetate, lactate (2 mM) increased t1/2 for 14CO2 clearance from a bolus injection of [1-14C]palmitate from 3.0 ± 0.4 minutes (n = 3) at control to 4.3 ± 0.2 minutes (n = 3, p < 0.01). Addition of acetate in nontracer amounts (0.5 or 5 mM) caused significant underestimation of TCA cycle flux when estimated with [1-14C]acetate. 14CO2 clearance accounted for 88–98% of total effluent 14C between 10 and 20 minutes after [1-14C]acetate bolus injection; rate constants for clearance of 14CO2 and total 14C clearance were very similar during this period, and these two rate constants did not differ significantly from each other under any conditions tested. It is concluded that radiolabeled acetate kinetics are insensitive to myocardial substrate supply and may thus provide the basis for quantitative estimation of myocardial TCA cycle flux, allowing 14C acetate to be used in vivo to assess directly myocardial oxidation metabolism with positron emission tomography. (Circulation Research 1988;63:628–634)

Positron emission tomography (PET) offers a unique potential for the noninvasive study of regional myocardial metabolism in vivo. Currently, no tracer is available to assess overall myocardial oxidative metabolism by PET. The tissue kinetics of [1-14C]palmitate have been used to assess qualitatively myocardial oxidative metabolism, the rate constant for initial clearance of palmitate from myocardium being decreased in ischemia.1–4 Clearance of palmitate from reperfused or "stunned" myocardium was also slowed but returned to normal over time, correlating well with normalization of mechanical function.5 However, quantitative assessment of myocardial oxidative metabolism and function by measurement of [1-14C]palmitate kinetics is complicated by the sensitivity of long-chain fatty acid oxidation to changes in substrate supply.6–8 Since all major myocardial oxidative fuels, including fatty acids, glucose, lactate, pyruvate, ketone bodies, and some amino acids are oxidized via conversion to acetyl coenzyme A (CoA) and passage through the tricarboxylic acid (TCA) cycle, a tracer of TCA cycle flux might allow a more readily quantifiable assessment of overall oxidative metabolism in myocardium. Acetate is readily oxidized via the TCA cycle, and in myocardium is not metabolized via any other major pathways.9–12

Initial studies in dogs12 and human subjects13 with...
[1-\textsuperscript{14}C]acetate have demonstrated monoexponential clearance of \textsuperscript{14}C activity from myocardial tissue. Clearance rates were slower in ischemic relative to normal tissue. This qualitative relation between oxidative metabolism and tissue clearance kinetics supported the potential usefulness of [1-\textsuperscript{14}C]acetate for myocardial imaging and studies of myocardial oxidative metabolism. However, in the initial studies cited, no attempt was made to relate quantitatively acetate kinetics to TCA cycle flux. The present study was designed to investigate the relations between acetate oxidation, acetate kinetics, and TCA cycle flux in the isolated perfused rat heart, and to assess the effects of different myocardial substrates on acetate kinetics. The results support the potential of acetate for noninvasive assessment of myocardial oxidation by PET in vivo.

\textbf{Materials and Methods}

Langendorf perfusion of hearts from male Sprague-Dawley rats, 225-350 g, fed ad libitum was performed as described previously using a constant flow rate of 10 ml/min.\textsuperscript{14} The pulmonary artery was cannulated for sampling cardiac effluent and for continuous monitoring of oxygen consumption using an on-line Clark-type oxygen electrode. The perfusate, Krebs-Henseleit bicarbonate buffer,\textsuperscript{15} contained glucose, 5 mM, and insulin, 10 mU/ml; the calcium chloride concentration was 1.25 mM. For specific study protocols, additional substrates and hormones were administered by an infusion syringe into the perfusion line. Potassium chloride arrest was achieved by infusion of potassium chloride to give a perfusate concentration of 20 mM.

In washout experiments [1-\textsuperscript{14}C]acetate (15 \textmuCi; specific activity, 10 mCi/mmole) was given as a bolus (0.3 ml) over 10 seconds. Effluent perfusate samples were collected for determination of \textsuperscript{14}CO\textsubscript{2} and total \textsuperscript{14}C activity. \textsuperscript{14}CO\textsubscript{2} was determined by acidification of effluent perfusate samples in sealed 25-ml flasks and by trapping of \textsuperscript{14}CO\textsubscript{2} with phenethylamine followed by liquid scintillation counting as described previously.\textsuperscript{16} An aliquot of the acidified perfusate was scintillation-counted for determination of non-\textsuperscript{14}CO\textsubscript{2} radioactivity. An aliquot of [1-\textsuperscript{14}C]acetate was also acidified in a flask and apparent \textsuperscript{14}CO\textsubscript{2} counts determined to correct for volatility of acetate;\textsuperscript{11} the correction was 0.5-1.5\% of [1-\textsuperscript{14}C]acetate radioactivity. [1-\textsuperscript{14}C]palmitate washout experiments were similarly performed; [1-\textsuperscript{14}C]palmitate (specific activity 20 mCi/mmole) was bound to bovine serum albumin, 0.1\% wt/vol; a 0.25 ml bolus containing 20 \textmuCi was injected.

TCA cycle flux was estimated from oxygen consumption. Because the partition of oxygen for TCA cycle/noncycle oxidations is similar for palmitate and carbohydrate (16/7 and 2/1, respectively), TCA cycle flux (in \textmu mol acetyl CoA units) was estimated as one third of \textsuperscript{18}O consumption.\textsuperscript{11} Because acetate consumes oxygen exclusively via the TCA cycle, TCA cycle flux was estimated as the acetate oxidation rate + (oxygen consumption - 2 \times acetate oxidation rate).\textsuperscript{3,11,17} When acetate was present in nontracer quantities, acetate oxidation rates were estimated by steady-state perfusion of hearts with known concentrations and specific activities of [1-\textsuperscript{14}C]acetate and collection of effluent \textsuperscript{14}CO\textsubscript{2} as described above. This method will slightly underestimate acetate oxidation because incorporation of the label into TCA cycle intermediates is neglected. However, the error is normally small (~10\%).\textsuperscript{10,11}

\textsuperscript{14}CO\textsubscript{2} and total \textsuperscript{14}C tissue clearance were analyzed by monoexponential or biexponential least-squares fitting routines as appropriate. The relative sizes of the two components in biphasic \textsuperscript{14}CO\textsubscript{2} washout curves were estimated by extrapolating the curve component to the time of maximum \textsuperscript{14}CO\textsubscript{2} production. Tissue clearance half-times (t\textsubscript{1/2}) were determined from the clearance rate constant (k) by the relation t\textsubscript{1/2} = ln 2/k.

Results are expressed per gram dry weight; hearts were weighed after drying overnight at 150°C.

Results are given as mean ±SD. Statistically significant differences were assessed by analysis of variance followed by Students t test with the Bonferroni method. A p<0.05 was considered statistically significant. Regression analysis was by weighted (1/y) least-squares.

\textbf{Results}

The time course of effluent perfusate \textsuperscript{14}CO\textsubscript{2} and total \textsuperscript{14}C activity following a bolus injection of [1-\textsuperscript{14}C]acetate into a representative rat heart perfused with glucose and insulin under control conditions is shown in Figure 1A. Peak \textsuperscript{14}CO\textsubscript{2} efflux occurred at 3.0±0.8 minutes (n = 4), followed by a biexponential clearance of \textsuperscript{14}CO\textsubscript{2}. The initial \textsuperscript{14}CO\textsubscript{2} clearance component had a t\textsubscript{1/2} of 3.1±0.5 minutes (n = 4). A second, slower component, with t\textsubscript{1/2} of approximately 17 minutes was also observed. The initial rapid component of \textsuperscript{14}CO\textsubscript{2} production accounted for approximately 98% of total \textsuperscript{14}CO\textsubscript{2} effluent. Total \textsuperscript{14}C activity, after clearance of the initial vascular component closely paralleled \textsuperscript{14}CO\textsubscript{2} production; during the initial phase of \textsuperscript{14}CO\textsubscript{2} clearance between 10 and 20 minutes after injection of acetate, \textsuperscript{14}CO\textsubscript{2} accounted for approximately 97% of total \textsuperscript{14}C activity.

Figure 1B demonstrates the effect of hypoxia on myocardial acetate kinetics. Peak \textsuperscript{14}CO\textsubscript{2} production occurred later, at 4.6±0.5 minutes (n = 4), and was followed by a clearance curve that, up to 50 minutes, could be fitted monoexponentially with a half-time of 7.2±1.2 minutes (n = 4; p<0.001 vs. control). However, in two experiments that were continued to 75 minutes, a second kinetic component was observed; due to the small amount of radioactivity in this component accurate quantitation was not possible, but t\textsubscript{1/2} was approximately 20 minutes, similar to normoxia, although the relative size was greater, approximately 10% of the total. Since curve-stripping was not performed on hypox-
Figure 1. Efflux of $^{14}$CO$_2$ (●) and total $^{14}$C (○) from isolated perfused rat hearts following bolus injection of [1-$^{14}$C]acetate. All hearts were perfused with glucose, 5.5 mM, and insulin, 10 mU/ml. Curves were fitted by monoexponential or biexponential least-squares curve fitting as appropriate. A, control: normoxic buffer, oxygen concentration 750 μM. Line equations for $^{14}$CO$_2$ and total $^{14}$C clearance were $y=2.5 \times 10^7 \cdot e^{-0.0201x} + 5.7 \times 10^4 \cdot e^{-0.0075x}$, respectively ($r=0.999$). B, hypoxia: oxygen concentration 220 μM. Injection of [1-$^{14}$C]acetate was made 5 minutes after switching from normoxic perfusate ($y=2.6 \times 10^5 \cdot e^{-0.0532x}$, $r=0.999$ and $y=2.7 \times 10^5 \cdot e^{-0.0077x}$, $r=0.998$). C, + phenylephrine, 10^{-5}M. [1-$^{14}$C]acetate bolus injection was at 5 minutes after start of phenylephrine infusion ($y=1.9 \times 10^7 \cdot e^{-0.0615x} + 4.8 \times 10^4 \cdot e^{-0.0043x}$, $r=0.997$ and $y=1.7 \times 10^7 \cdot e^{-0.0215x} + 5.0 \times 10^4 \cdot e^{-0.0011x}$, $r=0.999$). Curves shown are representative experiments.

In hearts using long-chain fatty acid and carbohydrate as energy sources, TCA cycle flux (in micromoles acetyl CoA units) can be estimated as one third of oxygen consumption (or two thirds in oxygen equivalents) since the non-TCA cycle oxygen consumption of these substrates is very similar. When acetate is present in nontracer amounts, acetate oxidation must be accounted for because 100% rather than 67% of the oxygen consumed by acetate is via the TCA cycle. Consequently, acetate (5 mM) stimulates TCA cycle flux in the perfused heart by 48–68%.$^{11,16}$ The oxidation rate of acetate was therefore investigated as a function of acetate concentration (Figure 2). As the acetate concentration in the perfusate was increased from 36 μM to 5 mM, acetate decarboxylation increased progres-
deviation from the relation between TCA cycle flux and \( k \).

Palmitate, and DL-3-hydroxybutyrate were without significant effect on either TCA cycle flux or \( k \), while as observed in Figure 3A, the addition of acetate in nontracer amounts caused divergence from the relation between TCA cycle flux and \( k \).

Acetate kinetics were also measured in KC\(\text{I}^{-}\)-arrested hearts, where a significant increase in \( t_{1/2} \) (Table 1) was observed (\( p<0.01 \)). Acetate (0.5 mM and 5 mM) progressively decreased \( t_{1/2} \) (Table 1), similar to M\( ^{14}\)CO\(\text{2} \) washout from [\( \text{1-}^{14}\text{C} \)]acetate; during this time, efflux was approximately monoexponential in all experiments. TCA, tricarboxylic acid.

\( *p<0.001 \) compared with control group.

To compare the sensitivity of acetate kinetics with alternate substrates in the perfusate with that of palmitate, washout experiments were performed with [\( \text{1-}^{14}\text{C} \)]palmitate in the presence and absence of lactate (2 mM). Figure 4 demonstrates that clearance of \( ^{14}\text{CO}_{2} \) from hearts following a bolus injection of [\( \text{1-}^{14}\text{C} \)]palmitate was biexponential. However, in this case, the second, slower phase was much larger than observed with [\( \text{1-}^{14}\text{C} \)]acetate, approximately 15%, and is believed to reflect extensive esterification of long-chain fatty acids in myocardium and slow turnover of the pool.\(^1\) In control hearts (Figure 4A), the initial rapid \( ^{14}\text{CO}_{2} \) phase cleared with \( t_{w} \) 3.0±0.4 minutes (\( n=3 \)), similar to \( ^{14}\text{CO}_{2} \) washout from [\( \text{1-}^{14}\text{C} \)]acetate (3.1±0.5 minutes). However, in the presence of lactate (2 mM), although acetate kinetics were unaltered, reflecting unchanged TCA cycle flux, \( ^{14}\text{CO}_{2} \) produc-

\( \text{TABLE 1. Acetate Kinetics, Oxygen Consumption, and TCA Cycle Flux in Perfused Rat Hearts} \)

<table>
<thead>
<tr>
<th>Condition</th>
<th>( t_{1/2} ) ( ^{14}\text{CO}_{2} ) (min)</th>
<th>( t_{1/2} ) total ( ^{14}\text{CO}_{2} ) (min)</th>
<th>( ^{14}\text{CO}_{2} ) % total</th>
<th>( M_{\text{VO}_{2}} ) (( \mu \text{mol/g/min} ))</th>
<th>TCA cycle flux (( \mu \text{mol/g/min} ))</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.1±0.5</td>
<td>3.0±0.5</td>
<td>95.1±3.0</td>
<td>28.8±0.6</td>
<td>9.6±0.2</td>
<td>4</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>7.2±1.2*</td>
<td>7.4±1.0*</td>
<td>87.1±4.1</td>
<td>10.7±1.5*</td>
<td>3.6±0.5*</td>
<td>3</td>
</tr>
<tr>
<td>KCl arrested</td>
<td>6.0±0.3*</td>
<td>6.2±0.9*</td>
<td>87.7±5.1</td>
<td>15.2±1.3*</td>
<td>5.1±0.3*</td>
<td>3</td>
</tr>
<tr>
<td>+ Lactate (2 mM)</td>
<td>3.3±0.8</td>
<td>3.4±1.1</td>
<td>95.3±2.7</td>
<td>24.3±4.4</td>
<td>8.1±1.5</td>
<td>3</td>
</tr>
<tr>
<td>+ Palmitate (0.1 mM)</td>
<td>3.1±0.4</td>
<td>3.1±0.3</td>
<td>97.3±1.3</td>
<td>24.8±1.4</td>
<td>8.2±0.5</td>
<td>4</td>
</tr>
<tr>
<td>+ 3-Hydroxybutyrate (20 mM)</td>
<td>3.2±0.8</td>
<td>3.2±0.7</td>
<td>89.4±6.8</td>
<td>26.5±3.5</td>
<td>8.8±1.2</td>
<td>4</td>
</tr>
<tr>
<td>+ Phenylephrine (10 ( \mu \text{M} ))</td>
<td>2.2±0.2</td>
<td>2.1±0.2</td>
<td>93.3±2.8</td>
<td>33.0±3.6</td>
<td>11.0±2.2</td>
<td>7</td>
</tr>
<tr>
<td>+ Acetate (0.5 mM)</td>
<td>3.1</td>
<td>3.1</td>
<td>98.2</td>
<td>24.9</td>
<td>10.1</td>
<td>2</td>
</tr>
<tr>
<td>+ Acetate (5 mM)</td>
<td>2.5±0.4</td>
<td>2.5±0.2</td>
<td>93.9±3.2</td>
<td>29.4±5.1</td>
<td>13.8±1.7*</td>
<td>5</td>
</tr>
</tbody>
</table>

\( \text{Clearance half-time (} t_{1/2} \text{) for} ^{14}\text{CO}_{2} \) and total \( ^{14}\text{C} \) in effluent perfusate was determined by monoexponential curve fitting of the initial kinetic component. \( ^{14}\text{CO}_{2} \) as a percentage of total integrated \( ^{14}\text{C} \) efflux was determined between 10 minutes and 20 minutes after bolus injection of [\( \text{1-}^{14}\text{C} \)]acetate; during this time, efflux was approximately monoexponential in all experiments. TCA, tricarboxylic acid.

\( *p<0.001 \) compared with control group.

To compare the sensitivity of acetate kinetics with alternate substrates in the perfusate with that of palmitate, washout experiments were performed with [\( \text{1-}^{14}\text{C} \)]palmitate in the presence and absence of lactate (2 mM). Figure 4 demonstrates that clearance of \( ^{14}\text{CO}_{2} \) from hearts following a bolus injection of [\( \text{1-}^{14}\text{C} \)]palmitate was biexponential. However, in this case, the second, slower phase was much larger than observed with [\( \text{1-}^{14}\text{C} \)]acetate, approximately 15%, and is believed to reflect extensive esterification of long-chain fatty acids in myocardium and slow turnover of the pool.\(^1\) In control hearts (Figure 4A), the initial rapid \( ^{14}\text{CO}_{2} \) phase cleared with \( t_{w} \) 3.0±0.4 minutes (\( n=3 \)), similar to \( ^{14}\text{CO}_{2} \) washout from [\( \text{1-}^{14}\text{C} \)]acetate (3.1±0.5 minutes). However, in the presence of lactate (2 mM), although acetate kinetics were unaltered, reflecting unchanged TCA cycle flux, \( ^{14}\text{CO}_{2} \) produc-

\( \text{FIGURE 3. Concentration curve for decarboxylation of} [\( \text{1-}^{14}\text{C} \)]acetate in perfused rat heart. Increasing [\( \text{1-}^{14}\text{C} \)]acetate concentrations were infused sequentially into isolated perfused hearts and \( ^{14}\text{CO}_{2} \) production determined. Each concentration was infused for 15 minutes, during which time steady-state \( ^{14}\text{CO}_{2} \) production was reached. Results are mean±SD for three hearts.} \)
FIGURE 3. Relation between TCA cycle flux and $[1^{14}C]$ activity clearance. A, correlation between $k$, the rate constant for the first component of $^{14}CO_2$ efflux and TCA cycle flux. The solid line represents the weighted regression line for experiments with tracer acetate only (•). The equation for the line was $y = 0.0256x + 0.003$ ($r = 0.93; p < 0.001$). The dashed line is the fit for experiments with nontracer acetate concentrations (0.5 mM and 5 mM) (△) and control experiments. The equation for the line was $y = 0.013x + 0.101$ ($r = 0.71; p < 0.025$). The difference in slopes was significant ($p < 0.01$).

B, comparison of $k$ and TCA cycle flux under varying metabolic conditions. Results are mean±SD for 2–7 hearts.

Discussion

In the presence of tracer acetate concentrations, a close correlation was observed between the rate of the initial rapid phase of $^{14}CO_2$ clearance from myocardium and TCA cycle flux, as estimated from oxygen consumption. This finding supports the potential of labeled acetate for developing an in vivo radioassay method for determining regional TCA cycle flux. $T_{1/2}$ for the initial $^{14}CO_2$ clearance component, 3.1 ± 0.5 minutes, is similar to that observed for clearance of $^{14}CO_2$ from $[2-{^14}C]$pyruvate ($T_{1/2}$, 2.7 minutes), assumed to represent TCA cycle decarboxylation. In the presence of 5 mM acetate, TCA cycle flux is stimulated by about 60%, partly due to increased oxygen consumption, but mainly due to the absence of reduced nicotinamide adenine dinucleotide generation by non-TCA cycle reactions when acetate serves as a substrate. In vivo radioassay method for determining regional TCA cycle flux. $T_{1/2}$ for the initial $^{14}CO_2$ clearance component, 3.1 ± 0.5 minutes, is similar to that observed for clearance of $^{14}CO_2$ from $[2-{^14}C]$pyruvate ($T_{1/2}$, 2.7 minutes), assumed to represent TCA cycle decarboxylation. In the presence of 5 mM acetate, TCA cycle flux is stimulated by about 60%, partly due to increased oxygen consumption, but mainly due to the absence of reduced nicotinamide adenine dinucleotide generation by non-TCA cycle reactions when acetate serves as a substrate.$^{11,17}$ It can be seen from Figure 3 that in the presence of acetate, $k_1$, the rate constant for the initial $^{14}CO_2$ clearance component, was not increased proportionately with increased TCA cycle flux. Thus, in the presence of 5 mM exogenous acetate, $k_1$ underestimates TCA cycle flux by approximately 30% or about one half the stimulation of TCA cycle flux by acetate. Acetate infusion has been demonstrated to lead to a large increase in tissue acetyl CoA concentration in perfused hearts, and it is possible that the underestimation of TCA cycle flux by $k$ is related to the acetyl CoA pool size expansion. Since the cytosolic acetyl CoA pool has been proposed to act as an overflow when mitochondrial acetyl CoA is elevated,$^{19}$ the inclusion or expansion of an additional cytosolic acetyl CoA pool may account for the decrease in $k$.

Metabolism of acetate in myocardial tissue is less complex than that of long-chain fatty acids because 80–90% of acetate extracted by myocardium is oxidized.$^{10,11}$ Activation of acetate in myocardial tissue by acetyl CoA synthase takes place primarily in the mitochondrial matrix, in contrast to liver and adipose tissue where the primarily cytoplasmic location of acetyl CoA synthase reflects extensive use of acetate for long-chain fatty acid biosynthesis.$^{20,21}$ The inner mitochondrial membrane is impermeable to acetyl CoA, but acetyl CoA can be transferred to the cytosol via a carnitine-mediated acetyltransferase system.$^{22,23}$ Williamson demonstrated that in normoxic perfused hearts, less than 0.1% of acetate was incorporated into tissue lipid. Anoxia has been shown to increase esterification of $^{14}C$-acetate 12-fold in perfused rat hearts$^{24}$; however, the extent of esterification was small, 1.3 μmol · hr$^{-1}$ per heart in anoxia, which can be calculated to represent less than 2% of the acetate supplied.

The major alternative to direct oxidation of acetate in myocardial tissue is transamination to glutamate and aspartate via the TCA cycle intermediates 2-oxoglutarate and oxaloacetate respectively, with
glutamate representing the larger component.\textsuperscript{10,11,25} The second kinetic component for \textsuperscript{14}C washout observed in the present study (t_{1/2} approximately 17 minutes) may represent incorporation of the \textsuperscript{14}C label into these amino acid pools and subsequent turnover, although this possibility has yet to be confirmed.

Plasma acetate levels are generally much lower than 5 mM. Normal levels reported in the literature for human plasma acetate concentration range from 30–250 \textmu M.\textsuperscript{26,27} Corresponding acetate oxidation rates in perfused rat heart are 0.5–3 \textmu mol/g/min (Figure 2), which can be calculated to result in increases in TCA cycle flux of 2–10% compared with calculations based simply on M\textsubscript{VO}_2.\textsuperscript{3} Thus, under normal physiological conditions, underestimation of TCA cycle flux by \textit{k}\textsubscript{i} is likely to be insignificant. Since [1-\textsuperscript{14}C]acetate can be synthesized at high specific activity (100–300 Ci/mmols), only \textasciitilde 50 nmol of [1-\textsuperscript{14}C]acetate need be injected for imaging purposes (∼10 mCi). After ethanol administration however, blood acetate concentrations may reach 0.6–1 mM and remain elevated for several hours.\textsuperscript{27,28}

PET measures only tissue concentrations of radiolabel rather than individual chemical species. The relation between clearance of labeled CO\textsubscript{2} and total radioactivity clearance is therefore of paramount importance if tissue clearance rates are to give accurate estimates of TCA cycle flux. It can be seen in Table 1 that total effluent \textsuperscript{14}CO\textsubscript{2} closely parallels \textsuperscript{14}CO\textsubscript{2} efflux over a wide range of oxygen consumption, reflecting the small contribution of non-\textsuperscript{14}CO\textsubscript{2} labeled component to the total. In this series of experiments the relative contribution of back diffusion of \textsuperscript{14}C acetate and of metabolites to non-\textsuperscript{14}CO\textsubscript{2} activity was not determined. Further studies are underway to address this issue.

Central to the potential of acetate as an in vivo TCA cycle tracer is the demonstration that the alternate substrates of palmitate, lactate, and DL-3-hydroxybutyrate were without effect on \textit{k}\textsubscript{i}. In control hearts to which [1-\textsuperscript{14}C]palmitate was administered, \textit{k}\textsubscript{i} was similar to that observed with [1-\textsuperscript{14}C]acetate, suggesting that under these conditions palmitate could be used as a TCA cycle tracer. However, inclusion of lactate (2 mM) caused a dissociation between \textit{k}\textsubscript{i} for palmitate and TCA cycle flux. Although TCA cycle flux was unaltered, \textit{k}\textsubscript{i} was significantly decreased, reflecting inhibition of long-chain fatty acid oxidation by lactate at a step prior to TCA cycle oxidation of acetyl CoA.

In conclusion, these results and the preliminary study of Brown et al in perfused rabbit heart support the potential of [1-\textsuperscript{14}C]acetate as a quantitative tracer of regional myocardial oxidative metabolism for use with PET. In addition, compared with [1-\textsuperscript{14}C]palmitate, kinetics of [1-\textsuperscript{14}C]acetate are relatively straightforward and insensitive to changes in myocardial substrate supply with the possible exceptions discussed above. This would allow the use of a rather simple tracer kinetic model to quantitate myocardial oxidative metabolism with [1-\textsuperscript{14}C] acetate.

Acknowledgments

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**KEY WORDS** • acetate • positron emission tomography • oxidative metabolism