Baroreceptor Sensitivity During the Compensatory Phase of Left Ventricular Overloading

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This study focused on the role of the baroreceptor-mediated control during the compensatory process after acute left ventricular overloading induced by aortic regurgitation (AR). Baroreceptor-heart rate sensitivity was measured using a phenylephrine-induced increase in blood pressure according to the steady state method before, 1 day, 1 week and 4 weeks after production of AR in 7 rabbits, and compared with 6 other rabbits that underwent a sham operation. Blood pressure was monitored noninvasively using Finapres in the unanesthetized state. Four weeks after the procedure, the left ventricular diameters of both end-diastole and end-systole were larger in the rabbits with AR than in the sham-operated rabbits. There was no difference in the left ventricular end-diastolic pressure or cardiac output. Left ventricular weight was higher in the rabbits with AR than in the sham-operated rabbits. Myocardial β-adrenergic receptor density and norepinephrine content were comparable between the two groups. Baroreceptor-heart rate sensitivity significantly decreased 1 week after production of AR, and this alteration in sensitivity was partially restored 4 weeks after production of AR. These findings suggested that the altered baroreceptor-heart rate sensitivity was reversible, relating to the compensatory process after acute left ventricular overloading, and that these changes had some role in its pathophysiology. (Jpn Circ J 1998; 62: 773–778)

**Key Words:** Aortic regurgitation; Baroreceptor sensitivity; β-Adrenergic receptor; Heart failure

Neurohumoral activations, especially the plasma norepinephrine level, are important determinants of life expectancy in patients with heart failure! Recent large scale trials on the survival in congestive heart failure have confirmed this issue. It is noted that some agents, including angiotensin converting enzyme inhibitors and digitalis glycosides which modulate baroreceptor sensitivity, have a significant impact on morbidity and mortality, while other cardiotonic agents, which accelerate the sympathetic-signal transduction pathway, deleteriously affect survival. Baroreceptor-mediated control and cardiopulmonary receptor-mediated control have a key role in maintaining the homeostasis of multiple neurohumoral factors. In congestive heart failure, alterations in the control result in excessive activation of neurohumoral factors, and therefore accelerate left ventricular dilatation and lead to shortened survival in patients with heart failure.

There is accumulating evidence suggesting that arterial baroreceptor-mediated control and cardiopulmonary receptor-mediated control are altered in both human and experimental heart failure but there are few studies concerning the compensatory process after acute left ventricular overloading. We previously demonstrated that left ventricular function progressively deteriorated, along with myocardial β-adrenergic receptor down-regulation, depletion of catecholamines and an increase in plasma norepinephrine level, during the first week after production of aortic regurgitation (AR) in rabbits. The left ventricular function recovered and, as well, there was reversal of the β-adrenergic receptor down-regulation, repletion of catecholamines and a decrease in plasma norepinephrine concentration, relating to the development of cardiac hypertrophy 4 weeks after the procedure. We hypothesized that baroreceptor-mediated control had some role in the pathophysiology of the compensatory process in this setting. Thus, the present study investigated the sequential changes in baroreceptor-heart rate sensitivity during the progression of and compensatory processes after acute left ventricular overloading.

**Methods**

**Experimental Animals**

The experiments were done in 19 female Japanese white rabbits, aged 3 months and weighing 2.5–3.5 kg. All experiments were performed in accordance with the ‘Guide for the Care and Use of Laboratory Animals’ published by the US National Institutes of Health.

**Production of Aortic Regurgitation (AR)**

AR was induced, as described previously, under anesthetization in 13 rabbits. In brief, aortic pressure was measured by a 5F catheter-tipped manometer (PC-350, Millar Instruments, Houston, TX, USA) inserted from the right carotid artery before production of AR. Aortic valves were perforated with a metallic catheter under fluoroscopic guidance. The successful production of AR was confirmed by auscultation and by a substantial reduction in aortic diastolic pressure. Our laboratory data showed that acute reduction of aortic diastolic pressure predicted subsequent...
left ventricular overloading in this animal model. After the procedure, the carotid artery was ligated and surgical suturing was done. Antibiotics were injected immediately after the procedure and the following day. For the sham operation, the right carotid artery was ligated without inducing AR in the remaining 6 rabbits.

**Measurement of Baroreceptor Sensitivity**

Rabbits were kept in a dark and quiet room for at least 30 min before taking the measurements, which were done between 9.00 h and 10.00 h without anesthesia. Steady-state increases in systemic arterial pressure were used to activate arterial baroreceptors. Phenylephrine hydrochloride was continuously administered intravenously, starting at the dose of 20 μg/kg per min up to 160 μg/kg per min, titrated every 90 sec to induce a gradual increase in systemic arterial pressure using an infusion syringe pump. The infusion was discontinued when the systolic pressure exceeded 200 mmHg. The Ohmeda Finapres 2300 NIBP device (Ohmeda Monitoring Systems, Englewood, CO, USA) was used to measure systemic arterial pressure and heart rate at the distal portion of the rabbit’s front legs, which had been previously shaved. Arterial pressure estimated by this method closely correlated with directly measured pressures in the distal portion of the rabbit’s front legs, which had been previously shaved. Arterial pressure estimated by this method closely correlated with directly measured pressures.

Baroreflex sensitivity was defined as the slope of the regression line relating the RR interval to systolic arterial pressure (msec/mmHg). Arterial pressure more than 160 mmHg was excluded from the analyses. Baroreflex sensitivity was measured sequentially before production of AR, and 1 day, 1 week and 4 weeks after production of AR. Three rabbits were excluded from the analysis because of inappropriate pressure recordings in at least 1 of the 4 measurement points and physical rest was not maintained during the examination in additional 3 rabbits. Complete data were obtained in the remaining 7 rabbits. The same measurements were done in the same manner in the sham-operated rabbits.

**Measurement of Hemodynamic Variables**

Four weeks after production of AR, the animals were anesthetized and ventilated via a tracheal incision. The chest was opened by a mid-sternal incision and the pericardium was dissected. The aortic root pressure was measured by a 5F catheter-tipped manometer inserted from the left carotid artery. Aortic flow was measured by an electromagnetic flowmeter (MFV120, Nihon-Kohden, Tokyo, Japan) placed around the ascending aortic root. The left ventricular pressure was measured by a 3F catheter-tipped manometer (PC-330, Millar Instruments) inserted from an apical incision. The left ventricular short axis diameter was measured by a pair of ultrasonic dimension gauges attached to the anterior and posterior epicardial surfaces.

**Postmortem Morphological Analysis**

After the hemodynamic variables were measured, the rabbits were killed by rapid injection of potassium chloride. The left and right ventricular free walls were isolated in an ice-cold sucrose buffer (0.25 mol/L sucrose, 1 mmol/L KHCO3, 1 mmol/L MgCl2, pH 7.2). Each ventricle was weighed.

**Biochemical Analysis**

Each heart was cleansed as much as possible of endocardium, epicardium, connective tissues and vessels. A small amount of myocardium was stored at −80°C for assay of myocardial norepinephrine concentration by high performance liquid chromatography. Myocardium was minced with scissors in ice-cold sucrose buffer and homogenized in 5 ml of sucrose buffer using the Physcotron homogenizer (Niti-On, Chiba, Japan). The homogenates were filtered through gauze and centrifuged at 7000xg for 10 min at 4°C. The resulting supernatant was centrifuged at 17000xg for 15 min at 4°C. The pellet was resuspended in Tris buffer (100 mmol/L Tris, 5 mmol/L MgCl2, 1 mmol/L EGTA, pH 7.4–7.5) and stored at −80°C until further analysis. The concentration of the membrane protein was determined by a modification of Lowry’s method and adjusted to 0.3 mg/ml for assay of β-adrenergic receptor.

The density of the myocardial β-adrenergic receptors was determined by a radioiodin binding assay using 125I-lodocyanopindolol (specific activity 74 TBq/mmol; Amersham Japan, Tokyo, Japan), as described previously.

Assays were performed in duplicate. For saturation analysis, 100 μl of membrane sample was added to 25 μl of 10 different concentrations of 125I-lodocyanopindolol ranging from 30 to 800 pmol/l with 25 μl of Tris buffer or 25 μl of 1.7 × 10−4 mol/L propranolol. The final volume of 150 μl was incubated for 60 min at 37°C. The reaction was terminated by the addition of 750 μl of ice-cold Tris buffer. Samples were filtered through Whatman GF/C glass fiber filters. The filters were washed rapidly twice by vacuum filtration (Milipore Corporation, Bedford, MA, USA). The radioactivity of the filters was counted in a gamma counter (ARC-600, Aloka, Tokyo, Japan) at an efficiency of 76.5%. Specific binding was determined by subtracting the nonspecific binding in the presence of propranolol from total binding. The number of maximal binding sites and the dissociation constant were calculated from Scatchard analysis.

**Data Analysis**

Hemodynamic data were collected with a thermal array recorder (Nihon-Kohden) at a paper speed of 200 mm/sec. Total forward stroke volume (TSV) and regurgitant volume (RV) were calculated by digitization (MYPAD-A3, Logitec digitizer model K-510, Tokyo, Japan) of the positive and negative components of aortic flow. Stroke volume was obtained by subtracting RV from TSV; the regurgitant fraction (RF) was calculated as follows:

\[ RF (%) = \left(\frac{RV}{TSV}\right) \times 100 \]

Fractional shortening (FS) was the percentage shortening of the left ventricular short axis dimension and was calculated as follows:

\[ FS (%) = \left(\frac{LVDd - LVDs}{LVDd}\right) \times 100 \]

Where LVDd was the left ventricular end-diastolic dimension, and LVDs was the left ventricular end-systolic dimension. All values were expressed as mean ± SD. An unpaired Student’s t-test was performed for comparison between groups. Two-way analysis of variance for repeated measurements, followed by a Fisher’s protected least significant difference as a post-hoc analysis was used to assess the serial changes in baroreceptor sensitivity between the rabbits with AR and the sham-operated rabbits. Linear regression analysis was performed for Scatchard analysis and determination of baroreceptor sensitivity using the least-square method. Statistical significance was set at p<0.05.
Results

The decrease in aortic diastolic pressure immediately after production of AR was 24+10 mmHg. There were no deaths during the observation period. Table 1 shows the hemodynamic data 4 weeks after production of AR. Aortic diastolic pressure was lower in the rabbits with AR than in the sham-operated rabbits. The RF was 52.1±5.8%. Both the end-diastolic and end-systolic dimensions were higher in the rabbits with AR than in the sham-operated rabbits. There was no difference in cardiac output or left ventricular end-diastolic pressure between the 2 groups.

Table 2 shows postmortem morphological data, myocardial ß-adrenergic receptor density and norepinephrine content 4 weeks after production of AR. The left ventricular weight was higher in the rabbits with AR than in the sham-operated rabbits. There was no difference in the maximal binding sites of the ß-adrenergic receptors or dissociation constant and the myocardial norepinephrine content was also similar between the rabbits with AR and the sham-operated rabbits.

Fig 1 shows the representative trace of the arterial pressure using both Finapres and an arterial cannula in the pilot study. There was no difference between the blood pressure measured by Finapres and the arterial pressure directly measured through the catheter placed in the carotid artery, if properly used. There was a close correlation in the blood pressure measurement between the 2 methods (r=0.975). Baroreceptor sensitivity calculated from the direct and indirect methods was also similar. Fig 2 shows the plots of the RR interval as the function of systolic arterial pressure in a rabbit with AR and in a sham-operated rabbit. Baroreceptor sensitivity was 2.32 msec/mmHg in the sham-operated rabbits, whereas it was 0.66 msec/mmHg in the rabbits with AR. Fig 3 shows the serial changes in the baroreceptor sensitivity during the observation period. There was a significant difference in the sensitivity between the AR and sham-operated rabbits (P<0.01). Baroreceptor sensitivity tended to be decreased in the first day after production of AR (0.86±0.27 msec/mmHg).

### Table 1 Hemodynamic Data 4 Weeks After Production of AR

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>AR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>3.18±0.17</td>
<td>3.12±0.23</td>
<td>0.575</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>231±27</td>
<td>234±48</td>
<td>0.841</td>
</tr>
<tr>
<td>Aortic pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>101±17</td>
<td>95±19</td>
<td>0.480</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76±16</td>
<td>56±16</td>
<td>0.018</td>
</tr>
<tr>
<td>Mean</td>
<td>83±16</td>
<td>69±15</td>
<td>0.101</td>
</tr>
<tr>
<td>Cardiac output (ml/min/kg)</td>
<td>165±35</td>
<td>140±29</td>
<td>0.173</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)</td>
<td>8.1±2.4</td>
<td>9.2±0.8</td>
<td>0.314</td>
</tr>
</tbody>
</table>

### Table 2 Morphological and Biochemical Data

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>AR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular weight (g/kg)</td>
<td>1.08±0.12</td>
<td>2.00±0.20</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Bmax (fmol/mg protein)</td>
<td>49.2±16.4</td>
<td>34.1±3.8</td>
<td>p=0.0795</td>
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<tr>
<td>Kd (pmol/L)</td>
<td>78.3±31</td>
<td>65.8±16</td>
<td>p=0.4545</td>
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<tr>
<td>Norepinephrine (μg/g tissue)</td>
<td>1.70±0.33</td>
<td>1.37±0.57</td>
<td>p=0.0591</td>
</tr>
</tbody>
</table>

Bmax and Kd, maximal binding sites and dissociation constant of myocardial ß-adrenoreceptor.
compared with that before production of AR (1.78±0.47 msec/mmHg). The sensitivity was significantly decreased in the first week after production of AR (0.83±0.32 msec/mmHg, p<0.05). Four weeks after production of AR (1.25±0.27 msec/mmHg), there was no difference compared with that before AR. For the sham-operated rabbits, there was no change in the sensitivity during the 4 weeks (before, 1.81±0.71 msec/mmHg; 1 day, 1.85±1.20 msec/mmHg; 1 week, 1.57±0.68 msec/mmHg; 4 weeks, 1.92±1.25 msec/mmHg).

Discussion

This study demonstrated that baroreceptor-heart rate reflex sensitivity decreased as early as 1 day after induction of AR. This alteration persisted over the first week, together with hemodynamic abnormalities. There was a recovery in the baroreceptor-heart rate sensitivity 4 weeks after induction of AR, which was associated with the development of cardiac hypertrophy and the reversal of the hemodynamic abnormalities, as well as the myocardial Ԑ-adrenergic receptor density and norepinephrine content.

Reliability of Blood Pressure Measurement Using Finapres

In this study, we used noninvasive equipment instead of direct measurement to monitor the blood pressure during perturbation. It is difficult to maintain an arterial cannula for as long as 4 weeks in rabbits, and the invasive procedure itself could alter the sensitivity of the reflex. Previous studies have showed that blood pressure measured by Finapres correlated well with that measured simultaneously in a brachial or radial artery.22,27–30 One study, however, reported that there was a significant underestimation of the increase in systolic blood pressure during vasoconstriction by phenylephrine infusion.22 This underestimation occurred when the blood pressure exceeded 200 mmHg. Although some studies employed mean arterial pressure to assess baroreceptor sensitivity, we assessed the sensitivity using systolic arterial pressure according to Aitken et al.31 who reported that systolic arterial pressure was preferable when assessing baroreceptor sensitivity using Finapres. Our data showed that there was a close correlation between baroreceptor sensitivity determined by Finapres and that determined by a direct measurement of arterial pressure.

Assessment of Baroreflex Sensitivity

In this study, we used phenylephrine-induced vasoconstriction by the steady-state increment to assess baroreceptor sensitivity. There are many methods for assessing baroreflex sensitivity including head-up tilt, Valsalva’s maneuver, vasodilatation induced by nitroprusside, lower body negative pressure, and neck chamber suction or pressure. Isolated perfusion of the carotid sinus is employed in experimental animals. Head-up tilt, Valsalva’s maneuver and lower body negative pressure reflect cardiopulmonary receptor-mediated control rather than arterial receptor-mediated control. We employed the most standard method in this regard. Heart rate response during phenylephrine-induced blood pressure increase may reflect arterial baroreceptor-mediated vagal afferent traffic32 although abnormalities in the baroreceptors itself, altered central mechanisms, impaired transmission of sympathetic and parasympathetic efferent neurons or end-organ responsiveness may be also involved. The cardiopulmonary receptor-mediated reflex cannot be excluded since vasoconstriction-induced changes in cardiac filling pressure occur simultaneously. However, receptor-mediated sensitivity should be less in heart failure than control despite the fact that elevation of the filling pressure must be higher in heart failure. This suggests that the response to phenylephrine-induced vasoconstriction mostly reflects arterial baroreceptor-mediated control. We employed a steady-state method to assess the reflex in order to minimize the respiratory effect on blood pressure. Niebauer and Zucker showed that baroreceptor gain was lower in dogs with chronic volume overload induced by aortocaval fistula than in control dogs for both the step and lowest ramp rate pressure changes.15 A steady-state method may have an advantage in assessing both vagal and sympathetic traffic whereas a ramp method essentially reflects vagal traffic.33 Although several previous animal studies were undertaken in the anesthetized state,6,15,16 we assessed baroreceptor-mediated reflex in the awake state to mini-
mimize the potential effect of anesthetization. We also took a great care with the conditions in which the experiments were done; that is, in a dark quiet room after a prolonged interval for acclimatization.

**Baroreceptor Sensitivity in Congestive Heart Failure**

It is well known that baroreceptor sensitivity is attenuated in congestive heart failure. Higgins et al reported that the bradycardia response to an increase in blood pressure caused by infusion of phenylephrine was reduced in dogs with heart failure produced by tricuspid regurgitation and pulmonic stenosis. Niebauer and Zucker examined the carotid sinus-discharge relationship by perfusing the carotid sinus with controlled pressure in dogs with aortocaval fistula, and found that the baroreceptor control of the heart rate remained depressed for as long as 8 months after reversal of heart failure induced by aortocaval fistula. By contrast, Olivier and Stephenson observed that the range and gain of baroreceptor control of blood pressure were substantially reduced as early as 3 days after induction of heart failure using rapid ventricular pacing and were partially restored within 7 days after cessation of the rapid ventricular pacing for 21 days in conscious dogs. Grima et al reported that baroreceptor-heart rate sensitivity assessed by hypotensive and hypertensive responses induced by phenylephrine and nitroprusside administration recovered as early as 48 h after cessation of rapid ventricular pacing along with hemodynamics and plasma norepinephrine, but ventricular dilatation persisted for 4 weeks. Previous studies were not concerned about the reversal of altered baroreceptor-heart rate sensitivity along with the compensatory process after acute left ventricular overloading. The present study demonstrated that alterations in the baroreceptor-mediated heart rate control were reversible, even in the compensatory hypertrophy after the induction of AR.

The cause-effect relationship between baroreceptor sensitivity and hemodynamics remains uncertain. Improvement of the hemodynamic status relating to cardiac hypertrophy may be responsible for the decrease in sympathetic tone, which results in a restoration of baroreceptor sensitivity. Alternatively, it is possible that the reversal of baroreceptor function inhibited further sympathetic overactivity, causing reversal of myocardial ß-adrenergic receptor down-regulation, norepinephrine depletion, and hemodynamic alterations. There is some evidence to support that altered cardiopulmonary reflex-mediated control has some role in the development of congestive heart failure. Kinugawa et al reported that abnormalities in the cardiopulmonary reflex appeared before gross cardiac dilatation was evident and before sympathetic function was augmented. These authors concluded that the abnormal cardiopulmonary reflex was responsible for neurohumoral activation and this may have been an important mechanism in the sympathetic activation in heart failure. By contrast, another study showed that plasma norepinephrine level was comparable between dogs with sinoaortic denervation and control dogs after induction of heart failure using chronic rapid pacing. There was also no difference in hemodynamics, or in the progression or severity of heart failure between the 2 groups. Although we cannot determine with certainty the role of the baroreceptor-mediated control in the progression and compensation after acute left ventricular overloading, alterations in the function may be, at least in part, related to the compensatory processes.

**Clinical Implications**

Alterations in baroreceptor sensitivity are reversible in congestive heart failure. Therapeutic interventions restoring that sensitivity are one of the promising future directions in patients with heart failure, although we cannot extrapolate animal data directly to human congestive heart failure. Long-term changes beyond 4 weeks after the left
ventricular overload, which were not determined in the present study, are another concern.

References
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