Effects of Long-term Pressure Overload on Regional Myocardial Glucose and Free Fatty Acid Uptake in Rats
A Quantitative Autoradiographic Study
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To investigate the effects of long-term pressure overload on regional myocardial substrate use, we performed quantitative autoradiography using 2-deoxy-D-[U-14C]glucose (14C-DG) and β-methyl[1-14C]heptadecanoic acid (14C-BMHD) in conscious rats with a 10-week ascending aortic constriction. Heart weight/body weight ratio increased by 27% in aortic-constricted rats as compared with sham-operated rats (p<0.01). Myocardial 14C-DG uptake increased (258±63 vs. 144±41 nCi/g, p<0.01, n=6 for each group); however, 14C-BMHD extraction decreased (251±69 vs. 342±75 nCi/g, p<0.05, n=7 for each group) in aortic-constricted rats as compared with sham-operated rats. In sham-operated rats, both 14C-DG and 14C-BMHD uptakes were higher in the left ventricular anterior and lateral walls as compared with the posterior wall or the interventricular septum. In aortic-constricted rats, 14C-DG uptake also increased in the interventricular septum, as well as in the left ventricular anterior and lateral walls, as compared with the posterior wall. There was, however, no regional difference in 14C-BMHD extraction among these four regions. Myocardial blood flow distribution determined by 4-[N-methyl-14C]iodoantipyrine or myocyte width showed no regional variations among the four regions, either in aortic-constricted or sham-operated rats. Regional interstitial fibrosis was small in either group. The present study suggests that myocardial substrate uptake is altered nonhomogeneously, and that the nonhomogeneity is not because of regional variations in blood flow distribution, myocyte hypertrophy, or interstitial fibrosis. The results of angiotensin II–induced acute pressure overloading in other sham-operated rats, in which a remarkable increase in myocardial 14C-BMHD extraction (n=3, p<0.01) and no difference in 14C-DG uptake (n=3) as compared with normotensive sham-operated rats were elicited, suggest that the findings in aortic-constricted rats are not direct responses to increased left ventricular pressure itself but rather should be explained by still unknown factors related to prolonged pressure overload. (Circulation 1990;81:1353–1361)

Long-term systemic hypertension produces prolonged left ventricular pressure overload, and cardiac hypertrophy occurs as an adaptive response.1 Several early studies suggested that myocardial substrate metabolism is altered in the habitually pressure-overloaded heart.2–5 Yonekura et al6 reported that increased glucose analogue uptake and decreased fatty acid analogue extraction were preferentially found in the subendocardial layer of the left ventricular free wall in salt-sensitive hypertensive Dahl rats. It is unclear, however, whether the nonhomogeneity of alteration in regional myocardial substrate use is similarly observed in any type of the pressure-overloaded myocardium. Further, there are no data concerning whether the nonhomogeneity is observed only between subendocardial and subepicardial layers. Moreover, it is not known whether this alteration is observed in acutely pressure-overloaded heart.
The purpose of this study was to investigate the effects of long-term left ventricular pressure overload on regional myocardial glucose and fatty acid use in conscious rats. We performed quantitative autoradiography of the heart in rats with 10-week ascending aortic constriction by using glucose and fatty acid analogues. Furthermore, blood flow distribution with quantitative autoradiography after a diffusible tracer injection and regional histological findings were examined. Finally, to determine whether the findings in aortic-constricted rats are because of metabolic abnormalities caused by prolonged pressure overload or because of direct responses to increased left ventricular pressure itself, we also performed quantitative autoradiography in acutely pressure-overloaded sham-operated rats by using angiotensin II, as was done in the long-term pressure-overloading study.

Methods

Animals

Six-week-old male Wistar rats were obtained from Funabashi Farms (Shizuoka, Japan), and maintained in our laboratory until they were subjected to the surgical procedure. The rats were fed normal rat chow and tap water ad libitum.

Surgical Procedure for Ascending Aortic Constriction

Ascending aortic constriction was induced by a modification of the techniques described by Isoyama et al. Each rat was anesthetized with sodium pentobarbital (50 mg/kg i.p.) at 8 weeks of age. After left thoracotomy under artificial ventilation, the ascending aorta was dissected from the pulmonary artery. A surgical thread (3-0 silk) was tied around both a needle (1.4 mm o.d.) and the ascending aorta. The needle was removed rapidly, and the thorax was closed. The ascending aorta was isolated but was not constricted in sham-operated rats.

Radiopharmaceuticals

Regional myocardial glucose uptake was assessed with 2-deoxy-D-[U-14C]glucose (14C-DG) (specific activity, 282 mCi/mmol) (New England Nuclear, Boston, Massachusetts). Regional myocardial free fatty acid uptake was determined with β-methyl[1-14C]heptadecanoic acid (14C-BMHDA) (specific activity, 57.66 mCi/mmol) (New England Nuclear). Relative regional myocardial blood flow distribution was assessed with 4-[N-methyl-14C]idoantipyrine (14C-IAP) (specific activity, 50 mCi/mmol) (New England Nuclear).

Protocols

The rats were anesthetized with ether 10 weeks after the aortic constriction or the sham operation. An arterial catheter (PE 50) was placed in the right femoral artery for measurement of arterial blood pressure (model MPU 0.5, Nihon Kohden, Tokyo, Japan) and for blood sampling. A venous catheter was inserted into the right femoral vein for the administration of radiopharmaceuticals. The rats were allowed 2 hours for recovery from the anesthesia, and then studied in the unrestrained condition in an open-top plastic cage. All rats were fasted for 3–3.5 hours before they were injected with radiopharmaceuticals. Thirty-seven rats (17 sham-operated rats and 20 aortic-constricted rats) were divided into three radiopharmaceutical groups as herein described.

In the first group (six sham-operated rats and six aortic-constricted rats), arterial blood was sampled for determination of the plasma concentrations of glucose, insulin, and free fatty acids. Ten μCi of 14C-DG diluted to 0.2 ml with 0.9% NaCl solution was injected intravenously for 30 seconds. Forty-five minutes after the injection, arterial blood was sampled to determine the plasma concentration of 14C-DG, and the rats were killed with 0.4 ml of saturated KCl solution. The hearts were removed rapidly, stripped of large vessels and atria, weighed, and frozen in dry ice. The hearts were then processed for quantitative autoradiography as herein described.

In the second group (seven sham-operated rats and seven aortic-constricted rats), 10 μCi of 14C-BMHDA dissolved in 0.2 ml of an aqueous solution of bovine serum albumin was injected intravenously for 30 seconds after arterial blood sampling to assess the plasma concentrations of glucose, insulin, and free fatty acids. Fifteen minutes after the injection, arterial blood sampling was performed to determine the plasma concentration of 14C-BMHDA, and the rats were then killed for quantitative autoradiography. The plasma concentrations of 14C-DG and 14C-BMHDA were determined with a liquid scintillation counter. In the 14C-DG and 14C-BMHDA protocols, those times for killing were selected, considering the time course of radioactivity in blood (clearance curve) and tissue (accumulation curve) obtained from our preliminary study and from earlier reports.

In the third group (four sham-operated rats and seven aortic-constricted rats), for assessment of relative regional myocardial blood flow distribution, 10 μCi of 14C-IAP dissolved in 0.6 ml of 0.9% NaCl solution was infused intravenously at a constant rate for 30 seconds with an infusion pump (STC-521, Terumo, Tokyo, Japan). Thirty seconds after the initiation of 14C-IAP infusion, the rats were killed with 0.4 ml of saturated KCl solution through another venous catheter inserted into the left femoral vein, and thereafter, quantitative autoradiography was performed.

Quantitative Autoradiography

For autoradiography, 20-μm-thick frozen sections taken perpendicular to the long axis of the left ventricle were prepared from the midventricular level by using a cryomicrotome (975C-Histostat Microtome, American Optical, Buffalo, New York). The slice was dried on a hot plate at 60°C for 10 minutes and placed in contact with x-ray film (NMC-
Effects of Pressure Overload on Substrate Uptake

Kagaya et al

1, Eastman Kodak Co., Rochester, New York) along with 14C-graded standards of known radioactivities (C936, Amersham, England) for 5 weeks. The autoradiograph was analyzed using a computer-assisted image-processing system. This system consists of a densitometer (Chromoscan 3, Joyce Loebi, Gateshead, England), an image processor (Nexus 6400, Kashiwagi Research Corp., Tokyo, Japan), a minicomputer (model SS-3, Nippon UNIVAC, Tokyo, Japan), and a digitizer (model 68230S, Kashiwagi Research Corp., Tokyo, Japan). Positional resolution and scanning apertures of the densitometer are 50 μm. The optical density is digitized into 256 levels. The digitized values of the autoradiograph are converted to 14C content, pixel by pixel, by using the standard curve obtained from the graded 14C standards.

On the color monitor display of the image processor, we divided the autoradiographic image of the left ventricle into eight regions as follows: left-hand and right-hand halves of the interventricular septum, and inner and outer halves of the left ventricular anterior wall, lateral wall, and posterior wall. The regional concentration (nCi/g) of 14C-DG, 14C-BMHDHA, or 14C-IAP was determined by averaging the values of all pixels in the region of interest. Moreover, for each rat, regional 14C-IAP concentrations were normalized to the total concentration in the left ventricular wall to assess relative regional blood flow distribution.

Left Ventricular Pressure Measurement and Histological Study

To confirm left ventricular pressure overloading, and to assess regional differences in myocardial hypertrophy and interstitial fibrosis, we performed hemodynamic and histological studies in another 10 rats (four sham-operated rats and six aortic-constricted rats). Ten weeks after the ascending aortic constriction or sham operation, the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Polyethylene cannulas (PE 50) were inserted into the right carotid artery for the aortic pressure measurement. After a left thoracotomy under artificial ventilation, a 19-gauge needle connected to a pressure transducer (model TP 300-T, Nihon Kohden, Tokyo, Japan) was inserted from the apex into the left ventricular cavity. The left ventricular pressure and the aortic pressure were then recorded simultaneously. After the rat was killed with KCl solution, the heart was processed for histological study.

The heart was fixed in 10% formalin, dehydrated, and embedded in paraffin. Sections (6 μm) taken perpendicular to the long axis of the left ventricle were prepared from the midventricular level, and stained with hematoxylin-cosin (for measurement of the myocyte width) or with Masson-trichrome (for evaluation of regional interstitial fibrosis). A mean myocyte width was determined by measurement of transmural widths of random longitudinally oriented myocytes in the circular midwall muscle bundles14 with a calibrated microscope eyepiece reticle (12 cells for each region of the heart, i.e., interventricular septum, left ventricular anterior wall, lateral wall, and posterior wall) on random fields at a magnification of ×400. Regional interstitial fibrosis was semiquantitatively evaluated for each region of the heart as follows: score 0, no fibrotic lesion; score 1, minimal or focal fibrotic lesions; score 2, focal fibrotic lesions and subendocardial scars; score 3, multifocal fibrotic lesions or large subendocardial scars; score 4, generalized scarring. These histological analyses were performed by a single observer without knowledge of the other data.

Acute Pressure Overloading Study With Angiotensin II

Another six sham-operated rats were anesthetized with ether 10 weeks after the surgical procedure, and polyethylene cannulas were inserted into the right carotid artery and the right jugular vein. The rats were allowed 2 hours for recovery from the anesthesia. Arterial pressure was monitored using a pressure transducer (model TP 300-T, Nihon Kohden, Tokyo, Japan), and angiotensin II (5 μg/ml) was infused intravenously with an infusion pump (STC-521, Terumo, Tokyo, Japan) to maintain a systolic arterial pressure greater than 180 mm Hg. After the arterial blood pressure had reached a steady state (approximately 15–20 minutes after the initiation of angiotensin II infusion), arterial blood was sampled to determine the plasma concentrations of glucose, insulin, and free fatty acids, and the rats were then injected intravenously with 10 μCi of 14C-DG (n=3) or 14C-BMHDHA (n=3). The rats were subjected to the procedures for quantitative autoradiography.

Data Analysis

The plasma glucose concentration was determined by a modification15 of the glucose oxidase method,16 the plasma insulin concentration was measured by radioimmunoassay,17 and the plasma concentration of free fatty acids was determined by an enzymatic method.18 The data are presented as mean±SD. The statistical significance of differences in mean values between sham-operated and aortic-constricted rats was assessed with the unpaired Student’s t test. The two-factor analysis of variance with repeated measures on one factor19 was applied to compare regional profiles between sham-operated and aortic-constricted rats. Differences among mean values of the four different regions of left ventricular wall in sham-operated or aortic-constricted rats were assessed with the Newman-Keuls method. The Mann-Whitney test for nonparametric testing and the Quade test20 were used to assess differences in mean values of fibrotic scores.

Results

Changes in Heart Weight, Hemodynamics, and Plasma Concentrations of Glucose, Insulin, and Free Fatty Acids

Both the heart wet weight and the heart wet weight/body weight ratio increased in aortic-constricted rats...
as compared with sham-operated rats in each radiopharmaceutical group. The heart wet weight and the heart wet weight/body weight ratio in aortic-constricted rats did not differ significantly among the three radiopharmaceutical groups (Table 1). The heart rate and the mean femoral arterial pressure in the \(^{14}\text{C-DG}\) and \(^{14}\text{C-IAP}\) groups did not differ between sham-operated and aortic-constricted rats. The mean femoral arterial pressure in the \(^{14}\text{C-BMHDA}\) group, however, decreased in aortic-constricted rats as compared with sham-operated rats. In both sham-operated and aortic-constricted rats, no significant difference in these values was found among the three radiopharmaceutical groups (Table 1).

Hemodynamic data for anesthetized open-chest rats are summarized in Table 2. Peak systolic left ventricular pressure increased in aortic-constricted rats as compared with sham-operated rats. The peak-to-peak pressure difference between the left ventricle and aorta was 104±37 mm Hg in aortic-constricted rats. Left ventricular end-diastolic pressure increased in aortic-constricted rats as compared with sham-operated rats.

The plasma concentrations of glucose, insulin, and free fatty acids in the \(^{14}\text{C-DG}\) and \(^{14}\text{C-BMHDA}\) groups are summarized in Table 1. No significant difference was found between sham-operated and aortic-constricted rats in either radiopharmaceutical group. The plasma concentration of insulin in the \(^{14}\text{C-BMHDA}\) group was higher than that of the \(^{14}\text{C-DG}\) group, in both sham-operated and aortic-constricted rats. The reason for the higher insulin levels in \(^{14}\text{C-BMHDA}\) group is not clear. The experimental feeding conditions might be different between the two groups.

### Table 1. Heart Weight, Hemodynamic Data, and Plasma Concentrations of Glucose, Insulin, and Free Fatty Acids

<table>
<thead>
<tr>
<th>Data</th>
<th>(^{14}\text{C-DG})</th>
<th>(^{14}\text{C-BMHDA})</th>
<th>(^{14}\text{C-IAP})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>AoC</td>
<td>Sham</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>308±32</td>
<td>304±38</td>
<td>300±8</td>
</tr>
<tr>
<td>Heart wet weight (mg)</td>
<td>696±59</td>
<td>879±44*</td>
<td>668±21</td>
</tr>
<tr>
<td>Heart weight/body weight</td>
<td>2.28±0.27</td>
<td>2.93±0.39*</td>
<td>2.23±0.08</td>
</tr>
<tr>
<td>Mean femoral arterial pressure (mm Hg)</td>
<td>118±5</td>
<td>115±5</td>
<td>118±8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>434±34</td>
<td>408±66</td>
<td>433±29</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>149±15</td>
<td>161±24</td>
<td>149±11</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>19.0±4.4</td>
<td>20.3±1.2</td>
<td>28.7±9.0‡</td>
</tr>
<tr>
<td>Free fatty acids (mEq/l)</td>
<td>1.39±0.20</td>
<td>1.47±0.20</td>
<td>1.45±0.24</td>
</tr>
</tbody>
</table>

Values are mean±SD. Sham, sham-operated rats; AoC, aortic-constricted rats; \(^{14}\text{C-DG}\), \(^{14}\text{C-BMHDA}\), and \(^{14}\text{C-IAP}\) indicate rats injected with \(^{14}\text{C-2-deoxyglucose}, \(^{14}\text{C-β-methylheptadecanoic acid}, and \(^{14}\text{C-iodoantipyrine}, respectively. Sham, sham-operated rats; AoC, aortic-constricted rats. *p<0.01, †p<0.05 vs. sham-operated rats. ‡p<0.05 vs. rats injected with \(^{14}\text{C-2-deoxyglucose}.

### Table 2. Hemodynamic Data of Anesthetized Open-Chest Rats

<table>
<thead>
<tr>
<th>Hemodynamic data</th>
<th>Sham</th>
<th>AoC</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Peak systolic LVP (mm Hg)</td>
<td>122±30</td>
<td>215±42*</td>
</tr>
<tr>
<td>End-diastolic LVP (mm Hg)</td>
<td>3.2±2.2</td>
<td>6.0±1.5†</td>
</tr>
<tr>
<td>Peak systolic AoP (mm Hg)</td>
<td>120±28</td>
<td>110±22</td>
</tr>
<tr>
<td>Mean AoP (mm Hg)</td>
<td>89±18</td>
<td>81±20</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>446±30</td>
<td>443±32</td>
</tr>
</tbody>
</table>

Values are mean±SD. Sham, sham-operated rats; AoC, aortic-constricted rats; LVP, left ventricular pressure; AoP, aortic pressure. *p<0.01, †p<0.05 vs. sham-operated rats.
show a difference in regional profile between sham-operated and aortic-constricted rats.

The ratios of the concentrations of radiopharmaceuticals in the inner half and the outer half of the left ventricular wall (inner/outer ratio) are shown in Figure 3. The $^{14}$C-DG inner/outer ratio tended to decrease, and that of $^{14}$C-BMHDA significantly decreased (0.86±0.05 vs. 0.92±0.03, $p<0.05$) in

![Figure 1. Autoradiographs obtained from hearts of sham-operated rats injected with $^{14}$C-2-deoxyglucose (DG), $^{14}$C-β-methylheptadecanoic acid (BMHDA), and $^{14}$C-iodoantipyrine (IAP) (A, B, and C, respectively), and of aortic-constricted rats injected with DG, BMHDA, and IAP (D, E, and F, respectively). In sham-operated rats, both DG and BMHDA uptakes are higher in left ventricular anterior and lateral walls as compared with posterior wall or interventricular septum. In aortic-constricted rats, DG uptake was also higher in interventricular septum, as well as in left ventricular anterior and lateral walls, as compared with posterior wall. Although there was no difference in BMHDA uptake among four regions of left ventricle, uptake decreased in inner half as compared with outer half of left ventricular wall. IAP distribution was homogeneous in both sham-operated and aortic-constricted rats.](image)

**Table 3. Results of Quantitative Autoradiography**

<table>
<thead>
<tr>
<th></th>
<th>$^{14}$C-DG</th>
<th>$^{14}$C-BMHDA</th>
<th>$^{14}$C-IAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>AoC</td>
<td>Sham</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Septum</td>
<td>136±41</td>
<td>260±75*</td>
<td>324±71</td>
</tr>
<tr>
<td>Anterior</td>
<td>154±40</td>
<td>278±62*</td>
<td>365±85</td>
</tr>
<tr>
<td>Lateral</td>
<td>152±42</td>
<td>260±54*</td>
<td>359±85</td>
</tr>
<tr>
<td>Posterior</td>
<td>133±42</td>
<td>228±64†</td>
<td>323±66</td>
</tr>
<tr>
<td>Inner half of septum</td>
<td>143±43</td>
<td>235±75†</td>
<td>303±66</td>
</tr>
<tr>
<td>Inner half of anterior</td>
<td>158±40</td>
<td>242±49†</td>
<td>344±31</td>
</tr>
<tr>
<td>Inner half of lateral</td>
<td>169±49</td>
<td>285±61*</td>
<td>349±80</td>
</tr>
<tr>
<td>Inner half of posterior</td>
<td>147±50</td>
<td>246±67†</td>
<td>309±60</td>
</tr>
<tr>
<td>Outer half of septum</td>
<td>131±40</td>
<td>269±77*</td>
<td>335±76</td>
</tr>
<tr>
<td>Outer half of anterior</td>
<td>152±42</td>
<td>295±72*</td>
<td>378±89</td>
</tr>
<tr>
<td>Outer half of lateral</td>
<td>143±39</td>
<td>248±52*</td>
<td>365±86</td>
</tr>
<tr>
<td>Outer half of posterior</td>
<td>126±37</td>
<td>219±66†</td>
<td>334±68</td>
</tr>
</tbody>
</table>

*Values are mean±SD. $^{14}$C-IAP concentrations are normalized to total concentration in left ventricular wall in each rat. $^{14}$C-DG, $^{14}$C-BMHDA, and $^{14}$C-IAP indicate rats injected with $^{14}$C-2-deoxyglucose, $^{14}$C-β-methylheptadecanoic acid, and $^{14}$C-iodoantipyrine, respectively. Left-hand and right-hand halves of interventricular septum are shown as inner and outer halves of septum, respectively. Sham, sham-operated rats; AoC, aortic-constricted rats; septum, interventricular septum; anterior, lateral, and posterior indicate left ventricular anterior, lateral, and posterior walls, respectively.

* $p<0.01$, † $p<0.05$ vs. sham-operated rats.
aortic-constricted rats compared with sham-operated rats. The \(^{14}\text{C}\)-IAP inner/outer ratio, however, did not differ between the two experimental groups.

The plasma concentration of \(^{14}\text{C}\)-DG determined by arterial blood sampling just before killing was 20±10 nCi/g in sham-operated rats and 19±10 nCi/g in aortic-constricted rats. The plasma concentration of \(^{14}\text{C}\)-BMHDA was 21±5 nCi/g in sham-operated rats and 39±21 nCi/g in aortic-constricted rats.

**Histological Analysis**

As shown in Table 4, myocyte width in aortic-constricted rats increased in all four regions of the left ventricle as compared with sham-operated rats. There is no difference in myocyte width among the four different regions of the left ventricle in either sham-operated or aortic-constricted rats. The fibrosis score in aortic-constricted rats was not significantly different from that in sham-operated rats in each region of the left ventricle. The fibrosis score of the left ventricular lateral wall was higher than that of the interventricular septum or anterior wall in aortic-constricted rats.

**Results of Acute Pressure Overloading Study**

Peak systolic arterial blood pressure in rats continuously infused with angiotensin II was 186±7 mm Hg. Mean arterial blood pressure was 155±15 mm Hg and was increased as compared with normotensive sham-operated rats (\(p<0.01\)). The plasma concentrations of glucose, insulin, and free fatty acids were 166±81 mg/dl, 23.8±7.1 \(\mu\)IU/ml, and

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**TABLE 4. Histological Analysis**

<table>
<thead>
<tr>
<th>Interventricular septum</th>
<th>LV anterior wall</th>
<th>LV lateral wall</th>
<th>LV posterior wall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>AoC</td>
<td>Sham</td>
</tr>
<tr>
<td>Myocyte width ((\mu)m)</td>
<td>10.7±1.2</td>
<td>15.5±2.9*</td>
<td>10.7±1.2</td>
</tr>
<tr>
<td>Fibrosis score</td>
<td>0.3±0.5</td>
<td>0.5±0.5‡</td>
<td>0.3±0.5</td>
</tr>
</tbody>
</table>

Values are mean±SD. LV, left ventricular; Sham, sham-operated; AoC, aortic-constricted rats.

*\(p<0.05\), †\(p<0.01\), statistical significance of differences between mean values in sham-operated rats (\(n=4\)) and aortic-constricted rats (\(n=6\)). §\(p<0.05\), ‡\(p<0.01\), statistical significance of differences between mean values in left ventricular lateral wall and different regions in aortic-constricted rats.
1.44±0.12 mEq/l, respectively, and did not differ from those concentrations of normotensive sham-operated rats.

Left ventricular 14C-DG uptake was 181±51 nCi/g and did not differ from that of normotensive sham-operated rats. Left ventricular 14C-BMHDA extraction was 666±116 nCi/g and was increased (p<0.01) as compared with that of normotensive sham-operated rats (Figure 4). These results are definitely in contrast with those of the habitually pressure-overloaded state. 14C-BMHDA uptake in the four regions of the left ventricle were 681±117 nCi/g in the interventricular septum, 685±122 nCi/g in the anterior wall, 657±109 nCi/g in the lateral wall, and 641±114 nCi/g in the posterior wall. Uptake in the posterior wall was lower as compared with the interventricular septum or anterior wall (p<0.05 for each). In contrast, there was no difference in 14C-DG uptake among the four regions of the left ventricle. The 14C-DG inner/outer ratio was 1.16±0.10, and that of 14C-BMHDA was 0.94±0.02. These uptake ratios did not differ as compared with those of normotensive sham-operated rats.

Discussion

We observed that left ventricular 14C-DG uptake increased and, in contrast, 14C-BMHDA extraction decreased in aortic-constricted rats. Additionally, the pattern of regional differences in the uptake of these substrate analogues was quite different from the pattern in sham-operated rats. There was no regional difference, however, in 14C-IAP distribution in either sham-operated or aortic-constricted rats. Furthermore, in contrast to habitually pressure-overloaded left ventricle produced by ascending aortic constriction, myocardial 14C-BMHDA uptake was remark-

ably increased in the acutely pressure-overloaded left ventricle with angiotensin II.

Methodological Considerations

The heart weight/body weight ratio in aortic-constricted rats increased by 27% as compared with sham-operated rats. This value is comparable with those in earlier studies, in which the aorta was banded to induce long-term pressure overload.5,7 In the present study, there were no rats with ascites, or dilation of the left ventricular cavity. Therefore, our rats are considered to be a model of long-term left ventricular pressure overload and compensated cardiac hypertrophy.

We did not use the ascending aortic constriction to induce acute left ventricular pressure overload. This was because rats might require several days to recover from the surgery, and hypertrophic responses occur during that period.21 Furthermore, the initial peak-to-peak pressure difference between the left ventricle and aorta in aortic-constricted rats was approximately 40 mm Hg in our preliminary study, and more severe aortic constriction resulted in a very high mortality rate. Angiotensin II infusion can increase sympathetic tone.22 It is unlikely, however, that the difference in results between angiotensin II–infused and normotensive sham-operated rats is because of the difference in sympathetic discharge because the plasma concentration of free fatty acids did not differ between the two groups.

We measured the regional concentrations of the radiopharmaceuticals with a computer-assisted image-processing system. This system permits quantitative metabolic mapping with higher spatial resolution than the tissue-sampling method. We do not show the 14C-DG or 14C-BMHDA uptake normalized to the total injection dose/body weight ratio or to the final plasma radioactivity because these normalizations do not elicit any different results.

Glucose and free fatty acids are two of the major substrates for myocardial energy metabolism.23 Myocardial glucose and free fatty acid use is influenced by the experimental feeding conditions.24 The contribution of glucose to myocardial energy metabolism might be negligible after a period of prolonged fasting. We did not fast the rats overnight but only for 3–3.5 hours before injection with radiopharmaceuticals, to assess both myocardial glucose and free fatty acid use.

Glucose and Free Fatty Acid Use in Habitually Pressure-Overloaded Heart

14C-DG shares the transport system across plasma membrane with glucose and is phosphorylated to 14C-DG–6-phosphate, which is almost inert metabolically.8 We could not determine which factor, accelerated glycolysis or increased myocardial glycogen synthesis, is related to the accelerated myocardial 14C-DG uptake. Our data suggest, however, that the myocardium of aortic-constricted rats depends
more on exogenous glucose as an energy source than does the myocardium of sham-operated rats.

\[^{14}\text{C}-\text{BMHDA}\] was synthesized first by Livni et al.\(^{10}\)

This fatty acid analogue was designed to inhibit the \(\beta\)-oxidation process by preventing the formation of the corresponding \(\beta\)-ketoacylSCoA. The main metabolic fates of free fatty acids extracted by the myocardium are \(\beta\)-oxidation and the synthesis of triglycerides or phospholipids. Abendschein et al.\(^{25}\) reported that 85% of extracted \(^{11}\text{C}-\text{BMHDA}\) was accumulated as triglycerides or phospholipids although only 5% of that was oxidized.

Wittels and Spann\(^{2}\) and Vasdev et al.\(^{26}\) reported increased incorporation of free fatty acid into triglycerides or phospholipids in the hypertrophic heart. Reibel et al.\(^{27}\) demonstrated that myocardial total phospholipid concentration did not decrease in aortic-constricted rats. Revis and Cameron\(^{28}\) showed increased myocardial triglyceride content in the hypertrophic heart induced by aortic banding. Although we did not measure cardiac triglycerides or phospholipid content, it is unlikely that the decreased \(^{14}\text{C}-\text{BMHDA}\) uptake in aortic-constricted rats demonstrated in our study represents decreased synthesis of myocardial triglycerides or phospholipids. We believe, rather, that our data suggest that some process between fatty acid uptake and the initial stage of \(\beta\)-oxidation is impaired in aortic-constricted rats. Reibel et al.\(^{27}\) reported decreased myocardial coenzyme A and carnitine contents in pressure-overloaded heart. These findings might partially explain the decreased fatty acid analogue extraction in aortic-constricted rats in our experiment. Additionally, accelerated glucose use in aortic-constricted rats might be explained as a compensatory response for impaired fatty acid use.

There are also other possible mechanisms for the decreased myocardial \(^{14}\text{C}-\text{BMHDA}\) uptake in aortic-constricted rats. First, interstitial lipid deposition as well as circulating triglycerides might be increased in aortic-constricted rats. Although the plasma concentration of free fatty acids did not differ from that of sham-operated rats, these two factors could possibly reduce the ratio between \(^{14}\text{C}-\text{BMHDA}\) and nonlabeled free fatty acids in the interstitial space, and dilute the tracer function of \(^{14}\text{C}-\text{BMHDA}\). Second, Fox et al.\(^{29}\) reported 6% of \(^{14}\text{C}-\text{palmitate}\) extracted by canine myocardium back-diffused within 10 minutes. It is possible that the myocardial free fatty acid content in aortic-constricted rats was increased, and that back-diffusion of extracted \(^{14}\text{C}-\text{BMHDA}\) was accelerated. Further study is needed to determine the precise mechanism of decreased \(^{14}\text{C}-\text{BMHDA}\) uptake in aortic-constricted rats.

Interestingly, the alteration in myocardial substrate use is dependent on the duration of pressure overload, that is, \(^{14}\text{C}-\text{DG}\) uptake did not increase and \(^{14}\text{C}-\text{BMHDA}\) extraction was remarkably increased in the acute phase of pressure overload. In other words, the findings in the aortic-constricted rats previously described are not direct responses to increased left ventricular pressure itself, and are suggested to be brought about by unknown factors related to long-term pressure overload.

Meerson et al.\(^{13}\) reported increased incorporation of \(^{14}\text{C}-\text{glucose}\) into myocardial glycogen in the long-term pressure-overloaded heart induced by abdominal aortic constriction. Yonekura et al.\(^{16}\) demonstrated increased myocardial \(^{14}\text{C}-\text{DG}\) uptake and decreased \(^{14}\text{C}-\text{BMHDA}\) extraction in salt-sensitive hypertensive Dahl rats. In these studies, however, the arterial levels of glucose, insulin, and free fatty acids, which influence myocardial glucose and free fatty acid use,\(^{30-32}\) were not examined. Therefore, we could not speculate why those metabolic alterations were induced after long-term pressure overload. In our study, there are no differences in plasma concentrations of glucose, insulin, or free fatty acids between sham-operated and aortic-constricted rats. Therefore, the humoral factors previously mentioned cannot explain the difference in myocardial substrate use between the two groups.

**Nonhomogeneity of Alteration in Regional Myocardial Substrate Use in Habitually Pressure-Overloaded Heart**

Yonekura et al.\(^{16}\) reported that accelerated \(^{14}\text{C}-\text{DG}\) uptake and decreased \(^{14}\text{C}-\text{BMHDA}\) extraction were more remarkably demonstrated in the subendocardial layer of the left ventricular free wall in salt-sensitive hypertensive Dahl rats. In our study, however, \(^{14}\text{C}-\text{BMHDA}\) and \(^{14}\text{C}-\text{DG}\) inner/outer uptake ratios in aortic-constricted rats decreased or tended to decrease as compared with those in sham-operated rats (Figure 3). The difference between their results and ours might be because of differences in animal model of pressure overload, stage of cardiac hypertrophy, or both. Because these inner/outer uptake ratios did not differ significantly between acutely pressure-overloaded sham-operated and normotensive sham-operated rats, duration of pressure overload is an important determinant factor of the myocardial substrate inner/outer uptake ratio.

Interestingly, the patterns of regional difference in substrate analogue uptake in aortic-constricted rats were different from those of sham-operated rats, in which we cannot explain the mechanism of nonhomogeneity of substrate use. Regional myocardial glucose or fatty acid metabolism alters in myocardial ischemia.\(^{33}\) Myocardial blood flow distribution determined by \(^{14}\text{C}-\text{IAP}\), however, showed no unevenness either in sham-operated or aortic-constricted rats. Regional differences in the degree of myocyte hypertrophy cannot explain the nonhomogeneous alteration of myocardial substrate uptake in aortic-constricted rats because there was no difference in myocardial cell width among the four regions of the left ventricle. Histological analysis showed that interstitial fibrosis score, although very small, was highest in the left ventricular lateral wall in aortic-constricted rats. Therefore, regional differences in interstitial fibrosis cannot explain the nonhomogeneous alter-
ation of substrate uptake. Lew and LeWinter reported that circumferential shortening was significantly greater in the anterior than posterior wall in the normal canine left ventricle. These regional differences in segment shortening might influence regional myocardial energy metabolism and alter regional myocardial substrate use. Although mechanisms that can explain the present data are still unclear, as suggested by the findings in sham-operated rats, it is likely there is some regional factor influencing myocardial glucose and free fatty acid uptake; and as suggested by the findings in aortic-constricted rats, it is likely long-term pressure overloading alters this factor nonhomogeneously.

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References


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