Detection of Myocardial Ischemia before Infarction, Based on Accumulation of Labeled Pyruvate

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To determine whether ischemic, but not irreversibly injured myocardium, can be differentiated from normal tissue based on accumulation of labeled pyruvate, isolated hearts were perfused with buffer containing [^{14}C]pyruvate under conditions of normal or low flow. Fifteen minutes after the hearts were exposed to labeled material, myocardial radioactivity was fourfold greater in ischemic compared to control hearts, due to accumulation of label in sequestered lactate produced from the pyruvate. Open-chest rabbits subjected to coronary occlusion exhibited a 1.73:1 ratio of radioactivity in ischemic compared with normal myocardium 15 min after systemic injection of [^{14}C]pyruvate. The results obtained suggest that zones of myocardial ischemia should be detectable in vivo by positron tomography after systemic administration of [^{11}C]pyruvate as well.

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Decreased uptake of radioagents, such as potassium analogs, reflects myocardial ischemia but also occurs in regions of infarction (*1*). Identification of regions of ischemia as opposed to regions of irreversible injury—particularly with tracers that accumulate selectively in such zones potentially permitting imaging of these particular areas would be helpful not only for improved detection of myocardial ischemia but also to facilitate objective assessment of interventions designed to modify favorably the evolution of myocardial ischemic injury.

Under aerobic, physiological conditions, oxidative metabolism of fatty acids provides most of the energy required by the heart. Pyruvate is generally present in concentrations of only 0.1 m*M* and does not generally serve as a major substrate. Nevertheless, when it is present in higher concentrations, extraction of pyruvate by the heart is marked, and pyruvate oxidation competes with oxidation of fatty acid (*2,3*). In contrast, when oxygen supply is limited, glycolysis becomes the major energy source, with consequent accumulation of lactate from metabolism of pyruvate (*4*). Accordingly, we reasoned that extraction of labeled pyruvate from blood, with myocardial clearance of the labeled material and its metabolic products, should be rapid in well-oxygenated myocardium but that if ischemia supervenes radioactivity should accumulate as a result of entrapment in the lactate produced. We have previoulsy characterized accumulation of selected C-14-labeled physiological substrates (such as fatty acids) in isolated hearts as an initial approach to selecting corresponding C-11-labeled tracers for positron emission tomography in vivo, and found the C-14 results to presage those obtained subsequently with C-11-labeled agents utilized in more complex systems (*5–7*). In the present study, we evaluated the extraction, accumulation, and metabolism of labeled pyruvate in isolated hearts and in vivo, to determine whether C-11-labeled pyruvate is likely to permit delineation of zones of myocardial ischemia in vivo, detectable by positron tomography.

METHODS

Isolated hearts. Twenty-nine isolated hearts from male rabbits weighing 2–2.5 kg were perfused retrograde

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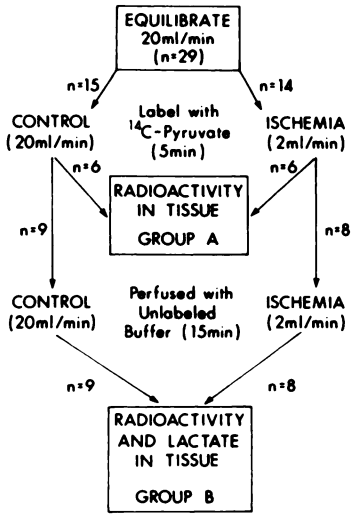

FIG. 1. Flow diagram of experimental protocol used for isolated hearts.

(modified Langendorff preparation) at a constant flow of 20 ml per min through an aortic cannula. Hearts were equilibrated for 15 min with Krebs-Henseleit solution containing 5 m*M* D-glucose, 10 m*M* sodium pyruvate, and insulin (70 μU/ml), and oxygenated with O_2/CO_2 (95:5) at pH 7.4 and 37°. Heart rate was maintained constant at 180 beats per min with a right-ventricular pacing electrode. A saline-filled, compliant latex balloon was inserted into the left ventricle through the left atrium for continuous monitoring of isovolumetric pressure. Left-ventricular diastolic pressure was maintained at 10 mm Hg. After 15 min of equilibration, hearts were randomized to control flow (20 ml/min) or low flow (2 ml/min, ischemic hearts) and perfused with Krebs-Henseleit buffer containing 1-[^{14}C]pyruvate (48,100 dpm/ml, Fig. 1). Some hearts (Group A) were labeled for 5 min, removed quickly from the perfusion apparatus, and blotted dry. Septal samples were frozen rapidly and subsequently used for determination of radioactivity. Others (Group B) were labeled in an identical manner but subsequently perfused at the same flow for 15 min with medium devoid of radiopyruvate. Myocardial samples (250 mg) were solubilized in 1.5 ml of Protosol† and heated to 55° for 18 hr. One hundred microliters of 30% hydrogen peroxide were added and the sample was heated again for 30 min at 55°. After cooling, 150 μl of acetic acid were added followed by 10 ml of scintillation fluor. The sample was counted 60 min later in a liquid scintillation counter (*8*). Myocardial lactate was mea-

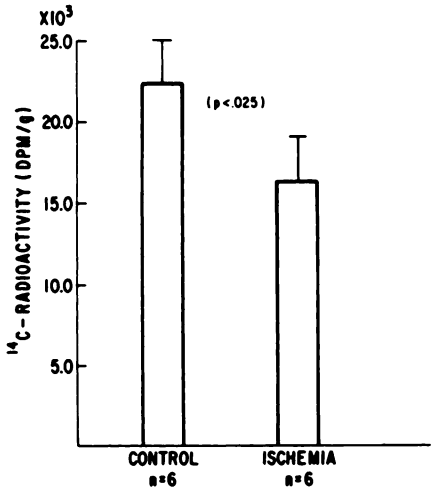

FIG. 2. C-14 pyruvate activity in isolated hearts immediately after labeling under conditions of control or low flow (ischemia). Values are means ± s.e.m.

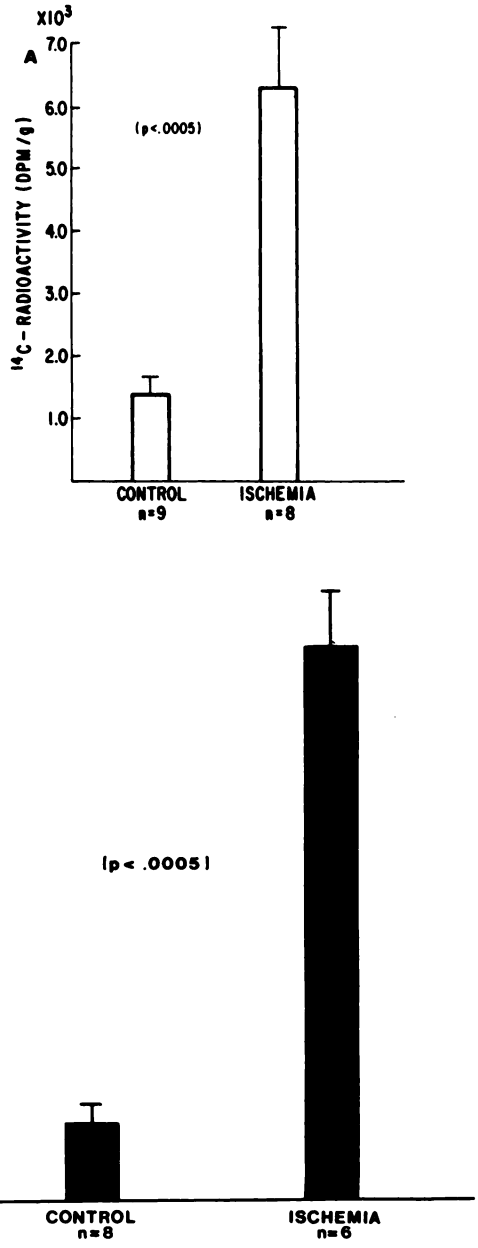

FIG. 3. (A) C-14 pyruvate activity in isolated hearts 15 min after labeling had been completed. (B) Concentrations of lactate in control and ischemic hearts. Values are means ± s.e.m.

sured conventionally by a spectrophotometric, enzymatic method using lactate dehydrogenase (*9*).

Experiments in vivo. To determine whether results in isolated perfused hearts were paralleled by those in vivo, additional studies were performed with male rabbits anesthetized with sodium pentobarbital (30 mg/kg), ventilated with a respirator on room air, and subjected to a median sternotomy. A saline-filled catheter was inserted into the left atrium, and the left anterior descending coronary artery was ligated. Five microcuries of 1-[^{14}C]pyruvate were injected through the left-atrial catheter 30 sec after coronary occlusion. Fifteen minutes later (an interval known to precede the onset of irreversible injury) myocardial samples were taken from the ischemic anterior left-ventricular wall, and from the normal posterior left-ventricular wall, for measurement of tissue radioactivity as described above.

To determine whether radioactivity within the blood pool might interfere with the detection of ischemic or normal zones of myocardium during imaging in vivo, C-14 pyruvate was injected and blood samples collected initially at 30-sec intervals for 5 min, then at 1-min intervals for 25 min. The blood time-activity curve was thereby characterized in each animal.

Statistical methods. Comparisons among isolated perfused hearts were made using Student's t-test for unpaired data. For studies in vivo, each heart served as its own control and data were analyzed with Student's t-test for paired samples. Values expressed are means ± standard error.

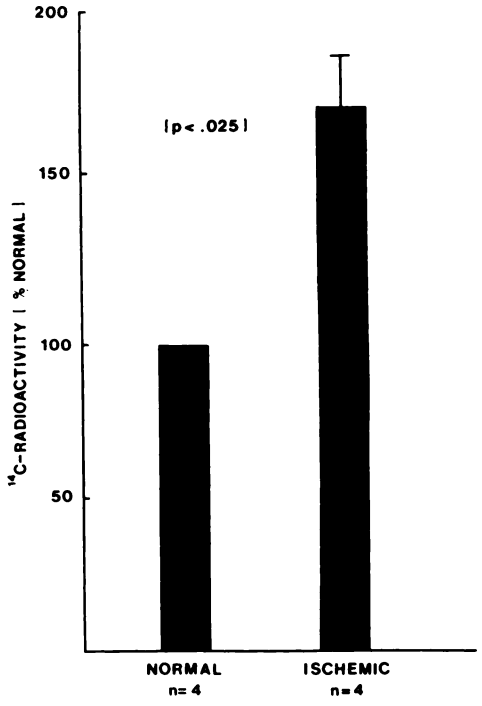

FIG. 4. C-14 pyruvate activity in ischemic and nonischemic zones of rabbit hearts after coronary occlusion. Values in nonischemic zones are normalized, with control values at 100%.

RESULTS

Isolated perfused hearts. Figure 2 depicts radioactivity in control and ischemic hearts in which activity was measured immediately after labeling (Group A). Activity averaged 22,100 ± 1000 dpm/g wet weight in control, and 15,800 ± 2300 in ischemic hearts ($p < 0.025$). The higher activity in control hearts probably reflected the increased delivery of tracer due to higher flow.

Figure 3 illustrates activity in hearts labeled for 5 min at control and at low flow and subsequently perfused with medium devoid of radiopyruvate for 15 min (Group B). In contrast to results in Group A, radioactivity averaged 6,240 ± 1050 dpm/g wet weight in ischemic hearts and only 1,440 ± 223 in controls ($p < 0.0005$). Thus, radioactivity in ischemic hearts was more than four times that in controls. Lactate in these ischemic hearts was considerably greater than in controls, averaging 10.7 ± 1.9 μ mols/g wet weight compared with 1.5 ± 0.02 ($p < 0.005$) (Fig. 3B).

Studies in vivo. Radioactivity in anterior-wall myocardium subjected to ischemia in vivo averaged 10,300 ± 1540 dpm/g wet weight, compared with 6000 ± 440 in nonischemic myocardium from the same hearts ($p < 0.025$, Fig. 4). Thus, in vivo as well as in vitro ischemia led to retention of C-14 pyruvate despite diminished delivery of the tracer due to low flow. Radioactivity in the blood pool was cleared within 1 min in each animal after left-atrial injection of labeled pyruvate (Fig. 5). Thus, blood-pool radioactivity would be unlikely to interfere with external detection of labeled pyruvate in normal and ischemic regions of the heart in vivo after intravenous administration of a tracer such as C-14 pyruvate.

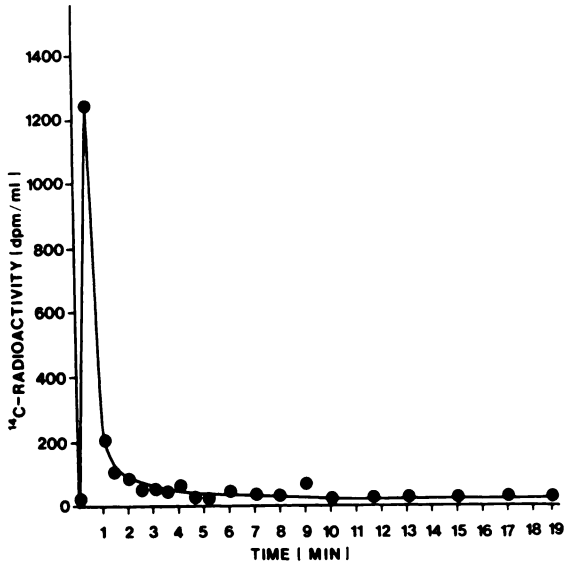

FIG. 5. Typical time-activity curve of C-14 activity after left-atrial injection of C-14 pyruvate.

DISCUSSION

The foregoing results demonstrate that 15 min after reduction of flow, myocardial retention of radioactivity from labeled pyruvate is substantially greater than that in tissue perfused normally. The accumulation of radioactivity occurs despite markedly diminished delivery of tracer. The low level of retained radioactivity under control conditions probably results from unimpaired, aerobic utilization of pyruvate with rapid liberation of carbon dioxide (*10*). Despite the low flow in ischemic tissue, net extraction of labeled pyruvate is likely to be facilitated by the prolonged residence time of tracer. Since metabolism of the labeled pyruvate is aborted at the lactate step, accumulation of radioactivity in lactate from label initially present in pyruvate results (*10*). As shown in Fig. 3, increases in lactate parallel retention of radioactivity.

In open-chest rabbits subjected to coronary ligation, retention of radioactivity from pyruvate in ischemic zones was similar to retention observed in ischemic isolated hearts. The ratio of radioactivity in ischemic compared with control regions was not as marked as the corresponding ratio in vitro, probably in part because of the unavoidable inclusion of some well-perfused tissue in the samples of ostensibly ischemic myocardium. In previous studies with C-14-labeled palmitate, a 1.2:1 ratio of radioactivity in zones of normal myocardium, compared with zones of infarction, was sufficient to presage quantitative delineation of infarcts by positron emission tomography after intravenous administration of the tracer in vivo (*7*). In the present study, the observed ratio of 1.73:1 in ischemic compared with normal myocardium suggests that analogous tomographic studies with C-11 pyruvate should permit delineation and focal increased activity imaging of ischemic myocardium as well, but not after irreversible injury.

FOOTNOTE

† New England Nuclear, North Billerica, MA.

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