Evaluation of the cardiac autonomic nervous system in spontaneously non-insulin-dependent diabetic rats by $^{123}$I-metaiodobenzylguanidine imaging

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**Objective:** To evaluate the sensitivity of $^{123}$I-labeled metaiodobenzylguanidine ($^{123}$I-MIBG) scintigraphy in detecting diabetic autonomic nervous system disorders.

**Materials and Methods:** Thirty-one-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of spontaneous non-insulin-dependent diabetes mellitus, were maintained for 8 weeks with or without 30% sucrose solution as a drinking water (n = 3 each). Long-Evans Tokushima Otsuka (LETO) rats (n = 3), served as controls. Plasma glucose and insulin levels were measured, and $^{123}$I-MIBG scintigraphy was performed with a gamma camera equipped with a pinhole collimator for animals. Plasma and cardiac tissue catecholamine levels were also determined.

**Results:** Plasma glucose levels of OLETF rats with and without sucrose loading (554 ± 106 and 141 ± 1.5 mg/dl respectively) were significantly higher than those of LETO rats (116 ± 3.7 mg/dl). Norepinephrine concentrations in heart and plasma tended to be lower in diabetic rats. The washout rate of $^{123}$I-MIBG in diabetic rats was significantly higher than the rate in control rats. Cardiac uptake of $^{123}$I-MIBG, calculated as % dose/g of tissue, was significantly lower in diabetic rats than in control rats.

**Conclusion:** These results suggest that myocardial $^{123}$I-MIBG scintigraphy is suitable for assessing cardiac sympathetic activity noninvasively in diabetic states, even in the early stages.

**Key words:** $^{123}$I-MIBG, OLETF rats, diabetes mellitus, catecholamine, autonomic nervous system

**INTRODUCTION**

Diabetes mellitus is frequently associated with microangiopathy, such as diabetic retinopathy, nephropathy and neuropathy, but also with coronary artery disease. Diabetics may have myocardial infarction due to multivessel lesions, which are sometimes asymptomatic, but abnormalities of the myocardium due to diabetes (diabetic cardiomyopathy) may exist independently of diabetic atherosclerosis. Impairement of left ventricular function and cardiomegaly may be characteristic features. Although the pathogenesis of diabetic cardiomyopathy remains to be clarified, mechanisms proposed have included small vessel disease and metabolic alterations. In addition, changes in the cardiac autonomic nervous system have been considered to be one mechanism of pathogenesis (based on a high incidence of congestive heart failure and sudden death in diabetics). Ewing’s sign and/or the achilles tendon reflex, in addition to subjective symptoms, have been used to indicate diabetic neuropathy, but these tests sometimes do not detect neuropathy in its early stage. For detection of cardiac autonomic neuropathy, various electrocardiogram-based cardiac reflex tests, such as heart rate variation at rest and during deep breathing (CVR-R) and the heart rate and blood pressure response on standing, have been used. Recently, low-frequency and high-frequency fluctuations measured by 24-hour ECG have also been reported, but these assess
cardiac autonomic function in diabetes only indirectly. Recently single-photon emission tomography (SPET) with $^{123}$I-labeled metaiodobenzylguanidine ($^{123}$I-MIBG), an analog of guanethidine which has been used for the detection of pheochromocytoma, has been introduced as a direct and noninvasive method for measuring global and regional distributions of cardiac sympathetic dysinnervation in ischemic heart diseases. It has been observed that the myocardial uptake of $^{123}$I-MIBG is severely impaired in diabetics with cardiac autonomic neuropathy detected on ECG. Even in diabetic subjects without changes on ECG, a subtle change in cardiac $^{123}$I-MIBG uptake has been reported. In addition, $^{123}$I-MIBG uptake in diabetic patients is not homogeneous throughout the myocardium but shows significant regional variation, but it remains to be clarified whether this uptake correlates with the severity of the disease or reflects the efficacy of the treatment. The present study was therefore designed to evaluate: 1) whether or not $^{123}$I-MIBG uptake is related to the degree of glucose intolerance; and 2) whether or not $^{123}$I-MIBG uptake and washout rates are useful for the early diagnosis of impairment of the cardiac sympathetic nervous system. We used Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of spontaneous non-insulin-dependent diabetes mellitus (NIDDM) with obesity, which were developed by the Otsuka Pharmaceutical Company (Tokushima, Japan) in 1992.

MATERIALS AND METHODS

**Animals**

Male Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Long-Evans Tokushima Otsuka (LETO) rats (body weight 120–140 g, 5 weeks of age) were supplied by Tokushima Research Institute, Otsuka Pharmaceutical Company (Tokushima, Japan). The animals had free access to rat chow and tap water, were housed two to a cage and were kept at a temperature of 23 ± 1°C and a humidity of 50±10% with a 12-hour light-dark cycle. All procedures conformed with the principles of laboratory animal care (NIH publication revised 1985).

All the rats were given an intraperitoneal glucose tolerance test (IPGTT) at 31 weeks of age. After 16 hours of fasting, they were given an intraperitoneal injection of 20% glucose solution (2 g/kg). Plasma glucose and insulin levels were determined before, 1 hour after and 2 hours after glucose administration in blood samples obtained from tail veins. Glucose levels were determined by the glucose-oxidase method and insulin concentrations measured by specific radioimmunoassay (Phadesef Insulin kit, Pharmacia, Sweden) with a rat insulin as a standard. The area under the curve was calculated for plasma glucose and insulin levels. After IPGTT, the rats were divided into 3 groups as follows: Group A: LETO rats (control rats, n = 3); Group B: OLETF rats (n = 3); Group C: OLETF rats + 30% sucrose loading (n = 3). Group C rats were given tap water containing 30% sucrose (w/v) for 8 weeks. Body weight was measured at the ages of 31, 35, 39 and 40 weeks, respectively.

At the age of 39 weeks, blood pressure was measured by a tail cuff method.

### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>31 wk (g)</th>
<th>35 wk (g)</th>
<th>39 wk (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LETO</td>
<td>491 ± 12.5</td>
<td>493 ± 11.5</td>
<td>500 ± 12.3</td>
</tr>
<tr>
<td>OLETF</td>
<td>589 ± 19.0*</td>
<td>586 ± 22.5*</td>
<td>600 ± 27.7*</td>
</tr>
<tr>
<td>O + S</td>
<td>612 ± 5.6*</td>
<td>636 ± 8.9*</td>
<td>534 ± 4.7*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

LETO: Long-Evans Tokushima Otsuka rats (control)
OLETF: Otsuka Long-Evans Tokushima Fatty rats
O + S: OLETF rats with 30% sucrose loading

*p < 0.05 compared to LETO

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>BS (mg/dl)</th>
<th>Insulin (ng/ml)</th>
<th>Insulin/BS</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLETF</td>
<td>533.0 ± 23.0*</td>
<td>4.27 ± 1.32*</td>
<td>0.080 ± 0.040*</td>
</tr>
<tr>
<td>LETO</td>
<td>252.5 ± 4.8</td>
<td>1.32 ± 0.04</td>
<td>0.052 ± 0.002</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

LETO: Long-Evans Tokushima Otsuka rats (control)
OLETF: Otsuka Long-Evans Tokushima Fatty rats

*p < 0.05 compared to LETO

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>FBS (mg/dl)</th>
<th>Insulin (ng/ml)</th>
<th>Insulin/BS</th>
<th>1,5 AG (μg/ml)</th>
<th>Fructosamine (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LETO</td>
<td>116 ± 3.7</td>
<td>1.02 ± 0.07</td>
<td>0.088 ± 0.004</td>
<td>7.067 ± 0.18</td>
<td>193.7 ± 6.23</td>
</tr>
<tr>
<td>OLETF</td>
<td>140 ± 1.5*</td>
<td>1.84 ± 0.41*</td>
<td>0.131 ± 0.03*</td>
<td>16.167 ± 4.78</td>
<td>185.0 ± 3.79</td>
</tr>
<tr>
<td>O + S</td>
<td>554 ± 106.2*</td>
<td>3.88 ± 0.82*</td>
<td>0.077 ± 0.02</td>
<td>2.367 ± 0.29***</td>
<td>278.0 ± 7.75***</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

LETO: Long-Evans Tokushima Otsuka rats (control), OLETF: Otsuka Long-Evans Tokushima Fatty rats
O + S: OLETF rats with 30% sucrose loading
FBS: fasting blood sugar, 1,5 AG: 1,5-anhydro glucose
t *p < 0.05 compared to LETO rats, **p < 0.05 compared to OLETF rats
Coefficient of variation of the R-R interval
At the age of 40 weeks, the coefficient of variation of the R-R interval (CVR-R) on the electrocardiogram was determined, as reported previously. Briefly, the rats were anesthetized lightly with diethyl ether and the electrocardiogram was recorded with a Labo-System ZS-501 (Fukuda ME, Tokyo, Japan). The data obtained for 1 min were analyzed to determine if the heart rate exceeded 350 beats/min just before the rat awoke.

Table 4  Heart rate and blood pressure of each rat

<table>
<thead>
<tr>
<th>No.</th>
<th>HR (bpm)</th>
<th>HR mean ± SEM</th>
<th>BP (mmHg)</th>
<th>BP mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>LETO1</td>
<td>477</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LETO2</td>
<td>434</td>
<td>118</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LETO3</td>
<td>484</td>
<td>465.1 ± 15.8</td>
<td>121</td>
<td>123.0 ± 3.5</td>
</tr>
<tr>
<td>OLETF1</td>
<td>403</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLETF2</td>
<td>444</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLETF3</td>
<td>428</td>
<td>425.0 ± 11.8</td>
<td>139</td>
<td>136.3 ± 2.9*</td>
</tr>
<tr>
<td>O + S1</td>
<td>413</td>
<td>134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O + S2</td>
<td>399</td>
<td>132</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O + S3</td>
<td>486</td>
<td>432.9 ± 26.8</td>
<td>141</td>
<td>135.8 ± 2.6*</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to LETO.

123I-MIBG imaging
The rats were anesthetized with pentobarbital (30-40 mg/kg, intraperitoneally), and received 74 MBq (2 mCi) 123I-MIBG (kindly provided by Daiichi Radiisotope Laboratories, Ltd., Tokyo, Japan) intravenously via a catheter indwelled into a femoral vein. Planar imaging was performed with a single-headed gamma camera, PRISM2000 X P equipped with a 2 mm pin-hole collimator (Picker Co., Cleveland, Ohio, U.S.A./Shimadzu Co., Kyoto, Japan). Each rat was subjected to early imaging, (20 min after injection) and delayed imaging (4 h after injection). The data were collected with a matrix size of 256 x 256, MAG2, and the total imaging time was 10 min.

For global uptake indices, we measured the myocardial uptake ratio of 123I-MIBG. Regions of interest in the heart were recorded during both the early (20 min) and delayed (4 h) imaging sessions. After correction for the physical decay of 123I, as follows:

\[ WR = (\text{early count} - \text{delay count}) / \text{early count} \times 100\%

After the delay imaging, each rat was sacrificed, the heart weighed, the radioactivity in the heart was measured, and percentage dose/g was calculated.

Table 5  % dose/g, washout rate, heart and plasma catecholamine, blood sugar and insulin of each rat

<table>
<thead>
<tr>
<th>No.</th>
<th>% dose/g</th>
<th>Washout rate (%)</th>
<th>Cardiomyocyte (ng/g)</th>
<th>Plasma (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epinephrine</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Epinephrine</td>
</tr>
<tr>
<td>LETO1</td>
<td>1.109</td>
<td>48.8</td>
<td>44.4</td>
<td>1186</td>
</tr>
<tr>
<td>LETO2</td>
<td>1.367</td>
<td>44.5</td>
<td>36.7</td>
<td>1114</td>
</tr>
<tr>
<td>LETO3</td>
<td>1.317</td>
<td>47.8</td>
<td>46.7</td>
<td>1136</td>
</tr>
<tr>
<td>OLET1</td>
<td>0.904</td>
<td>57.2</td>
<td>28.9</td>
<td>832</td>
</tr>
<tr>
<td>OLET2</td>
<td>0.899</td>
<td>59.3</td>
<td>34.5</td>
<td>1026</td>
</tr>
<tr>
<td>OLET3</td>
<td>0.681</td>
<td>59.2</td>
<td>24.1</td>
<td>940</td>
</tr>
<tr>
<td>O + S1</td>
<td>0.807</td>
<td>56.2</td>
<td>19.1</td>
<td>881</td>
</tr>
<tr>
<td>O + S2</td>
<td>0.874</td>
<td>59.4</td>
<td>10.7</td>
<td>897</td>
</tr>
<tr>
<td>O + S3</td>
<td>0.775</td>
<td>56.6</td>
<td>13.2</td>
<td>917</td>
</tr>
</tbody>
</table>

Fig. 1  A and B. Plasma norepinephrine and epinephrine levels in LETO rats, OLETF rats and OLETF rats with sucrose loading. A: Norepinephrine (pg/ml), B: Epinephrine (pg/ml). *p < 0.05 compared to LETO rats.
RESULTS

As shown in Table 1, the body weight of OLETF rats (group B and C) was significantly greater than that of control LETO rats (group A), but sucrose administration for 8 weeks in group C significantly decreased body weight from 612 ± 5.6 to 534 ± 4.7 g. The area under the curve measured during IPGTT was much higher in OLETF rats than LETO rats (533 ± 23 vs. 253 ± 4.8 mg/dl x h, p < 0.05; Table 2). With sucrose administration, fasting plasma glucose levels were significantly higher (554 ± 106 mg/dl in group C and 140 ± 1.5 mg/dl in group B (Table 3)). Plasma levels of fructosamine were significantly higher and 1,5 AG was significantly lower in group C. Fasting insulin levels in groups A, B and C at the age of 39 weeks were 1.02 ± 0.07, 1.84 ± 0.41 and 3.88 ± 0.82 ng/ml, respectively. The ratio of insulin to glucose concentration in group B OLETF rats, 0.131 ± 0.03, was much higher than in control LETO rats (0.088 ± 0.004). The ratio of insulin to glucose tended to be diminished by sucrose administration (0.077 ± 0.02 in group C). The heart rate was similar in all three groups, and blood pressure of OLETF rats was significantly higher than that of LETO rats (Table 4). The values for % dose/g, washout rate, plasma and heart catecholamines, blood sugar and insulin are shown in Table 5.

As shown in Figure 1, plasma norepinephrine levels in OLETF rats were significantly lower than in the control LETO rats. Plasma epinephrine concentrations also tended to be lower in OLETF rats in group B, but significantly lower in OLETF rats given sucrose (group C). Cardiac norepinephrine and epinephrine levels were also significantly lower in diabetic OLETF rats (groups B and C), as shown in Fig. 2. There was no difference among the three groups in the plasma or cardiac dopamine level.

The coefficient of variation of R-R in group B (2.33 ±
0.75%) was lower, but not significantly, than in controls (3.03 ± 0.54%). With sucrose administration, CVR-R was significantly lower, at 1.27 ± 0.29% (Fig. 3). 123I-MIBG uptake, as calculated by % dose/g tissue, was significantly lower in diabetic OLETF rats with or without sucrose administration, as shown in Fig. 4A. The washout rate was significantly higher in both group B and group C than in control group A (Fig. 4B). Typical early and delayed imagings are shown in Figs. 5A, B and C.

DISCUSSION

OLETF rats are a new animal model of non-insulin-dependent diabetes.13 At the age of 24–28 weeks, male OLETF rats have mild obesity, hyperinsulinemia, hypertriglyceridemia and hypertension.13,15 These features are characteristics of insulin resistance, but with age (that is, after 50 weeks of age), insulin secretion diminishes. Diabetic complications, such as Kimmelstiel-Wilson-like lesions, appear at the age of 60 weeks. Regarding cardiac changes, Yagi et al.16 reported diminished systolic function in OLETF rats at the age of 30 weeks, possibly because of perivascular fibrosis and increased β-myosin heavy chain (MHC) mRNA associated with decreased α-MHC mRNA. CVR-R has been proven to be an easy and useful method to evaluate autonomic nervous systems and reported to be decreased in autonomic nervous disorders including diabetic neuropathy. Hotta et al. reported that administration of 30% sucrose decreased CVR-R in this strain.14 In the present study we used the same method to increase glucose intolerance. Administration of 30% sucrose for 8 weeks increased fasting plasma glucose from 140 mg/dl to 554 mg/dl associated with a further rise in plasma insulin concentrations. Hyperglycemia was also confirmed by a higher level of fructosamine and 1,5 AG.

123I-MIBG is an analog of norepinephrine, which has a specific affinity for postganglionic sympathetic nerve endings. High-affinity uptake of postganglionic sympathetic neurons (uptake 1) and a low-affinity extraneuronal uptake mechanism (uptake 2) have been demonstrated.17 123I-MIBG is metabolized by neither monoamine oxidase nor catechol-O-methyltransferase and is rapidly excreted into urine. In other words, 123I-MIBG is stored in secretory granules18-20 and released by exocytosis. Since uptake 1 is dependent on Na-ATPase, an impairment in energy production results in a reduced uptake of 123I-MIBG. 123I-MIBG scintigraphy depends on blood flow to some extent since 123I-MIBG is administered via a vein, but in patients with myocardial infarction, 123I-MIBG scintigraphy sometimes shows a discordant decrease in uptake in comparison with 201TI-myocardial blood flow scintigraphy. In addition, such cases are frequently associated with arrhythmia.21 123I-MIBG scintigraphy reflects not only blood flow but also cardiac sympathetic innervation.

In the present study, 123I-MIBG uptake was significantly lower and the washout rate was significantly higher in diabetic OLETF rats with or without sucrose administration. These results are consistent with a previous study by Dubois et al. of streptozotocin (STZ)-induced diabetic Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).22 They reported that the washout rate of diabetic WKYs was higher than that of control WKYs, but there was no significant difference between STZ-SHRs and SHR. It is well known that STZ selectively destroys pancreatic β cells and results in a deficiency in insulin secretion. STZ-induced diabetic rats are therefore an animal model of insulin-dependent diabetes mellitus (IDDM). In patients with NIDDM and IDDM, diminished
Fig. 5  A, B and C. A typical early and delayed $^{123}$I-MIBG scintigraphy. A: LETO rat, B: OLETF rat, C: OLETF rat with sucrose loading.
cardiac $^{123}$I-MIBG uptake, especially in the posteriorinferior wall, and increased washout rate have been shown.$^{7,8}$ In the present study, evaluation of localization of $^{123}$I-MIBG in the myocardium was difficult because of small size of the animals. In addition, the amount of myocardial tissue was not enough for pathological investigation. Myocardial $^{123}$I-MIBG uptake reflects the neuronal integrity of the sympathetic nervous system of the heart, and the washout rate is presumed to be related to the sympathetic activity. The low uptake of $^{123}$I-MIBG in OLETF rats suggests neuronal dysfunction. These findings are in accordance with data in humans.$^{7,8,23}$

Plasma catecholamine levels have been reported to be higher in patients with congestive heart failure or dilated cardiomyopathy.$^{25}$ Moreover, it is well known that the higher the plasma norepinephrine concentration, the more severely impaired is cardiac function.$^{26}$ In addition, a higher $^{123}$I-MIBG washout rate has been demonstrated in patients with dilated cardiomyopathy associated with congestive heart failure,$^{11}$ suggesting that systolic dysfunction may induce an increased in the turnover rate and/or secretion of catecholamines from the nerve endings. In diabetic ketoacidosis, plasma catecholamine levels in response to exercise have been reported to be often very high,$^{27}$ but mean basal and posturally stimulated plasma norepinephrine and epinephrine concentrations have been reported to be normal$^{26}$ or sometimes decreased.$^{26}$ Thus, previous studies have sometimes been contradictory regarding plasma catecholamine levels, possibly because of differences in the duration of diabetes, differences in glycemic control, or the severity of diabetic complications such as neuropathy. Myocardial $\beta$-adrenergic receptor densities have been reported to be decreased in diabetic (STZ-induced and obese Zucker) rats.$^{22,30}$ In the present study, OLETF rats were considered to be insulin resistant as evidenced by a higher ratio of insulin/blood sugar. OLETF rats with sucrose loading were also insulin resistant because of a marked hyperinsulinemia, although they might be associated with a relative insulin deficiency resulting in a marked hyperglycemia. Peripheral insulin resistance has been shown to increase sympathetic activity,$^{24}$ but it is not likely in the present study, since plasma and myocardial norepinephrine concentrations in OLETF rats (with or without sucrose administration) were significantly lower than in nondiabetic LETO rats. Alternatively, an increased washout rate may be due to dysfunction of reuptake and/or the pooling mechanism considering that the change in catecholamine concentrations correlates with the washout rate of $^{123}$I-MIBG.

Mild hyperglycemia with fasting plasma glucose levels of 140 mg/dl for 8 weeks in OLETF rats was sufficient to result in such changes. The fasting plasma glucose level in OLETF rats receiving sucrose loading was about 550 mg/dl, which is similar to that in rats with STZ-induced diabetes.$^{22}$ These results indicate that myocardial $^{123}$I-MIBG scintigraphy may be a very useful noninvasive tool for detecting an early change in diabetic cardiac function. This view is also supported by finding that CVR-R was not decreased in OLETF rats without sucrose feeding, whereas abnormalities in $^{123}$I-MIBG scintigraphy were observed. In OLETF rats with sucrose administration, abnormalities in the CVR-R shown on ECG paralleled the abnormal findings on myocardial $^{123}$I-MIBG scintigraphy.

In conclusion, myocardial $^{123}$I-MIBG scintigraphy is suitable for noninvasive assessment of cardiac sympathetic activity in the diabetic state, even in the very early stages.

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REFERENCES


