Dual-Tracer Assessment of Coupling Between Cardiac Sympathetic Neuronal Function and Downregulation of β-Receptors During Development of Hypertensive Heart Failure of Rats

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Background—Heart failure is associated with activation of the sympathetic nervous system and downregulation of β-receptors. However, the coupling between cardiac sympathetic neuronal function and the β-receptor during the development of hypertensive heart failure is not clear.

Methods and Results—We determined cardiac neuronal function and β-receptors with a dual-tracer method of [131I]metaiodobenzylguanidine (MIBG) and 125I-cyanopindolol (ICYP) in Dahl salt-sensitive (DS) and salt-resistant (DR) rats. The rats were fed an 8% NaCl diet after the age of 6 weeks. Blood pressure was raised to >200 mm Hg at 12 weeks in DS rats and remained elevated until 18 weeks, but only slightly in DR rats. Left ventricular (LV) function of DS rats was preserved at 12 weeks but deteriorated at 18 weeks. Despite a 56% reduction of cardiac norepinephrine (NE) content at 12 weeks in DS rats, neither MIBG nor ICYP uptake in DS rats was different from that of DR rats. At 18 weeks, both MIBG and ICYP uptakes decreased, by 52% and 39%, respectively, in association with 71% reduction of cardiac NE, in DS rats. MIBG uptake of the LV was homogeneous at 6 weeks but was lower in the LV endocardial regions at 18 weeks in DS rats.

Conclusions—The present results indicate that cardiac sympathetic neuronal function is relatively preserved at the compensated, hypertrophic stage of DS rats but deteriorates in association with β-receptor downregulation at the failing stage. The cardiac neuronal dysfunction occurs heterogeneously. A combination of scintigraphic portrayal of β-receptors with MIBG should provide valuable information regarding sympathetic nerve signaling in living hearts.

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Key Words: heart failure ■ hypertrophy ■ receptors, adrenergic, beta ■ nervous system, autonomic ■ radioisotopes

Heart failure is associated with neural and hormonal activation, including the sympathetic nervous system and the renin-angiotensin system. Initially adaptive activation of these systems may lead to the progression of heart failure and, consequently, increased mortality. In heart failure, increased cardiac NE spillover, myocardial catecholamine depletion, and downregulation of β-receptors are commonly seen. Delehanty et al reported that synaptic NE levels varied inversely with β-receptor densities in the failing heart. The synaptic NE concentration depends on circulating levels of NE, the amount of neuronal release, and subsequent inactivation by neuronal uptake. Circulating NE levels required to induce downregulation of β-receptors in an intact heart are much higher than those seen in heart failure. Thus, the neuronal function of cardiac sympathetic nerve terminals would affect β-receptor signaling and development of heart failure. However, conflicting data exist in terms of neuronal function in heart failure due to mechanical overload. Using MIBG, an analogue of NE, Rabinovitch et al showed that neuronal function was impaired in decompensated heart failure but not in the compensated stage. In contrast, Somsen et al reported no cardiac neuronal dysfunction in either compensated or decompensated mechanical overload heart failure despite the downregulation of β-receptors. Thus, alterations of sympathetic nerve signaling in a transition from the compensated stage, before overt heart failure, to advanced heart failure could not be fully elucidated.

The DS rat is an animal model that develops systemic hypertension depending on the amount of sodium supplied in the diet. This model has the advantage of allowing study of the progression from compensated LV hypertrophy to overt congestive heart failure in a relatively short period. A high-salt diet after 6 weeks of age induces concentric LV hypertrophy with normal LV systolic function at the age of 11

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to 12 weeks, followed rapidly by marked LV dilation and impaired LV function without significant myocardial loss.\textsuperscript{15}

The purpose of the present study was therefore to elucidate a relationship between cardiac sympathetic neuronal function and \( \beta \)-receptors in the transition from the compensated hypertrophic stage to decompensated, dilated heart failure in Dahl rats. For this purpose, we established a dual-tracer technique to assess sympathetic neuronal function with MIBG and \( \beta \)-receptors with ICYP.

### Methods

The present study was undertaken in accordance with the guidelines for animal experimentation at Toyama Medical and Pharmaceutical University.

### Experimental Animals

A total of 139 male rats of the DS and DR strains were used. The rats were fed a 0.3% NaCl diet (low salt) until 6 weeks of age, then they were fed an 8% NaCl diet (high salt). The special diet and tap water were given ad libitum throughout the experiment. Systolic arterial pressure was measured every 2 weeks with an indirect tail-cuff method (BP-98A, Softron). The rats were divided into four groups. The first group was used for hemodynamic study and for measurement of plasma and cardiac tissue catecholamines. The second group was for assessments of cardiac MIBG and ICYP accumulation. The third group was for cardiac autoradiography to evaluate ventricular distribution of MIBG and ICYP. The fourth group was for \( \beta \)-receptor binding study in a crude membrane preparation to validate the cardiac ICYP accumulation after intravenous injection. Data were collected at the age of 6, 12, and 18 weeks in the first three groups and at 6 and 18 weeks in the fourth group.

### Hemodynamic Study

Under ether anesthesia, a 2F micromanometer-tipped catheter (Millar Instruments) was inserted into the right carotid artery and advanced into the LV to measure LV pressure. With the rat lightly anesthetized and breathing spontaneously, LV pressure and ECG were recorded on a multichannel thermal recorder (WR3151, Nihon Kohden). These signals were digitized on-line at 2-ms intervals and analyzed with a signal processing computer system (7T-18, NEC San-Ei).

After the hemodynamic study, blood was drawn from the carotid artery for an analysis of plasma catecholamines. The sample was immediately centrifuged at 4°C for separation of plasma. Then, pentobarbital sodium (70 mg/kg) was injected intraperitoneally. The chest was opened, and the heart was quickly removed. The LV was dissected from the atria and the RV, rinsed in ice-cold saline, and weighed. Plasma and tissue samples were stored at −80°C for later analyses. Catecholamines were measured by automated high-performance liquid chromatography.

### MIBG and ICYP Accumulation

A dose of 20 \( \mu \)Ci of MIBG was injected into the external jugular vein under anesthesia with pentobarbital sodium (30 mg/kg IP). Two hours later, 10 \( \mu \)Ci of ICYP was given intravenously. Rats were killed with an additional injection of pentobarbital sodium 1 hour after the ICYP injection. The heart was removed from the chest. LV counts of MIBG and ICYP were determined with an auto-well gamma counter (ARC 2000, Aroka). When radiolabeled ICYP was given intravenously, binding was predominantly to \( \beta \)-receptors in the heart.\textsuperscript{16} In our preliminary study of rats, cardiac accumulation of ICYP injected intravenously increased linearly at doses from 5 to 150 \( \mu \)Ci, and the increase was suppressed at a dose of 400 \( \mu \)Ci. Administration of propranolol (0.05 mg IV) just before the ICYP injection decreased cardiac ICYP accumulation by 56%. Cardiovascular accumulation reached relatively constant levels 3 hours after ICYP injection and 30 minutes after ICYP injection. These observations are consistent with previous studies.\textsuperscript{16,17}

To assess the sympathetic neuronal function of failing hearts, an \( \alpha \)-agonist, guanabenz, which suppresses neuronal release of NE, and a neuronal uptake-1 blocker, desipramine hydrochloride, were given separately to the rats at 18 weeks. A 3-mg/kg dose of guanabenz was administered intraperitoneally 60 minutes before ICYP injection, or a 10 mg/kg dose of desipramine was given 30 minutes before ICYP injection. Three hours after ICYP injection, the hearts were removed and cardiac MIBG activities were determined.

The MIBG preparation used (Daichi Radioisotope Laboratory) had a specific activity of 65 Ci/mmol and a chemical purity of >98%. The specific activity of the ICYP preparation used (Daichi Radioisotope Laboratory) was 2200 Ci/mmol and the purity >97%. The ICYP counts were determined 60 days later, after the decay of MIBG. The cross-talk from ICYP window to MIBG window was <3%, and therefore, we ignored the cross-talk between \( ^{125} \)I and \( ^{131} \)I. To normalize for differences in animal weight, tissue accumulations of MIBG and ICYP were expressed in percent kilogram dose per gram of LV wet weight.\textsuperscript{18}

### Dual-Tracer Autoradiography

In the study of dual-tracer autoradiography, animals were injected intravenously with 50 \( \mu \)Ci of MIBG and 2 hours later with an injection of 5 \( \mu \)Ci of ICYP. The hearts were removed 1 hour after the second injection. Serial transverse sections of the heart 20 \( \mu \)m thick were obtained after the specimens had been frozen in isopentane cooled in dry ice followed by embedding in methyl cellulose. The first autoradiographic exposure was made for 90 days and a second with an imaging plate (BAS-UR, Fuji) was carried out for 6 hours to reveal MIBG distribution. The second exposure was initiated 60 days later, after the decay of MIBG activity, and required 21 days for adequate image quality. In the single-tracer autoradiography with each tracer under the same conditions as the dual-tracer method, it was confirmed that ICYP density was <5% of MIBG density under the exposure conditions for MIBG imaging and that MIBG images were not visualized under the exposure conditions for ICYP imaging.

To quantify the myocardial distributions of MIBG and ICYP, a myocardial section at the level of the papillary muscles was divided into nine regions, ie, the epicardial and endocardial regions of LV anterior, posterior, lateral, and septal walls and the RV free wall. Distributions in the selective sections were quantified with a bioimaging analyzer (BAS3000, Fuji). The image data were recorded as the digitized values (PSL) of each pixel (50\( \times \)50 \( \mu \)m) in the analyzing unit of this system. To quantify the distribution of tissue radioactivity, the autoradiographic intensities [PSL−BG]/A, where BG is the PSL of the background and A is the area of each region in square millimeters, were obtained in each region. This was applied for an evaluation of distribution of radioactivities in each heart, and therefore, the data were expressed as a value relative to the density of the epicardial region of the LV lateral wall.

### \( \beta \)-Receptor Binding

After an injection of pentobarbital (70 mg/kg IP), hearts were quickly removed and rinsed in saline at 4°C. A part of the LV including the ventricular septum was dissected, cooled on dry ice, and stored in sealed containers at −80°C for later radioligand binding experiments.

Tissue membrane preparations were incubated with \([^{3}H] \)CGP12177 (specific activity 44.5 Ci/mmol, New England Nu-
clear) at 37°C for 60 minutes in borosilicate glass tubes in a total volume of 0.25 mL of 145 mmol/L NaCl, 0.1 mmol/L EDTA, 2 mmol/L MgCl₂, and 20 mmol/L Tris (pH 7.5). Incubations were stopped by dilution with 3 mL of ice-cold assay buffer, followed by rapid vacuum filtration onto Whatman GF/B filters, which were then washed twice with additional buffer. The radioactivity trapped on the glass filters was counted in a scintillation counter with an Aquazol II (Amersham). The nonspecific binding was defined as radioligand binding in the presence of an excess concentration (100 µmol/L) of dl-isoproterenol. Data from the saturation binding studies were analyzed by Scatchard analysis, giving the B₅₀ site and Kᵦ.

### Statistics

Results are expressed as mean±SD. Group comparisons were made with ANOVA, followed by a Bonferroni test to identify differences among various groups. A value of P<0.05 was considered statistically significant.

### Results

At the age of 6 weeks, blood pressure, body weight, and LV weight did not vary between DS and DR rats (Table 1). As in the previous study,³⁵ systolic blood pressure in DS rats with a high salt intake increased gradually with age, reaching ≥200 mm Hg at 12 weeks, and remained elevated until 18 weeks, whereas it increased only slightly in DR rats. Body weight increased similarly in both groups until 12 weeks, but not any more in DS rats after 14 weeks. At the age of 16 to 18 weeks, when heart failure developed, DS rats displayed labored respiration and decreased activity, and their general condition progressively deteriorated. In total, 14 DS rats died between 16 and 18 weeks before euthanasia at 18 weeks, but none of the DR rats died. LV weight at 12 weeks was greater in DS rats than in DR rats, and the difference became greater at 18 weeks. Therefore, the ratio of LV weight to body weight was significantly higher in DS rats than in DR rats at 12 and 18 weeks.

### Hemodynamic data are shown in Table 2. LV end-diastolic pressure increased slightly in DS rats at 12 weeks and markedly at 18 weeks, although it was unchanged in DR rats. dP/dtₘₐₓ at 12 weeks was greater in DS rats than in DR rats, associated with increased LV systolic pressure in DS rats. Despite the sustained elevation of LV systolic pressure in DS rats at 18 weeks, dP/dtₘₐₓ decreased to the same level as in DR rats. A dP/dtₙₐₓ normalized to the systolic LV pressure, dP/dtₚ, remained essentially unchanged until 12 weeks in both groups but decreased significantly at 18 weeks in DS rats. dP/dtₙₐₓ was similar between the two groups at 12 weeks, despite the marked increase in dP/dtₙₐₓ of DS rats, and dP/dtₙₐₓ decreased at 18 weeks in DS rats.

As shown in Figure 1, plasma NE levels were unchanged until 12 weeks and increased significantly at 18 weeks in DS rats. Cardiac NE contents decreased significantly at 12 weeks in DS rats. At 18 weeks, cardiac NE decreased further in DS rats, and the difference between the two groups became much greater.

### MIBG and ICYP Accumulation

MIBG accumulation of the LV did not vary between the two groups at 6 and 12 weeks, but it decreased markedly in DS rats at 18 weeks (Figure 2). At the age of 18 weeks, an intraperitoneal injection of guanabenz increased MIBG accumulation by 100% in DS rats (n=3) and 118% in DR rats (n=3). Desipramine increased MIBG accumulation by 38% in DS rats (n=2) and decreased it by 23% in DR rats (n=3).

### Table 1. Blood Pressure, LV Weight, and Body Weight of DS and DR Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>DS (n=5)</th>
<th>DR (n=5)</th>
<th>DS (n=8)</th>
<th>DR (n=7)</th>
<th>DS (n=6)</th>
<th>DR (n=6)</th>
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<tbody>
<tr>
<td>HR, bpm</td>
<td>426±22</td>
<td>414±16</td>
<td>439±48</td>
<td>393±38</td>
<td>433±29</td>
<td>400±28</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>117±10</td>
<td>108±10</td>
<td>210±14</td>
<td>119±10*</td>
<td>207±16</td>
<td>128±11*</td>
</tr>
<tr>
<td>LWW, g</td>
<td>0.44±0.04</td>
<td>0.43±0.04</td>
<td>1.11±0.08</td>
<td>0.90±0.09*</td>
<td>1.33±0.14</td>
<td>0.97±0.05*</td>
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<td>BW, g</td>
<td>166±14</td>
<td>153±9</td>
<td>319±45</td>
<td>358±27</td>
<td>347±28</td>
<td>408±24*</td>
</tr>
<tr>
<td>LWW/BW, g/kg</td>
<td>2.65±0.27</td>
<td>2.85±0.19</td>
<td>3.52±0.45</td>
<td>2.53±0.35*</td>
<td>3.86±0.56</td>
<td>2.38±0.19*</td>
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</table>

HR indicates heart rate; SBP, systolic blood pressure; LWW, LV weight; and BW, body weight. Values are mean±SD.

<table>
<thead>
<tr>
<th>Time</th>
<th>DS (n=5)</th>
<th>DR (n=6)</th>
<th>DS (n=5)</th>
<th>DR (n=7)</th>
<th>DS (n=6)</th>
<th>DR (n=6)</th>
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<td>2.53±0.35*</td>
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<td>2.38±0.19*</td>
</tr>
</tbody>
</table>

HR indicates heart rate; SBP, systolic blood pressure; LWW, LV weight; and BW, body weight. Values are mean±SD.

### Table 2. Hemodynamic Data of DS and DR Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>DS (n=4)</th>
<th>DR (n=4)</th>
<th>DS (n=5)</th>
<th>DR (n=6)</th>
<th>DS (n=6)</th>
<th>DR (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>408±27</td>
<td>396±15</td>
<td>423±42</td>
<td>413±23</td>
<td>395±46</td>
<td>403±45</td>
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<tr>
<td>LVP, mm Hg</td>
<td>115±12</td>
<td>112±9</td>
<td>179±11</td>
<td>120±10*</td>
<td>155±22</td>
<td>124±10*</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>1±1</td>
<td>1±1</td>
<td>4±2</td>
<td>1±1</td>
<td>12±5</td>
<td>2±1*</td>
</tr>
<tr>
<td>dP/dtₘₐₓ ×10⁻⁶ mm Hg/s</td>
<td>9.9±1.8</td>
<td>9.2±0.8</td>
<td>13.3±3.1</td>
<td>8.9±2.0*</td>
<td>7.9±1.8</td>
<td>7.4±1.0</td>
</tr>
<tr>
<td>−dP/dtₙₐₓ ×10⁻⁶ mm Hg/s</td>
<td>7.1±0.6</td>
<td>7.4±1.1</td>
<td>7.5±1.5</td>
<td>8.2±1.2</td>
<td>5.1±0.9</td>
<td>7.4±1.3*</td>
</tr>
<tr>
<td>dP/dtₚ, 1/s</td>
<td>87±12</td>
<td>83±8</td>
<td>74±15</td>
<td>74±13</td>
<td>51±10</td>
<td>69±11*</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVP, LV systolic pressure; LVEDP, LV end-diastolic pressure; dP/dtₘₐₓ and −dP/dtₙₐₓ, maximum and minimum values of rate of change in LV pressure; and (dP/dtₚ)/P, dP/dtₙₐₓ divided by the LV systolic pressure. Values are mean±SD.

*P<0.05 vs age-matched DS rats.
at 18 weeks. ICYP accumulation in the LV was similar in the two groups at 6 and 12 weeks, but it became 39% lower in DS rats than in DR rats at 18 weeks.

**Autoradiography**

Figure 3 shows representative examples of autoradiography with MIBG. The MIBG distribution was relatively homogeneous in DS rats at 6 weeks. Along with advancing age, MIBG accumulation decreased progressively but inhomogeneously in DS rats. At 12 weeks, the accumulation was lower in the LV endocardial region than in the LV epicardial region or RV. At 18 weeks, it decreased markedly in the whole heart, although the decrease was greater in the LV endocardial region. In contrast, MIBG accumulation in the LV was relatively homogeneous at any stage in DR rats. Average data of ventricular MIBG distribution are shown in Table 3 and Figure 4. The MIBG accumulation was slightly lower in the posterior and septal walls than in the anterior wall in both groups, although the LV distribution pattern varied somewhat in each heart. The ratio of MIBG accumulation of the LV endocardial region to epicardial region decreased with age in DS rats but was unchanged in DR rats, although the accumulation was slightly lower in the endocardial region than in the
epicardial region in DR rats at all stages. The MIBG accumulation was greater in the RV than in the LV in both groups (Figure 4).

Figure 5 shows representative examples of autoradiography with ICYP. The ICYP distribution in a heart was homogeneous at all stages in both groups, although the ICYP uptake was slightly lower in the LV endocardial region in DS rats at 18 weeks (Table 4 and Figure 6).

### β-Receptor Binding in Membrane Preparation

The $B_{\text{max}}$ site and $K_d$ of β-receptors in the membrane preparation were not significantly different between DS and DR rats at 6 weeks (Table 5). At the age of 18 weeks, $B_{\text{max}}$ was 25% lower in DS rats than in DR rats, although $K_d$ was not different between the groups, a finding comparable to the decrease in ICYP accumulation in the LV (Figure 2).

### Discussion

The major findings of the present study are as follows. First, a neuronal dysfunction at the adrenergic nerve terminal was associated with β-receptor downregulation in DS rats. Second, MIBG accumulation in the LV was relatively homogeneous in the normal hearts but became heterogeneous in the hypertrophic and failing hearts. Third, MIBG accumulation did not always reflect the cardiac NE level and would depend on the neuronal function. Finally, assessment of β-receptor density obtained after an intravenous injection of ICYP was comparable to results obtained with a conventional radioligand binding assay in a membrane preparation.

### Transition From Compensated Hypertrophy to Heart Failure

High sodium intake develops hypertension in DS rats but not in DR rats. Recently, Inoko et al. observed that DS rats have a short life expectancy and rapidly develop congestive heart failure after LV concentric hypertrophy with normal systolic function when fed a high-salt diet starting at the age of 6 weeks. In the present study, at 16 to 18 weeks, DS rats fed the high-salt diet displayed labored respiration and decreased activity, with no more increase in body weight, although blood pressure remained elevated. Thirty percent of DS rats intended for the hemodynamic and radioisotope studies died before the final experiments at 18 weeks. Autopsy showed pleural effusion and ascites in most cases. These findings were consistent with the earlier study.

Many studies have demonstrated that alterations in sympathetic neural mechanisms play a critical part in the genetic predisposition to salt-induced hypertension in DS rats. Takeshita and Mark reported that elevated sympathetic nervous vasoconstrictor tone accounted for ~50% of the salt-induced increase in vascular resistance in DS rats. Chemical sympathectomy prevented the development of salt-induced hypertension. Central neural mechanisms might also be involved in the development of hypertension in DS rats. In the present study, cardiac NE content decreased markedly with an increase in blood pressure before the development of heart failure, suggesting the presence of an augmented cardiac sympathetic drive.

### Table 3. Ventricular Distribution of MIBG

<table>
<thead>
<tr>
<th></th>
<th>6 Weeks</th>
<th>12 Weeks</th>
<th>18 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS</td>
<td>DR</td>
<td>DS</td>
</tr>
<tr>
<td>Lateral-endo</td>
<td>1.01±0.09</td>
<td>0.89±0.08</td>
<td>0.65±0.10</td>
</tr>
<tr>
<td>Septal-epi</td>
<td>1.05±0.05</td>
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<td>0.81±0.26</td>
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<td>Septal-endo</td>
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<tr>
<td>Anterior-epi</td>
<td>1.19±0.16</td>
<td>1.12±0.05</td>
<td>1.02±0.18</td>
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<td>Anterior-endo</td>
<td>0.96±0.11</td>
<td>0.84±0.06</td>
<td>0.66±0.25</td>
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<tr>
<td>Posterior-epi</td>
<td>1.05±0.13</td>
<td>0.89±0.09</td>
<td>0.76±0.22</td>
</tr>
<tr>
<td>Posterior-endo</td>
<td>0.94±0.13</td>
<td>0.77±0.16</td>
<td>0.54±0.11*</td>
</tr>
<tr>
<td>RV</td>
<td>1.31±0.19*</td>
<td>1.10±0.25</td>
<td>1.07±0.09</td>
</tr>
</tbody>
</table>

Endo indicates endocardial region. Data are expressed relative to the density of epicardial (epi) region of LV lateral wall on autoradiography. n=4 at each stage in both groups.

*P<0.05 vs epicardial region of LV lateral wall.
MIBG Accumulation

MIBG is thought to share the same uptake, storage, and release mechanisms as NE in the adrenergic nerve terminals. Extravesicular accumulation of MIBG at the adrenergic neuron terminal decreases rapidly after the injection, whereas intravesicular accumulation reaches a plateau at 3 hours after the injection. A recent rat experiment using MIBG with a high specific radioactivity revealed that 80% to 90% of MIBG accumulated in cardiac neurons 3 hours after injection and 70% to 80% of MIBG was present in adrenergic vesicles (M.I., unpublished data). In the present study, we used a high specific radioactivity of 65 mCi/mmol. An α₂-agonist, a suppressant of the neuronal release of NE, increased cardiac MIBG accumulation in both DS and DR rats. Myocardial MIBG accumulation determined 3 hours after the injection, therefore, could be regarded as a reflection of cardiac adrenergic function.

Cardiac MIBG accumulation did not decrease in the DS rats at 12 weeks, despite a significantly low level of cardiac NE. Although MIBG accumulation is considered to reflect the level of cardiac NE, the present result implies that cardiac MIBG accumulation is not always in parallel with cardiac NE content, a finding consistent with

![Figure 5. Representative examples of ventricular ICYP distribution obtained by a second exposure after decay of MIBG.](image)

**TABLE 4. Ventricular Distribution of ICYP**

<table>
<thead>
<tr>
<th></th>
<th>6 Weeks</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>DR</td>
<td>DS</td>
<td>DR</td>
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<td>DR</td>
</tr>
<tr>
<td>Lateral-endo</td>
<td>1.11±0.11</td>
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<td>0.93±0.07</td>
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<td>0.97±0.16</td>
<td>0.86±0.17</td>
<td>0.95±0.08</td>
<td>0.89±0.12</td>
<td>1.02±0.14</td>
</tr>
<tr>
<td>Anterior-epi</td>
<td>1.15±0.15</td>
<td>1.03±0.04</td>
<td>1.00±0.07</td>
<td>1.02±0.10</td>
<td>1.00±0.07</td>
<td>1.10±0.17</td>
</tr>
<tr>
<td>Anterior-endo</td>
<td>1.09±0.17</td>
<td>0.98±0.12</td>
<td>0.88±0.07</td>
<td>0.99±0.07</td>
<td>0.90±0.14</td>
<td>0.93±0.22</td>
</tr>
<tr>
<td>Posterior-epi</td>
<td>1.11±0.18</td>
<td>1.04±0.05</td>
<td>0.81±0.10</td>
<td>1.09±0.09</td>
<td>1.00±0.31</td>
<td>0.91±0.10</td>
</tr>
<tr>
<td>Posterior-endo</td>
<td>1.12±0.15</td>
<td>0.99±0.14</td>
<td>0.85±0.12</td>
<td>1.02±0.05</td>
<td>0.91±0.08</td>
<td>0.94±0.11</td>
</tr>
<tr>
<td>RV</td>
<td>1.03±0.18</td>
<td>0.96±0.21</td>
<td>0.95±0.14</td>
<td>1.03±0.08</td>
<td>1.03±0.25</td>
<td>1.09±0.18</td>
</tr>
</tbody>
</table>

Endo indicates endocardial region. Data are expressed relative to the density of epicardial (epi) region of LV lateral wall on autoradiography. n=4 at each stage in both groups.
the study of Somsen et al. Although a decrease in cardiac NE may be a marker of increased adrenergic activity, this could also be due to a decrease in adrenergic neuron density and/or a decrease in functional uptake-1 sites. However, the overall LV accumulation of MIBG did not decrease in association with developing LV hypertrophy in DS rats at 12 weeks, although the MIBG accumulation in the LV endocardial region was slightly lower in DS rats at 12 weeks. Therefore, we speculate that a cardiac sympathetic activation may have occurred at 12 weeks in DS rats.

At the age of 18 weeks in DS rats with depressed LV function, the MIBG accumulation decreased in association with a marked decrease in cardiac NE content and increase in plasma NE level. An $\alpha_2$-agonist, guanabenz, increased MIBG accumulation in DR rats as well as in DS rats at 18 weeks. This effect would be due to less competition from released NE for neuronal reuptake as a result of the reduction in NE release. Although increases in MIBG accumulation relative to each control value with guanabenz were similar between DS and DR rats, this would not necessarily imply a preserved function of uptake-1 in DS rats, because the control value was markedly less in DS rats than in DR rats at 18 weeks. The uptake-1 blocker desipramine should reduce MIBG uptake at the uptake-1 site. This occurred in DR rats but not in DS rats at 18 weeks. Desipramine may also interfere with central sympathetic outflow, which could lead to an increase in MIBG accumulation due to less competition at uptake sites. This inhibitory effect of desipramine might be more pronounced in DS rats at 18 weeks with an augmented sympathetic activity. Previous studies reported that the function of uptake-1 in nerve terminals was impaired and/or reduced in failing hearts with decreased cardiac NE contents. In the disturbed energy status of the sympathetic neuron, nonexocytotic NE release from the sympathetic nerve ending into extracellular space occurred via uptake-1 carrier. Desipramine also interfered with this nonexocytotic release and therefore decreased the overflow of NE. Our data suggest that impaired function of the sympathetic nerve ending and nonexocytotic NE release may contribute to decreased MIBG accumulation in DS rats at 18 weeks.

 Autoradiography showed that MIBG accumulation of DS rats at the age of 18 weeks was lower in LV endocardial regions than in the epicardial regions, although it was relatively homogeneous at 6 weeks. Adrenergic nerve fibers run along the coronary arteries and are distributed from the epicardial region to the endocardial region in the perfusion areas of the associated coronary arteries. In pressure-overload hypertrophy, LV endocardial dysfunction precedes the epicardial dysfunction. Our results would support the speculation that the function of sympathetic nerve terminals is more severely impaired in the endocardial region.

MIBG accumulation in both DS and DR rats was higher in the RV than in the LV, as in the previous study, a consistent finding that NE content of the RV is greater than that of the LV in the normal heart. Whether the increased innervation in the RV is of any physiological significance is unknown.

Pentobarbital sodium may suppress NE release and therefore may affect MIBG accumulation. An inhibition of NE release by pentobarbital may produce an increase in MIBG accumulation, as was found with the administration of $\alpha_2$-agonist. However, MIBG accumulation markedly decreased at the failing stage, in which sympathetic activity would be augmented, in DS rats. Therefore, we think that the effect of pentobarbital did not seriously influence the present results.

**ICYP Accumulation and $\beta$-Receptor Binding Assay**

We compared the ICYP accumulation obtained by intravenous injection with the results from a radioligand receptor assay of membrane preparations. In our preliminary study, administration of propranolol (0.05 mg IV) decreased ICYP accumulation by 56%. This does not indicate that 44% of the injected ICYP is bound to sites other than $\beta$-receptors, because this dose of propranolol may not be enough to completely occupy $\beta$-receptor sites. A 39% reduction in accumulation in DS rats at the age of.
18 weeks compared with age-matched DR rats was comparable to the 25% reduction of Bmax in DS rats at 18 weeks. Although the method of intravenous injection of radioisotope does not give Bmax or Kd of the receptor, it could be a simple and easy way to evaluate cardiac β-receptors and their distribution in the heart.16

ICYP accumulation was not impaired in DS rats at 12 weeks with a normal LV function despite a decreased cardiac NE content. However, it decreased at 18 weeks in association with decreased LV function and MIBG accumulation. An increased adrenergic drive would have been sustained during the course of developing heart failure of DS rats, but dysfunction of adrenergic nerve terminals may occur in the failing stage. The downregulation of β-receptors may be caused by increased NE levels in the synaptic cleft due to increased release and impaired reuptake of NE.32,36 Thus, the dual-tracer method would provide information on the relationship between cardiac neuronal function and β-receptors during development of heart failure.

The autoradiographic ICYP distribution was relatively homogeneous at all stages in both DS and DR rats. Murphee and Saffitz29 showed that the downregulation of β-receptors was marked in the failing heart, especially in the endocardial regions. In the present study, ICYP accumulation tended to decrease in the endocardial region of DS rats only at the age of 18 weeks.

In conclusion, cardiac sympathetic neuronal function deteriorates heterogeneously in association with β-receptor downregulation in the failing stage in DS rats. The dual-tracer technique using MIBG and ICYP is useful for simultaneous determination of cardiac sympathetic neuronal function and β-receptor densities. Further studies are required for the development of in vivo imaging with dual tracers.

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References


Dual-Tracer Assessment of Coupling Between Cardiac Sympathetic Neuronal Function and Downregulation of β-Receptors During Development of Hypertensive Heart Failure of Rats

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