Effect of Flow-Independent Reduction of Metabolism on Regional Myocardial Clearance of \(^{11}\)C-Palmitate

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SUMMARY Recent studies with sequential positron-emission tomography have demonstrated that early clearance of activity from myocardium after i.v. carbon-11 \(^{11}\)C-palmitate is decreased in regions of ischemia. To determine whether the reduced clearance is a reflection of decreased washout of labeled substrate or its metabolites, or a reflection of decreased metabolism labeled fatty acid, we characterized the effects of restricted oxygen supply on regional \(^{11}\)C clearance rates in vivo under two conditions: hypoxia without concomitant reduction of flow and hypoxia induced by reduction of flow (ischemia). In 21 open-chest dogs, the left anterior descending coronary artery (LAD) was cannulated and perfused by an extracorporeal bypass system. In each dog two regional time-activity curves (90 minutes apart) were recorded with a \(\beta\)-detector probe after intracoronary injection of \(^{11}\)C-palmitate. In control dogs \((n = 7)\), no intervention was imposed between the two studies. In the experimental dogs, oxygen supply was reduced 15 minutes before the second injection of \(^{11}\)C-palmitate by either reducing LAD flow by an average of 76\% (ischemia group, \(n = 7\)) or by perfusing the LAD bed at normal flow rate with venous blood, resulting in an average reduction in oxygen content of 66\% (hypoxia group, \(n = 7\)). Myocardial blood flow in the LAD-perfused region determined based on washout of H\(^{14}\)O did not change in either the control or hypoxia group, but decreased by an average of 64\% \((p < 0.025)\) in the ischemia group. Similarly, \(^{11}\)C clearance rates did not change from the first to the second study in control dogs. However, the \(^{11}\)C clearance rate was reduced by an average of 52\% with hypoxia despite maintenance of flow \((p < 0.02)\) as well as by ischemia itself \((61\%, p < 0.005)\). Thus, clearance of \(^{11}\)C-activity after extraction of \(^{11}\)C-palmitate by myocardium is consistently reduced in regions rendered hypoxic despite persistence of perfusion, supporting the hypothesis that the metabolic attenuation induced by hypoxia or ischemia per se can be detected in patients based on sequential and quantitative analysis of regional time-activity curves obtained by positron-emission tomography.

DECREASED OXIDATIVE METABOLISM of fatty acids is an early consequence of ischemia.\(^{1}\)\(^{4}\) Since palmitate labeled with the positron-emitting isotope carbon-11 \(^{11}\)C has the same biochemical properties as its physiologic counterpart and since positron-emission tomography permits quantitative determination of regional myocardial uptake and release of positron-emitting isotopes noninvasively, positron-emission tomography with \(^{11}\)C-palmitate has been suggested as an approach for delineation of ischemic myocardium based on its altered fatty acid metabolism.\(^{5}\)\(^{9}\) However, Schelbert et al.\(^{10}\) suggested that results would be distorted because of altered residence time or that altered washout would mask detection of impaired metabolism induced by hypoxia.

We showed that viable but ischemic myocardium supplied by vessels with critical coronary stenosis in dogs extract \(^{11}\)C-palmitate.\(^{4}\) In ischemic zones, the monoeponential decline of activity 5-15 minutes after administration of tracer has a lower rate constant than that in normal regions.\(^{8}\) At least two factors might account for this: decreased production of labeled diffusible metabolites due to impaired oxidative metabolism, and reduced washout of unmetabolized \(^{11}\)C-palmitate or labeled products of intermediary metabolism due to reduced myocardial blood flow per se. Based on results in vitro, metabolism itself appears to be a major determinant of this early phase of clearance.\(^{11}\) However, myocardial metabolism and perfusion change concomitantly in vivo,\(^{12}\) complicating the differentiation of the relative importance of metabolism and flow on observed changes in clearance of tracer. We used an experimental preparation designed to permit independent control of coronary flow to selected regions of myocardium in vivo and to permit independent control of oxygen delivery without reduction of flow by perfusion of the same regions with hypoxic blood.

The purpose of the present study was to determine whether reduced oxidative metabolism induced either by reduction of coronary flow (ischemia) or perfusion with hypoxic blood at physiologic flow rate (hypoxia) influences clearance of \(^{11}\)C-palmitate extracted by the myocardium independent of flow itself.

Methods

Twenty-one dogs (18-25 kg) were anesthetized with sodium thiopental, 12 mg/kg i.v., followed by \(\alpha\)-chloralose, 90 mg/kg, and urethane, 375 mg/kg i.v. After intubation, each dog was ventilated with a Harvard respirator (model 607) with room air enriched with oxygen to maintain arterial \(P_O_2\) greater than 90 mm Hg. Catheters were advanced from a right femoral cutdown into the ascending aorta for con-
Continuous pressure recording and into the inferior vena cava for administration of drugs. A left thoracotomy was performed in the fifth intercostal space and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated distal to the first diagonal branch. A polyethylene 20-gauge catheter (Argyle Intrimedicut) was inserted into a vein distal to the isolated artery segment. An electrode was sutured on the epicardium in an area perfused by the distal LAD for recording of the ECG. After i.v. heparin, 10,000 U, and lidocaine, 40 mg/kg, the LAD was ligated and cannulated distal to the ligature by a polyethylene cannula (0.8 mm i.d.). Perfusion was then initiated with blood from the femoral artery through the extracorporeal bypass system. Interruption of coronary flow for cannulation did not exceed 3 minutes.

Figure 1 is a diagram of the extracorporeal bypass system. Polyethylene catheters joined by a Y-connector were inserted into the descending aorta and the vena cava distal to the renal veins from a left femoral cutdown to permit perfusion with either arterial or venous blood. The bypass system included a calibrated occluding roller pump (Buchler Instruments), a heat exchanger to maintain temperature of the blood entering the LAD at 37°C, and a side arm for pressure measurements. Tracers were administered through a T-connector sealed with a rubber membrane inserted just proximal to the coronary cannula. Pump calibration included documentation of independence of delivered flow rates from inflow and outflow pressure as high as 200 mm Hg.

Aortic and coronary perfusion pressures were measured with Statham P23Db transducers. Perfusion pressure measurements were corrected for the small pressure gradient at the coronary cannula, determined during each experiment at the flow rates used with each dog's own blood. Pressures and a bipolar ECG recording obtained with the reference electrode on the left leg were recorded on a Gould recorder (model 2200).

Hemoglobin concentration and hemoglobin oxygen saturation in samples from the bypass system and from the coronary vein were assayed with an Instrumentation Laboratory Oximeter (model IL 182), $P_{O_2}$, $P_{CO_2}$ and pH were measured with an Instrumentation Laboratory blood gas analyzer (model 213). Blood samples were analyzed for plasma lactate and free fatty acid content enzymatically and colorimetrically.

Detection of Regional Time-Activity Curves

Myocardial time-activity curves after intracoronary injection of $^{13}$C-palmitate (half-life of $^{13}$C, 20.4 minutes) or oxygen-15-labeled water ($H_2^{15}O$) (half-life of $^{16}$O, 2.07 minutes) were determined with a recently developed and characterized $\beta$-detector probe positioned above the epicardial surface in a region between two arterial branches perfused by the cannulated LAD. The $\beta$-probe head contains a 3-mm-thick cylinder of scintillation plastic covered at the window opening by aluminum (0.05 mm thick), resulting in high efficiency for beta radiation compared to gamma radiation.

The fraction of recorded events attributable to gamma photons was estimated for $^{13}$C by counting test sources with selected amounts of $^{13}$C with and without interposition of a 0.5-mm lead, sufficient to absorb positrons completely but insufficient to attenuate gamma photons with an energy above the discriminator level of the single-channel analyzer by more than 15%. Interposition of a 0.5-mm lead sheet between the source and the detector reduced the count rate to 3.1 ± 0.4% (± SEM) (11 determinations). Accordingly, the contribution of gamma radiation elicited from positron annihilation to total count rate observed was less than 5%. The probe was connected to an Ortec amplifier (model 485) and an Ortec single-channel analyzer (model 488). Count rates were monitored with a rate meter and collected with a MITs microcomputer. The computer provided correction of raw data for physical decay of the isotope as well as printouts of regional time-activity curves.

Analysis of Regional Time-Activity Curves

Calculations of rate constants (k), half-times and correlation coefficients (r) for characterization of selected monoexponential portions of $H_2^{18}O$- and $^{13}$C-palmitate time-activity curves were performed with a microcomputer.

The $^{13}$C-palmitate clearance rate was determined for the early monoexponential component occurring from 3–7 minutes after injection of tracer. This early monoexponential phase was selected based on findings that suggest a close relation of this phase to ox-
Myocardial blood flow was determined from the initial mono-
exponential slope of $H_2^{18}$O washout curves 0.3–1.5
minutes after tracer injection. Flow was calculated by
dividing the rate constant of monoexponential clear-
ance by 1.05 (average specific weight of myo-
dium), assuming a blood/tissue partition coeffi-
cient for water of 1.0.16

Preparation of $H_2^{18}$O and $^{11}$C-palmitate

The $H_2^{18}$O and $^{11}$CO$_2$ were produced in the Wash-
ington University Medical Center Cyclotron (Allis
Chalmers).11,17 The $^{11}$C-palmitate was prepared from
$^{11}$CO$_2$ as previously described.11 Radiochemical purity
of $^{11}$C-palmitate was verified by high-performance li-
quid chromatography with a Waters M-Bondpak fatty
acid column.

Protocol

After cannulation, flow into the LAD was adjusted
so that perfusion pressure matched aortic diastolic
pressure. After this initial adjustment, coronary perfu-
sion was maintained constant throughout each experi-
ment except under conditions in which effects of
ischemia were studied. After a stabilization interval of
15 minutes, 0.5–1.5 mCi of $H_2^{18}$O in 0.1–0.3 ml of
saline were injected as a bolus just proximal to the
coronary cannula. Regional activity was monitored for
5 minutes after administration of tracer. When
counts had returned to background (consistently by 10
minutes after the $H_2^{18}$O injection), 2–5 mCi of $^{11}$C-
palmitate bound to 4% human albumin in 2–5 ml of
saline were infused within 15 seconds through the in-
jection port. The $^{11}$C-palmitate time-activity curves
were recorded for 20 minutes after each injection of
tracer. Arterial and coronary venous blood samples
for fatty acid, lactate and blood gas determinations
were withdrawn 10 minutes after the injection of $^{11}$C-
palmitate.

Eighty minutes after the first injection of $^{11}$C-palmit-
ate, an interval sufficient for counts from the first $^{11}$C-
palmitate injection to return to background, a second
study with a second injection of $H_2^{18}$O followed 10
minutes later by a second injection of $^{11}$C-palmitate
was performed. For the second tracor study, three
protocols were used:

1. In seven dogs (control group), the second study
was performed with perfusion continued with arterial
blood at the same flow rate as that used during the first
determination.

2. In seven dogs (ischemia group), 5 minutes
before the $H_2^{18}$O injection (15 minutes before the
$^{11}$C-palmitate injection) flow to the cannulated LAD
was reduced to approximately 30% of the initial flow rate.

3. In seven dogs (hypoxia group), 5 minutes before
the $H_2^{18}$O injection, perfusion was initiated with
venous rather than arterial blood while the flow rate
was maintained constant. The blood gas values of the
inferior vena caval blood used were $P_a = 29.6 \pm 2.6$
mm Hg (± SEM), $P_{CO_2} = 39.4 \pm 2.4$ mm Hg, and $pH$
= 7.24 ± 0.04, compared with $P_a = 110.9 \pm 10.8$
mm Hg, $P_{CO_2} = 30.1 \pm 2.4$ mm Hg, and $pH = 7.36 \pm$
0.02 in arterial blood.

Statistics

Values are mean ± SEM. The $t$ test for paired
samples was used to compare intragroup first and sec-
ond study values.

Results

Hemodynamic and Metabolic Data

The seven dogs in the control group showed no
difference in heart rate or in aortic or perfusion
pressure in the second compared with the first study
(table 1). No changes were observed in the epicardial
ECG. The coronary arteriovenous (A-V) difference in
oxygen and plasma free fatty acid content did not
change. Lactate was extracted by the myocardial
region of interest (table 2).

In the seven dogs in the ischemia group, flow in the
LAD perfused with arterial blood was reduced by an
average of 76% (from 18.1 ± 2.4 ml/min to 4.3 ± 0.64
ml/min) 15 minutes before the second injection of $^{11}$C-
palmitate (5 minutes before injection of $H_2^{18}$O). As a

<table>
<thead>
<tr>
<th>Table 1. Hemodynamic Data</th>
<th>Heart rate (beats/min)</th>
<th>Aortic pressure (mm Hg)</th>
<th>Perfusion pressure (mm Hg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>164.4 ± 10.9</td>
<td>126.9 ± 6.4</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>Control 1</td>
<td>159.1 ± 8.8</td>
<td>133.7 ± 7.4</td>
</tr>
<tr>
<td>$p$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ischemia</td>
<td>Control</td>
<td>162.7 ± 9.0</td>
<td>128.6 ± 7.6</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>Ischemia</td>
<td>157.3 ± 10.0</td>
<td>124.3 ± 10.1</td>
</tr>
<tr>
<td>$p$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Control</td>
<td>159.4 ± 5.9</td>
<td>127.4 ± 11.6</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>Hypoxia</td>
<td>148.9 ± 10.5</td>
<td>120.7 ± 12.1</td>
</tr>
<tr>
<td>$p$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Control 1 and 2 refers to values after the first and second injections of $^{11}$C-palmitate during normoxic perfusion at constant flow.
Table 2. Metabolic Data

<table>
<thead>
<tr>
<th></th>
<th>O2 content (ml/100 ml)</th>
<th>FFA (mM/l)</th>
<th>Lactate (mM/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Control 1</td>
<td>-12.4 ± 0.7 (3)†</td>
<td>-0.17 ± 0.03 (3)</td>
<td>-0.76 ± 0.44 (3)</td>
</tr>
<tr>
<td>Control Control 2</td>
<td>-10.9 ± 1.0 (3)</td>
<td>-0.19 ± 0.03 (3)</td>
<td>-0.61 ± 0.34 (3)</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ischemia Control</td>
<td>-13.9 ± 0.9 (6)</td>
<td>-0.19 ± 0.06 (5)</td>
<td>-0.59 ± 0.11 (6)</td>
</tr>
<tr>
<td>Ischemia</td>
<td>-18.1 ± 1.5 (6)</td>
<td>-0.23 ± 0.06 (5)</td>
<td>3.60 ± 1.53 (6)</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.005</td>
<td>NS</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Hypoxia Control</td>
<td>-10.2 ± 1.4 (6)</td>
<td>-0.10 ± 0.05 (4)</td>
<td>-0.45 ± 0.36 (6)</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>-3.5 ± 0.6 (6)</td>
<td>-0.13 ± 0.07 (4)</td>
<td>1.31 ± 0.54 (6)</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.02</td>
<td>NS</td>
<td>&lt; 0.02</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
†Difference between blood in the perfusion system and blood from the regional coronary vein.
Abbreviations: FFA = free fatty acid concentration; lactate = plasma lactate concentration.

Consequence of reduced flow, the average perfusion pressure was decreased by 65%, from 91.1 ± 6.4 mm Hg to 31.9 ± 4.5 mm Hg (p < 0.001). Heart rate and aortic pressure did not change (table 1). ST-segment elevation greater than 5 mV developed during ischemia in each experiment. The average A-V difference in oxygen content increased by 30% (p < 0.005), while the A-V difference in plasma fatty acid content did not change. Ischemic regions produced lactate (table 2).

In the seven dogs in the hypoxia group, average oxygen content decreased by 66%, from 16.7 ± 1.8 ml/100 ml to 5.7 ± 1.2 ml/100 ml. Although flow in the LAD was kept constant, the average perfusion pressure decreased by 44% (p < 0.02), indicating decreased coronary resistance. Heart rate and aortic pressure did not change (table 1). Changes in the epicardial ECG during hypoxia were less pronounced than those during ischemia. Nevertheless, ST-segment elevation of more than 1.5 mV occurred in every dog. The average A-V difference in oxygen content decreased by 66% (p < 0.02), while the A-V difference in fatty acid content did not change (table 2). As during ischemia, lactate extraction was converted to production (table 2).

Regional Myocardial Blood Flow (table 3)

Regional myocardial blood flow was determined from washout of myocardial activity after a bolus injection of H215O. The fit of the analyzed curve segments to a monoexponential was excellent, as indicated by the high correlation coefficients during control, hypoxic and ischemic conditions (> 0.99, > 0.99, and > 0.97, respectively). In the control group, myocardial blood flow did not change in the second compared with the first tracer study 90 minutes earlier. In the ischemia group, myocardial blood flow decreased by an average of 64%, from 126.2 ± 26.5 ml/min/100 g to 45.6 ± 9.9 ml/min/100 g (p < 0.025). In the hypoxia group, it increased slightly (NS), although flow in the LAD was kept constant, presumably due to reactive hyperemia mediated through collaterals.

Regional Clearance of 11C-Palmitate

Figure 2 shows regional time-activity curves from a control dog after two injections of 11C-palmitate 90

Table 3. Effect of Ischemia and Hypoxia on Myocardial H215O Clearance

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>k (min⁻¹)</th>
<th>MBF (ml/min · 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Control 1</td>
<td>-0.955 ± 0.004</td>
<td>-1.249 ± 0.205</td>
<td>118.9 ± 19.5</td>
</tr>
<tr>
<td>(n = 5) Control 2</td>
<td>-0.994 ± 0.004</td>
<td>-1.280 ± 0.216</td>
<td>121.8 ± 20.6</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ischemia Control</td>
<td>-0.96 ± 0.002</td>
<td>-1.325 ± 0.279</td>
<td>126.2 ± 26.5</td>
</tr>
<tr>
<td>(n = 7) Ischemia</td>
<td>-0.997 ± 0.010</td>
<td>-0.482 ± 0.103</td>
<td>45.6 ± 9.9</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>&lt; 0.025</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>Hypoxia Control</td>
<td>-0.994 ± 0.001</td>
<td>-1.220 ± 0.106</td>
<td>116.1 ± 10.1</td>
</tr>
<tr>
<td>(n = 6) Hypoxia</td>
<td>-0.995 ± 0.002</td>
<td>-1.590 ± 0.196</td>
<td>143.7 ± 18.7</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
Abbreviations: r = correlation coefficient for monoexponential fit of H215O-clearance curve; k = rate constant of monoexponential tracer clearance; MBF = myocardial blood flow calculated from H215O clearance.
FIGURE 2. Semilogarithmic plots of regional time-activity curves from a control dog after intracoronary injection of \(^{11}C\)-palmitate. Perfusion of the left anterior descending coronary artery (LAD) was maintained constant throughout the experiment. The two determinations were performed 90 minutes apart. Four phases of clearance of myocardial activity were consistently identified: (1) initial rapid clearance immediately after peak count rate, (2) a short leveling off between 1 and 3 minutes, (3) an early monoexponential phase between 3 and 7 minutes, and (4) a slow monoexponential phase later than 15 minutes. The two determinations exhibited excellent reproducibility, with rate constants for early monoexponential clearance \((k)\) agreeing closely. \(MBF = \) myocardial blood flow; \(SO_2 = \) perfusate saturation of oxygen in the blood entering the LAD; \(T_1/2 = \) half-time of early monoexponential clearance.

minutes apart. Four phases of myocardial \(^{11}C\) clearance can be identified. The first phase, immediately after peak count rate, represents a rapid decline in myocardial activity compatible with washout of unextracted tracer from the vascular and interstitial space. Between 1 and 3 minutes after injection, a short second phase occurs in which \(^{11}C\)-clearance transiently levels off. The third phase, 3–7 minutes after injection of \(^{11}C\)-palmitate, exhibits monoexponential decline of activity \((r = 0.99 \pm 0.003; \text{table 4})\). During the fourth phase, clearance decreases and conforms to a second monoexponential, evident 15 minutes and later after administration of tracer. Its half-time is longer than 40 minutes.

In the control group, the rate constants of the early monoexponential phase of myocardial \(^{11}C\)-clearance determined after each of the two \(^{11}C\)-palmitate injections were virtually identical \((k = -0.105 \pm 0.016 \text{ min}^{-1} \text{ and } -0.105 \pm 0.017 \text{ min}^{-1}; \text{NS; table 4})\).

Figure 3 shows regional time-activity curves from a dog subjected to ischemia. The \(^{11}C\)-palmitate is extracted by the myocardium despite 15 minutes of reduced coronary flow, as indicated by the activity remaining in the myocardium after the phase of initial vascular clearance.\(^6\) However, compared with the monoexponential clearance during perfusion at normal flow, the \(^{11}C\)-clearance rate is greatly decreased after reduction of coronary flow. Thus, the average rate of monoexponential \(^{11}C\)-clearance in the ischemia group was reduced by 61%, from a control value of \(-0.106 \pm 0.014 \text{ min}^{-1} \text{ to } -0.041 \pm 0.013 \text{ min}^{-1} (p < 0.005; \text{table 4}).

Figure 4 shows time-activity curves from a dog sub-

![Image](http://circ.ahajournals.org/)

TABLE 4. Effect of Ischemia and Hypoxia on \(^{11}C\) Clearance from Myocardium after Intracoronary Injection of \(^{11}C\)-Palmitate

<table>
<thead>
<tr>
<th>Condition</th>
<th>(r)</th>
<th>(k) ((\text{min}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(-0.990 \pm 0.003)</td>
<td>(-0.105 \pm 0.016)</td>
</tr>
<tr>
<td></td>
<td>((n = 7))</td>
<td>(p)</td>
</tr>
<tr>
<td>Ischemia</td>
<td>(-0.995 \pm 0.001)</td>
<td>(-0.106 \pm 0.014)</td>
</tr>
<tr>
<td></td>
<td>((n = 7))</td>
<td>(p)</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(-0.992 \pm 0.002)</td>
<td>(-0.098 \pm 0.017)</td>
</tr>
<tr>
<td></td>
<td>((n = 7))</td>
<td>(p)</td>
</tr>
</tbody>
</table>

Values are mean \(\pm\) SEM.

Abbreviations: \(r = \) correlation coefficient for monoexponential fits; \(k = \) rate constant of monoexponential \(^{11}C\) clearance.
FIGURE 4. Myocardial $^{11}$C-palmitate time-activity curves from a dog with hypoxia before (top panel) and after (bottom panel) the onset of perfusion with hypoxic blood. During hypoxia, the saturation of oxygen in the perfusate ($SO_2$) was reduced to 30%. Myocardial blood flow (MBF) in the left anterior descending coronary artery perfused region increased slightly. Similar to results with ischemia (fig. 3), there was a marked decrease in the rate of early monoeponential $^{11}$C-clearance during hypoxic perfusion.

Myocardial damage and, consequently, altered myocardial kinetics of $^{11}$C-palmitate. In the control group, no changes in the ECG, hemodynamics, or lactate metabolism were observed. In addition, myocardial blood flow determined from the rate of washout of $H_2^{18}$O did not change, and repeat determinations (90 minutes apart) of regional clearance of $^{11}$C-palmitate did not differ. Thus, under control conditions, the preparation was stable.

Venous blood differs from arterial blood not only in its reduced oxygen content, but also with respect to $PCO_2$ and pH. Thus, it might be argued that those factors might alter myocardial fatty acid metabolism independent of oxygen content. However, isolated rat hearts do not exhibit changes in fatty acid metabolism when pH in the perfusate is as low as 7.1 with or without a concomitant increase in $CO_2$ content.

We used a newly developed $\beta$-detector probe to monitor regional myocardial time-activity curves. In contrast to conventional gamma-probe systems, this approach eliminates distortion of regional time-activity curves by activity in ventricular blood, underlying or adjacent myocardium or that in other organs. The probe detects activity based on positrons themselves rather than that due to gamma radiation resulting from positron annihilation. The range of positrons in tissue is 4.1 mm for $^{11}$C and 7.1 mm for $^{18}$O. The actual thickness of myocardium contributing substantially to detected count rates is reduced even further because the most likely positron range is approximately one-third of the maximal range and positrons with low-energy are not detected due to absorption in the detector window or rejection by the discriminator. Accordingly, recorded count rates in the present study represent activity within a small cylindrical subepicardial region with a diameter of the detector window and thickness of approximately 3 mm.

Determination of myocardial blood flow in hearts of experimental animals based on washout of inert diffusible tracers has recently been replaced widely by use of the microsphere technique. However, for the purpose of the present study, determination of flow by analysis of washout of $H_2^{18}$O, a positron-emitting tracer, had several advantages: (1) determination of blood flow in virtually the identical myocardial region studied for $^{11}$C-palmitate clearance; (2) minimization of intrinsic limitations of washout techniques, such as the influence of recirculating tracer in ventricular blood and the influence of flow heterogeneity by use of a $\beta$-detector probe with high spatial resolution; (3) recognition of flow-independent alterations in passive washout potentially relevant to analysis of $^{11}$C-clearance; and (4) facilitation of repeated determinations without alteration in radiation background because of the desirable short half-life of $^{18}$O. Theoretically, the volume of myocardium monitored for $^{11}$C-activity is not necessarily entirely identical to the volume monitored for $^{18}$O activity because of the difference in positron range. However, since the major fraction of detectable activity is located within a 2–3-mm cylindrical field from the epicardial surface.

Discussion

Myocardial clearance of $^{11}$C-palmitate was consistently depressed after diminished delivery of oxygen, regardless of whether myocardial blood flow was reduced concomitantly.

Methodologic Considerations

The experimental preparation used in the present study was designed to permit reduction of oxygen supply to selected regions of myocardium, either by controlled reduction of flow (ischemia) or by perfusion with venous blood (hypoxia) without concomitant reduction of flow. It was initially important to characterize the hemodynamic and metabolic stability of the preparation throughout the experimental interval because factors such as duration of anesthesia and prolonged extracorporeal perfusion may have led to
for both isotopes, the possible differences in monitored volumes are negligible.

**11C-palmitate Clearance in Ischemic or Hypoxic Myocardium**

The time-activity curves under control conditions were almost identical to time-activity curves from isolated perfused rabbit hearts.\(^{11}\) The only apparent difference is a short phase in vivo after initial rapid washout in which \(^{11}\)C-clearance transiently levels off. Since this phase is more prominent and prolonged after i.v. \(^{11}\)C-palmitate (unpublished observation), it probably reflects concurrent uptake of recirculating \(^{11}\)C-palmitate, which is then rapidly cleared from blood.\(^{9}\) Studies in isolated perfused hearts elucidating the intracellular fate of \(^{14}\)C-labeled fatty acids extracted by myocardium from blood suggest that the monoeponential portion of the time-activity curve after injection of \(^{11}\)C-palmitate reflects primarily oxidative turnover of tracer incorporated into neutral lipid pools such as intracellular free fatty acids, mono-, di- and a part of triglycerides.\(^{24, 25}\) The subsequent slow component reflects turnover of tracer in phospholipids and other fractions of triglycerides exhibiting slow turnover as demonstrated by Stein and Stein.\(^{25}\)

In isolated rabbit hearts perfused at constant flow, the rate of monoeponential clearance of activity from \(^{11}\)C-palmitate extracted by the myocardium is closely correlated with determinants of myocardial oxygen consumption and \(^{14}\)CO\(_2\) production from concomitantly administered \(^{11}\)C-palmitate.\(^{11}\) These findings suggest that changes in oxidative metabolism of \(^{11}\)C-labeled fatty acids incorporated into myocardial neutral lipids can be detected externally based on analysis of regional time-activity curves after injection of \(^{11}\)C-palmitate. In the present study, monoeponential \(^{11}\)C-clearance was therefore characterized as a potential index of reduced aerobic metabolism induced by either ischemia or hypoxia and manifested independent of flow itself.

Early monoeponential \(^{11}\)C-clearance was reduced by 52% during hypoxic perfusion, a reduction of similar magnitude to the decrease induced by ischemia. Although flow in the LAD was maintained constant during hypoxic perfusion, myocardial blood flow in the subepicardial layers could have changed by altered collateral flow or redistribution of flow between subendocardial and subepicardial myocardial layers.\(^{11}\) L'Abbate et al.\(^{25}\) suggested that washout of diffusible labeled molecules can decrease without reduction of flow during vasodilatation, which was undoubtedly present in our study during hypoxic perfusion, as indicated by the significant decrease in perfusion pressure. However, the rate of clearance of \(H_2^{18}\)O increased slightly (although not significantly) during hypoxic perfusion. Therefore, reduced passive washout of labeled oxidation products or of unmetabolized palmitate could not be responsible for the decreased rate of \(^{11}\)C-clearance during hypoxic perfusion. However, oxidative metabolism was markedly reduced in the hypoxic perfused region, as indicated by the marked reduction in the A-V difference in oxygen saturation, the appearance of lactate production\(^{26}\) and the ST-segment elevation.\(^{27}\) Accordingly, decreased \(^{11}\)C-clearance rate is most compatible with decreased rate of oxidative fatty acid metabolism.

Since the average reduction of myocardial blood flow of 64% during ischemia was similar in magnitude to the reduction of blood oxygen content (66%) during hypoxic perfusion, restriction in oxygen supply was similar during both conditions. Thus, it is not surprising that the average reduction of \(^{11}\)C-clearance rate was also similar. Decreased washout of labeled oxidation products may have contributed to decreased \(^{11}\)C-clearance rate during ischemia, but since myocardium rendered hypoxic but not ischemic manifested comparable reduction of monoeponential \(^{11}\)C clearance, metabolism itself appears to be primarily responsible.

The results of this study are consistent with the decreased rate of clearance of \(^{11}\)C-activity during stress in myocardial regions supplied by a stenosed coronary artery in intact dogs.\(^{9}\)

Fatty acid metabolism in ischemic and hypoxic myocardium has been studied extensively. Uptake of fatty acids from blood into the myocardium is unchanged or slightly or severely depressed, depending on the severity and duration of oxygen restriction.\(^{26} \text{ }^{27}\) In contrast to the variable changes in fatty acid uptake, oxidation of fatty acids is consistently reduced in ischemic or hypoxic myocardium, with an increased fractional extraction of fatty acid incorporated into triglycerides.\(^{26} \text{ }^{27}\) Decreased uptake of \(^{11}\)C-palmitate in myocardium rendered ischemic for prolonged periods has been documented noninvasively in isolated perfused hearts,\(^{4}\) in dogs with coronary occlusion\(^{6} \text{ }^{7}\) and in patients with myocardial infarction.\(^{8}\) In contrast, no decrease in \(^{11}\)C-palmitate uptake was detectable in regions supplied by a critically stenosed coronary artery exhibiting ischemia without gross infarction.\(^{9}\) Consistent with the latter findings, results in the present study indicate that myocardium rendered ischemic or hypoxic for short intervals extracts fatty acids reflected by a persistent A-V fatty acid difference and a similar fraction of injected counts present at the beginning of the monoeponential clearance phase (figs. 2 and 3). Small changes in \(^{11}\)C-palmitate extraction cannot be excluded, however, because the tracer was injected over several seconds.\(^{6}\)

In summary, the present study demonstrates that reduced regional clearance of activity in vivo is not simply a flow-dependent phenomenon. \(^{11}\)C-clearance is consistently depressed in regions with restricted oxygen supply regardless of whether or not flow is concomitantly reduced. Since the magnitude of reduction of aerobic metabolism is not directly or exclusively related to the intensity of ischemia,\(^{4}\) analysis of regional time-activity curves after \(^{11}\)C-palmitate administration provides information qualitatively different from that obtainable from analysis of flow itself.

Our results support the concept that sequential positron-emission tomography with \(^{11}\)C-palmitate in
patients with ischemic and other myopathic forms of heart disease may permit identification and quantitative characterization of regions of viable but metabolically compromised tissue based on analysis of regional time-activity curves.

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