Heterogeneous fatty acid uptake early after reperfusion in rat hearts

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Abstract

Heterogeneous fatty acid uptake early after reperfusion in rat hearts. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H923–H929, 1998.—We determined whether spatial distributions of substrate uptake are heterogeneous within the area at risk during reperfusion. Quantitative autoradiography with imaging plates and two long-lived radioisotopes was applied to 15 open-chest, anesthetized rats subjected to 30 min of coronary artery ligation and 30 min of reperfusion. Regions showing increased β-methyl-[1-14C]heptadecanoic acid ([14C]BMHDA) uptake (166 ± 17% of that in the nonischemic area) appeared at the lateral borders and subepicardial layer within the area at risk, and 2-deoxy-o-[1-3H]glucose ([3H]DG; Amersham, Buckinghamshire, UK), with a specific activity of 640 GBq/mmol (4, 5, 29, 30). Regional myocardial glucose uptake was determined by regional coronary venous blood sampling (14, 17, 18) and positron emission tomography (10) may have failed to depict the heterogeneous distribution of substrate metabolism during reperfusion after acute regional ischemia.

METHODS

Radiopharmaceuticals. Regional myocardial free fatty acid uptake was assessed with a branched-chain fatty acid analog, β-methyl-[1-14C]heptadecanoic acid ([14C]BMHDA; NEN, Boston, MA), with a specific activity of 2.13 GBq/mmol (4, 5, 29, 30). Regional myocardial glucose uptake was determined with 2-deoxy-o-[1-3H]glucose ([3H]DG; Amersham, Buckinghamshire, UK), with a specific activity of 640 GBq/mmol (27, 29, 30). Regional myocardial blood flow was assessed with 4-[15N-methyl-1-14C]iodoantipyrine ([14C]IAP; NEN), with a specific activity of 2.2 GBq/mmol (23, 29).

Protocols. The purpose and nature of this study were approved by the Committee of Animal Experiments in the Cyclotron and Radioisotope Center of Tohoku University (Sendai, Japan). Fifteen male Wistar rats, weighing 283 ± 38 g (mean ± SD), were fed normal rat chow and tap water ad libitum until they were subjected to the surgical procedure, which was begun at about 2:00 PM. The rats were anesthe-
tized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg). Catheters (PE-50) were placed in the right common carotid artery for monitoring arterial blood pressure with an oscillograph (model MPU 0.5, Nihon Koden, Tokyo, Japan) and arterial blood sampling and in the right jugular vein for the administration of radiopharmaceuticals. After a left thoracotomy was performed under artificial ventilation, a snare (6-0 nylon) was placed around the left coronary artery. To confirm that the snare was positioned around the left coronary artery, we pulled both suture ends for a few seconds to determine whether myocardial cyanosis and wall motion abnormalities appeared. Arterial blood was sampled for the determination of plasma concentrations of glucose (9, 25), insulin (28), and free fatty acids (24). After 1 mg/kg lidocaine was injected to prevent ventricular arrhythmias, the left coronary artery was ligated. Thirty minutes after the initiation of the left coronary artery ligation, the snare loop was released.

In group A (n = 8), 5 min after reperfusion, 3.7 MBq of [3H]IDG were injected intravenously for 30 s, and 30 s later, 0.185 MBq of [14C]BMHDA dissolved in 0.2 ml of an aqueous solution of bovine serum albumin (11) was injected intravenously for 30 s. Thirty minutes after the [3H]IDG was injected, the left coronary artery was ligated again, and the rats were then injected intravenously with 2% methylene blue solution (0.5 ml) to demarcate the area at risk by negative staining (29, 30). Immediately afterward, the rats were killed by administration of saturated KCl solution (0.3 ml). The hearts were removed rapidly and frozen in dry ice. In group B (n = 7), 5 min after the reperfusion following 30 min of ligation of the left coronary artery, 3.7 MBq of [3H]IDG was injected intravenously for 30 s. Thirty minutes after the [3H]IDG injection, 0.185 MBq of [14C]AP dissolved in 1.8 ml of 0.9% NaCl solution was injected intravenously at a constant rate for 30 s with an infusion pump (STC-521, Terumo, Tokyo, Japan). To determine blood [14C]AP activities, we collected serial arterial blood samples at 0, 1, 3, 5, 8, 13, 18, 23, and 28 s after the initiation of the [14C]AP infusion. Activity-time curves of the blood [14C]AP concentrations were obtained using a liquid scintillation counter (2050CA, Packard, Downers Grove, IL). Thirty seconds after the initiation of the [14C]AP injection, the rats were killed by cutting the ascending aorta and the pulmonary trunk. The hearts were removed rapidly and frozen in dry ice.

Quantitative autoradiography. Quantitative double-tracer autoradiography with [3H] and [14C] was performed as described previously (29, 30). In brief, 20-μm-thick frozen heart sections taken perpendicular to the long axis of the left ventricle were prepared. The sections along with the [3H]- and [14C]-labeled graded standards (Amersham) were placed in contact with [3H]-sensitive imaging plates (Fuji Photo Film, Tokyo, Japan) for 2 wk and with general use imaging plates (Fuji Photo Film) for another 2 wk. The autoradiograms were analyzed using a computer-assisted imaging-processing system (BAS3000, Fuji Photo Film). The image data were recorded as digitized values of each pixel (50 × 50 μm) in the analyzing unit of this system. Discrimination between electrons emitted from [3H] and [14C] was possible because of their different energy distributions (30).

On the color monitor display of the image processor, circular regions of interest (the area of each region of interest was 0.0925 mm²) were placed throughout the left ventricular wall of one midventricular-level section in each rat. In each image, the numbers of the regions of interest in the nonischemic area and the area at risk were 133 ± 36 and 338 ± 80 (means ± SD, n = 8) in group A and 134 ± 23 and 343 ± 55 (means ± SD, n = 7) in group B, respectively. We traced the regions of interest of the general use-imaging plate image on a transparent film attached to the display. Using the traced film, we put the regions of interest on the [3H]-sensitive imaging plate at the same site as that of the general use-imaging plate image. The autoradiographic intensities of [3H]BMHDA and [14C]AP were determined by averaging the values of all pixels in the region of interest of the general use-imaging plate image. The autoradiographic intensities of [3H]DG were determined by subtracting the value of the region of interest of the general use-imaging plate image from that of the [3H]-sensitive imaging plate image, which was corrected using the [14C] calibration lines (30). For further analysis, we converted the autoradiographic intensities into tissue [3H] and [14C] contents using the calibration lines obtained from the [3H] and [14C]-labeled graded standards.

In group A, we determined both regional myocardial [3H]BMHDA uptake and [3H]DG uptake in each region of interest on the left ventricular wall. In group B, we determined both regional myocardial [3H]DG uptake and blood flow in each region of interest. Myocardial blood flow was obtained with myocardial [14C]AP activities and the time-activity curve of the blood [14C]AP concentrations (23). We obtained the percentages of [14C]BMHDA uptake, [3H]DG uptake, and blood flow by normalizing regional values to the mean value of all regions of interest in the nonischemic area in each rat. As a measure of heterogeneity, the percentage interregional coefficients of variation (%ICV), calculated as [(SD/mean) × 100] (26), in [3H]BMHDA uptake, [3H]DG uptake, and blood flow were determined in the nonischemic area and the area at risk in each rat. In group A, we determined the nonischemic area and the area at risk subjected to ischemia and reperfusion by positive and negative staining with methylene blue solution, respectively (29, 30).

In group B, the nonischemic area was determined as the area showing homogeneous distributions of both [3H]DG and [14C]AP in the interventricular septum and the left ventricular posterior wall. We confirmed that the ratio of the area at risk to the total area of the left ventricular wall in group B was comparable to that in group A (72 ± 5 vs. 71 ± 9%, means ± SD; n = 7 and 8, respectively).

Data analysis. The data are presented as means ± SD. The statistical significance of mean values between groups A and B was assessed with the two-tailed unpaired Student’s t-test. The statistical significance in mean values among regions and among the percentages of [14C]BMHDA uptake, [3H]DG uptake, and blood flow was assessed with the two-tailed paired Student’s t-test. The two-factor analysis of variance with repeated measures on one factor was applied to compare the increase in %ICV in the area at risk relative to the nonischemic area among [14C]BMHDA uptake, [3H]DG uptake, and blood flow. A value of P < 0.05 was considered significant.

RESULTS

No significant differences in the plasma concentrations of glucose (11.4 ± 2.8 vs. 12.9 ± 2.7 mmol/l), insulin (8.0 ± 4.4 vs. 7.8 ± 4.2 ng/ml), or free fatty acids (1.7 ± 0.8 vs. 2.1 ± 0.9 meq/l) were found between groups A and B. No significant differences in mean arterial pressure were found between groups A and B during the left coronary artery ligation (71 ± 18 vs. 79 ± 13 mmHg) or during the reperfusion (94 ± 29 vs. 100 ± 25 mmHg).

Fatty acid uptake, glucose uptake, and blood flow. Figures 1 and 2 demonstrate representative autoradiograms of the heart sections from rats in groups A and B,
Mean $^{14}$C-BMHDA uptake, mean $^{3}$H-DG uptake, and mean blood flow in the area at risk were lower than in the nonischemic area (0.93 ± 0.55 vs. 1.44 ± 0.36 Bq/mg, 82 ± 56 vs. 110 ± 64 Bq/mg, and 61 ± 27 vs. 91 ± 34 ml·100 g$^{-1}$·min$^{-1}$, respectively) (Fig. 3, A, C, and E). The %ICV in $^{14}$C-BMHDA uptake, $^{3}$H-DG uptake, and blood flow were higher in the area at risk than in the nonischemic area (76 ± 23 vs. 21 ± 7%, 39 ± 10 vs. 21 ± 7%, and 49 ± 19 vs. 14 ± 4%, respectively) (Fig. 3, B, D, and F). The increase in %ICV in the area at risk relative to the nonischemic area was more prominent in $^{14}$C-BMHDA uptake than in $^{3}$H-DG uptake ($P < 0.0005; n = 8$ and 15, respectively) and was more prominent in blood flow than in $^{3}$H-DG uptake ($P < 0.01; n = 7$ and 15, respectively). The increase in %ICV in the area at risk relative to the nonischemic area tended to be more prominent in $^{14}$C-BMHDA uptake than in blood flow ($P = 0.07; n = 8$ and 7, respectively).

In our preliminary experiments, $^{3}$H-DG and $^{14}$C-BMHDA were injected in sham-operated rats ($n = 2$) using the same procedure as that for rats in group A. In other sham-operated rats ($n = 2$), $^{3}$H-DG and $^{14}$C-IAP were injected using the same procedure as that for rats in group B. In the left ventricular wall in sham-operated rats, %ICV values in $^{14}$C-BMHDA uptake (19 ± 0%), $^{3}$H-DG uptake (25 ± 10%), and blood flow (18 ± 2%) were comparable to those in the nonischemic area in rats of groups A and B (Mann-Whitney U test). No significant differences in %ICV in $^{14}$C-BMHDA uptake, $^{3}$H-DG uptake, or blood flow were found among the interventricular septum, anterior wall, lateral wall, and posterior wall in sham-operated rats (Kruskal-Wallis test).

To depict the heterogeneous distributions of $^{14}$C-BMHDA uptake in the area at risk, we divided the area at risk into three regions using the mean and SD values of all regions of interest in the nonischemic area in each rat: high-BMHDA areas have percent values of $^{14}$C-BMHDA uptake of more than the mean + 2SD; mid-BMHDA areas have values from the mean − 2SD.
to the mean + 2SD; and low-BMHDA areas have values less than the mean – 2SD. The high-BMHDA area appeared at the lateral borders and subepicardial layer within the area at risk in seven of the eight rats (Fig. 4). The low-BMHDA area occupied the midmyocardial layer except at the lateral borders within the area at risk. In some rats, the subendocardial and subepicardial layers within the area at risk were also occupied by the low-BMHDA area. The high-, mid-, and low-BMHDA areas occupied 9 ± 8, 34 ± 18, and 58 ± 24% of the area at risk (n = 8 for each), respectively. The existence of high-BMHDA area was statistically significant (P < 0.005). As shown in Fig. 5, the percentage of [3H]DG uptake in the high-BMHDA area was comparable to that in the nonischemic area. However, in the low-BMHDA area, the percentage of [3H]DG uptake was lower than that in the nonischemic and high- and mid-BMHDA areas.

To depict the heterogeneous distributions of myocardial blood flow in the area at risk, we divided the area at risk into three regions using the mean and SD values of all regions of interest in the nonischemic area in each rat: high-blood-flow areas have percent values of blood flow of more than the mean + 2SD; mid-blood-flow areas have values from the mean – 2SD to the mean + 2SD; and low-blood-flow areas have values less than the mean – 2SD. The low-blood-flow area occupied the subendocardial and midmyocardial layers except at the lateral borders within the area at risk (Fig. 6). In some rats, the subepicardial layer within the area at risk was also occupied by the low-blood-flow area. The high-, mid-, and low-blood-flow areas occupied 7 ± 6, 40 ± 23, and 53 ± 29% of the area at risk (n = 7 for each), respectively. The existence of high-blood-flow area was statistically significant (P < 0.05) vs. Non-isch. However, the existence of mid- and low-blood-flow areas was not statistically significant (P > 0.05) vs. Non-isch.
statistically significant (P < 0.01). As shown in Fig. 7, the percentage of [3H]DG uptake in the high-blood-flow area was comparable to that in the nonischemic area. However, in the low-blood-flow area, the percentage of [3H]DG uptake was lower than that in the nonischemic and high- and mid-blood-flow areas.

DISCUSSION

In this study, we clearly demonstrated the heterogeneous distributions of myocardial fatty acid uptake, glucose uptake, and blood flow within the area at risk during reperfusion. Regions showing increased fatty acid uptake (166% of that in the nonischemic area) appeared at the lateral borders and subepicardial layer within the area at risk, and glucose uptake was 103% in these regions. These regions occupied 9% of the area at risk. Regions with decreased fatty acid uptake (28% of that in the nonischemic area) occupied the midmyocardial layer except at the lateral borders within the area at risk, and glucose uptake was 62% in these regions. Regions showing decreased blood flow (44%) appeared at the subendocardial and midmyocardial layers except at the lateral borders within the area at risk. The increase in heterogeneity in the area at risk relative to the nonischemic area was more prominent in fatty acid uptake and in blood flow than in glucose uptake.

Hale et al. (8) showed that, in rat hearts subjected to 20–60 min of coronary artery occlusion followed by 24 h of reperfusion, myocardial necrosis delineated by tetrazolium staining appeared first in the midmyocardium and developed toward the subepicardium, subendocardium, and the lateral borders within the area at risk as the duration of coronary artery occlusion increased. In rats with 30 min of coronary artery occlusion and 24 h of reperfusion, the area of necrosis as a percentage of the area at risk was 36% (8). We did not assess the infarct zone by using tetrazolium staining or perform histological studies in the present study, because 30 min of coronary artery ligation and 30 min of reperfusion may not be sufficient for accurate detection of necrosis. However, viable myocardium with various degrees of injury and necrotic tissue is assumed to have existed in the area at risk in this study. Heterogeneous myocardial injury may have lead to heterogeneous blood flow and substrate metabolism.

Miller et al. (16) measured the myocardial accumulation of another branched-chain fatty acid analog, [125I]-labeled 15-(p-iodophenyl)-β-methylpentadecanoic acid ([125I]BMIPP), in a canine model subjected to 15–60 min of coronary artery occlusion and 3 h of reperfusion. They showed that [125I]BMIPP uptake did not differ from control myocardium (108% of control) in segments within the area at risk that were stained with tetrazolium. In segments within the area at risk that were not stained with tetrazolium, [125I]BMIPP uptake was decreased to 79% of that in control segments. The present study demonstrated that regions showing high (166% of that in the nonischemic area), moderate (92%), and low (28%) levels of [14C]BMHDA uptake existed within the area at risk. Possibly because the spatial resolution in the present study (the area of each region of interest was 0.0925 mm²) is higher than that in the report by Miller et al. (16) (1 g for each myocardial segment), we could distinguish between regions with increased and decreased fatty acid uptake.

Saddik et al. (22) reported that fatty acid oxidation was increased in isolated rat hearts subjected to 30 min of global ischemia and reperfusion. They also showed (22) that triglyceride synthesis was significantly increased and that its lipolysis did not differ compared with control hearts, suggesting that triglyceride pools in myocardial cells are expanded during reperfusion. In isolated rat hearts subjected to 60 min of global ischemia and reperfusion, Görge et al. (7) showed that fatty acid oxidation was decreased. Fatty acid metabolism during reperfusion may depend on the degree of myocar-
dial injury. Fatty acid oxidation and/or its incorporation into lipid pools may have been increased in high-BMHDA areas during reperfusion in the present study. However, in low-BMHDA areas, fatty acid oxidation and/or its incorporation into lipid pools may have been decreased during reperfusion.

In this study, the area of low BMHDA, expressed as a percentage of the area at risk, varied from 23 to 83% (Fig. 4), probably because of individual differences in tolerance to myocardial ischemia. In rats with smaller low-BMHDA areas, low BMHDA was localized in the midmyocardial layer except at the lateral borders within the area at risk. In rats with larger low-BMHDA areas, low BMHDA occupied not only the midmyocardial layer but also the subendocardial layer, subepicardial layer, and lateral borders within the area at risk. It is assumed that a low-BMHDA area appears first in the midmyocardial layer and then expands toward the subendocardial layer, subepicardial layer, and lateral borders within the risk area as the duration of coronary artery occlusion increases. This is consistent with the process by which myocardial necrosis expands, as reported by Hale et al. (8). Decreased [14C]BMHDA uptake may reflect the severity of myocardial injury during reperfusion after transient regional ischemia.

Recently, another branched-chain fatty acid analog, 123I-labeled BMIPP, was introduced to assess myocardial fatty acid metabolism by single-photon emission computed tomography (12, 13). Franken et al. (6) performed scintigraphy with 123I BMIPP and echocardiographic studies in 18 patients 4–10 days after myocardial infarction. Fourteen of the eighteen patients received thrombolytic therapy and/or coronary angioplasty within 6 h after the onset of chest pain. Functional recovery assessed by echocardiography 6 mo after infarction was found in 75, 74, and 27% of the segments with 123I BMIPP uptake of 50–65%, 35–50%, and <35% of the maximal activity, respectively. These data also suggest that myocardial uptake of a branched-chain fatty acid analog is useful to estimate myocardial viability during reperfusion after acute myocardial infarction.

Although we did not perform double labeling with a fatty acid tracer and a blood flow tracer in this study, high-, mid-, and low-BMHDA areas might overlap high-, mid-, and low-blood-flow areas, respectively. The reasons are as follows. First, the areas of high, mid, and low BMHDA expressed as percentages of the area at risk were comparable to those of high, mid, and low blood flow (9 ± 8 vs. 7 ± 6%, 34 ± 18 vs. 40 ± 23%, and 58 ± 24 vs. 53 ± 29%, respectively). Second, the percentages of [3H]DG uptake in high-, mid-, and low-BMHDA areas were comparable to those in high-, mid-, and low-blood-flow areas (103 ± 24 vs. 98 ± 24%, 87 ± 8 vs. 92 ± 14%, and 62 ± 18 vs. 63 ± 17%, respectively). Further studies are needed to clarify the relationship between fatty acid uptake and myocardial blood flow during reperfusion.

Possible limitations. BMHDA is a branched-chain fatty acid analog that is transported into myocardial cells by the same long-chain fatty acid carrier protein mechanism that transports natural fatty acids, but it cannot be β-oxidized (4, 5). BMHDA is stored in lipid pools mainly as triglycerides, or it is cleared slowly as a result of limited α- and ω-oxidation and back diffusion of nonmetabolized BMHDA (1, 5). Abendschein et al. (1) obtained myocardial washout curves of β-methyl-[1-14C]-heptadecanoic acid ([14C]BMHDA) in dogs subjected to 50 min of left circumflex coronary artery ligation. They reported that the half-time value of the [14C]BMHDA washout curve was prolonged in the ischemic myocardium compared with that in the control myocardium (78 vs. 41 min). Nishimura et al. (19) reported that, in dogs undergoing 3 h of left anterior coronary artery ligation followed by 1 h of reperfusion, another branched-chain fatty acid analog, 123I BMIPP, was cleared more slowly in the reperfused myocardium than in the control myocardium. In the present study, the relative [14C]BMHDA uptake in the area at risk compared with that in the nonischemic area might be overestimated, because clearance of [14C]BMHDA activity might be prolonged in the area at risk compared with that in the nonischemic area.

The present study showed that spatial distributions of fatty acid uptake, glucose uptake, and myocardial blood flow are heterogeneous early after reperfusion following 30 min of left coronary artery ligation in rat hearts. The heterogeneous distributions of substrate uptake during reperfusion after regional ischemia may explain the controversial results of the previous studies using coronary venous blood sampling (14, 17, 18) and positron emission tomography (10).

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