Hypertrophic cardiomyopathy (HCM) characteristically has severe myocardial hypertrophy, predominantly involving the interventricular septum of the nondilated left ventricle. Echocardiography is useful for the differential diagnosis of HCM, but it can be difficult to distinguish HCM from hypertensive heart disease (HHD) with left ventricular (LV) hypertrophy. Although concentric LV hypertrophy is observed in patients with HCM and sometimes HCM patients present with hypertension as a complication, asymmetrical interventricular hypertrophy (ASH) is also observed in HHD. Recently, ultrasonic myocardial tissue characterization by integrated backscatter has provided additional information about the pathological changes; however, it is still unable to completely differentiate HCM from HHD.

It has been reported that various neurohumoral systems are accelerated in patients with HHD or HCM. In HHD, the plasma concentrations of catecholamines and norepinephrine are increased, and other trophic neurohumoral factors, such as angiotensin II and endothelin-1, are also increased. Moreover, it has been reported that atrial natriuretic peptide (ANP) is increased in patients with hypertension. With regard to HCM patients, it has been reported that plasma concentrations of catecholamines and angiotensin II are increased, as well as those of ANP and brain natriuretic peptide (BNP). However, there are no data on the differences in the neurohumoral profiles of HCM and HHD patients.

Thus, in the present study we measured the plasma concentrations of catecholamines (epinephrine and norepinephrine), angiotensin II, endothelin-1, and natriuretic peptides (ANP and BNP) in patients with HCM and HHD without LV dysfunction to clarify the neurohumoral profile of HCM and its clinical implications.
Determination of Neurohumoral Factors

We determined the plasma concentrations of neurohumoral factors (epinephrine, norepinephrine, angiotensin II, endothelin-1, ANP and BNP) after a 15-min rest in the supine position. The blood assayed for plasma ANP, BNP and endothelin-1 was transferred to a chilled tube containing EDTA and aprotinin, and then centrifuged at 3,000 rpm for 15 min at 4°C. Plasma ANP and BNP were determined using the Shionoria RIA and the S-1215 RIA kits, respectively. Plasma endothelin-1 was determined by radioimmunoassay using a specific rabbit anti-endothelin-1 serum. The blood assayed for plasma epinephrine, norepinephrine and angiotensin II was transferred to a chilled tube containing EDTA and apronin, and then centrifuged at 3,000 rpm for 15 min at 4°C. Plasma epinephrine and norepinephrine were determined by high-performance liquid chromatography according to the diphenylethylene diamine method, as previously described, and plasma angiotensin II was determined by radioimmunoassay according to the polyethylene glycol method, as previously described.

Statistical Analysis

All data were expressed as the mean ± SEM. Comparisons between 2 groups were determined by the paired or unpaired Student’s t-test as appropriate, and the values in multiple groups were assessed for significant differences by one-way ANOVA with Fisher’s post-hoc test. Linear regression analysis was used to determine the correlation between continuous variables. A p-value <0.05 was considered as statistically significant.

Table 1  Baseline Characteristics of the HCM Patients, HHD Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=15)</th>
<th>HHD patients (n=35)</th>
<th>HCM patients (n=40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56±2*</td>
<td>64±2*</td>
<td>66±2*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>M/F</td>
<td>9/6</td>
<td>23/12</td>
<td>26/14</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>72±4</td>
<td>68±2</td>
<td>68±2</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>127±7/74±4.5</td>
<td>139±4/77±2</td>
<td>136±3/73±2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*p<0.05 vs controls.

NS, not significant; HCM, hypertrophic cardiomyopathy; HHD, hypertensive heart disease.
Results

Echocardiographic Findings (Fig 1)
The IVSth and PWth were significantly higher in HCM patients (15.2±0.7 and 13.9±0.5 mm, respectively) than in HHD patients (12.2±0.3 and 11.4±0.3 mm, respectively) and controls (9.8±0.3 and 9.3±0.4 mm, respectively). The LVDd and LVEF were not significantly different among the 3 groups (data not shown). The LVMI was significantly higher in patients with HCM than in patients with HHD and controls (219.6±11.4, 166.3±7.2 and 104.7±7.4 g/m², respectively, p<0.05). LV diastolic dysfunction was observed in 24 HCM patients. There was no significant difference in the LVMI between HCM patients with and without LV diastolic dysfunction (224.1±17.6 and 214.1±13.7 g/m², respectively).

Neurohumoral Factors (Fig 2)
Although there were no significant differences in the plasma epinephrine concentrations among the 3 groups (data not shown), the plasma norepinephrine concentration was significantly higher in HCM patients than in HHD patients and controls, and it was significantly higher in HHD patients than in controls. Plasma angiotensin II (data not shown) and endothelin-1 concentrations were only slightly higher in HCM patients than in HHD patients and controls.

Regarding the natriuretic peptides, plasma ANP concentration ranged from 5.0 to 140 pg/ml in 35 HHD patients, and from 3.0 to 250 pg/ml in 40 HCM patients; plasma...
BNP ranged from 6.7 to 424 pg/ml in 35 HHD patients, and from 5.7 to 1,690 pg/ml in 40 HCM patients. The plasma concentrations of both ANP and BNP were significantly higher in HCM patients than in HHD patients and controls, and significantly higher in HHD patients than in controls. Plasma ANP and BNP were 1.7-fold and 3.3-fold, respectively, in HCM patients compared with HHD patients and 2.6-fold and 13.5-fold, respectively, compared with controls. When the plasma ANP concentration was 50 pg/ml or more, we could distinguish HCM from HHD with a sensitivity of 55% and a specificity of 80%, and when the plasma BNP concentration was 100 pg/ml or more, we could distinguish HCM from HHD with a sensitivity of 60% and a specificity of 83%.

Correlations Between LVMI and Neurohumoral Factors
Among the neurohumoral factors investigated in the present study, we evaluated the correlations between LVMI and norepinephrine, ANP and BNP because these were significantly higher in HCM patients than in the other 2 groups, but there were no significant correlations.

Correlations Between the Intraventricular Pressure Gradient and Neurohumoral Factors (Fig 3)
We also evaluated the correlations between the intraventricular pressure gradient and neurohumoral factors in the HCM patients. Although the plasma ANP concentration did not correlate with the intraventricular pressure gradient, that of norepinephrine had a weak, but significant correlation (r=0.33, p<0.05). In contrast, the plasma BNP concentration strongly correlated with the intraventricular pressure gradient (r=0.83, p<0.01).

LV Diastolic Dysfunction and Neurohumoral Factors (Fig 4)
We examined the relationship between the neurohumoral factors and LV diastolic dysfunction in HCM patients. There were no significant differences in the plasma concentrations of epinephrine, norepinephrine, angiotensin II or endothelin-1 between HCM patients with and without LV diastolic dysfunction. However, the plasma ANP and BNP concentrations in HCM patients with LV diastolic dysfunction (87.8±14.4 pg/ml and 349.0±86.7 pg/ml, respectively) were significantly higher than those in patients without LV diastolic dysfunction (35.4±7.4 pg/ml and 94.3±32.9 pg/ml, respectively).

Coronary Sinus Sampling (Protocol 2, Fig 5)
Finally, we examined the transcardiac production of neurohumoral factors in 12 HCM patients (6 HOCM and 6 HNCM) and 10 controls. There were no significant increases in the plasma concentrations of epinephrine, norepinephrine, angiotensin II or endothelin-1 in the samples.
collected from the coronary sinus compared with those in samples collected from the aortic root in the HOCM patients, HNCM patients or controls. However, significantly higher plasma concentrations of ANP and BNP were found in samples collected from the coronary sinus of both HCM patients and controls. The plasma ANP and BNP concentrations were significantly higher in the coronary sinus of the HCM patients (both HOCM and HNCM) than in the controls. Furthermore, the plasma BNP concentration, but not that of ANP, was significantly higher in the coronary sinus of HOCM patients (975.3±288.7 pg/ml) than in the HNCM patients (464.0±53.7 pg/ml). Transcardiac production (coronary sinus–aortic root) of BNP, but not ANP, was significantly higher in the HCM patients (450.8±63.1 pg/ml) than in the HNCM patients (279.1±24.9 pg/ml, p<0.05).

**Discussion**

Plasma concentrations of norepinephrine, ANP and BNP, but not epinephrine, angiotensin II or endothelin-1, were significantly higher in the present HCM patients than in the HHD patients and controls, and the plasma BNP concentration strongly correlated with the intraventricular pressure gradient, but not with LVMI. In addition, plasma concentrations of ANP and BNP were significantly higher in HCM patients with LV diastolic dysfunction than in patients without. Finally, the increases in the plasma BNP concentration in the coronary sinus were significantly higher in HOCM than in HNCM patients. These results describe the neurohumoral profiles of HCM patients in comparison with those of HHD patients and controls, and we suggest the possible mechanism of the increases in the plasma concentrations of ANP and BNP in HCM patients.

It has been previously reported that plasma neurohumoral factors, such as catecholamines and endothelin-1, are increased in HCM patients, but in the present study norepinephrine, but not epinephrine or endothelin-1, was increased in the HCM patients. The natriuretic peptides, especially BNP, are reported to have high predictive characteristics as a diagnostic test for heart disease and we found that their concentrations were remarkably increased in the patients with HCM compared with the HHD patients and controls, which is consistent with the previous studies. The increase in the plasma concentrations of ANP and BNP is the most significant difference in the neurohumoral profile of HCM patients compared with that of the HHD patients, and we were able to use these results to diagnose HCM. Thus, the plasma concentrations of natriuretic peptides, especially BNP, could serve as noninvasive markers of HCM.

The mechanisms of the increases in the plasma concentrations of ANP and BNP in HCM patients have not been clarified, although the size of the LV cavity has been reported as a major determinant of the plasma concentration of BNP in HCM patients. Nishigaki et al showed that the plasma BNP concentration was independent of echocardiographic and hemodynamic data. Hamada et al also reported that plasma ANP and BNP concentrations were significantly higher in HOCM patients than in HNCM patients, and that the increase in BNP was independent of echocardiographic and hemodynamic data.

Where is the source of ANP in HCM patients? ANP is mainly produced in the atrium and BNP is produced in the ventricle. In patients with congestive heart failure, however, the concentration of ANP, as well as that of BNP, increase remarkably and ANP production in the ventricle is significant in comparison with that of the atrium. In the present study, a significant relationship was observed between plasma ANP concentration and LV diastolic dysfunction, but not with the left atrial dimension (data not shown). Additionally, ANP mRNA has been detected in the ventricles of patients with HCM. Thus, the high plasma concentrations of ANP detected in HCM patients may have originated mainly from the left ventricle.

We cannot explain whether intraventricular obstruction and LV diastolic dysfunction account for the high plasma concentrations of ANP and BNP in all HCM patients. Although the plasma ANP and BNP concentrations were high in all patients with HOCM and LV diastolic dysfunction, high plasma concentrations of ANP (≥50 pg/ml) and BNP (≥100 pg/ml) were also observed in 2 and 4 patients, respectively, among the 15 HNCM patients with normal LV diastolic function in this study. Thus, in these HCM patients, high plasma concentrations of ANP and BNP seem to be independent of intraventricular obstruction or...
LV diastolic dysfunction. Takemura et al showed that, in a selected population of HCM patients, the ventricular ANP gene expresses in response to disease-specific changes (eg, myocardial fiber disarray, hypertrophy of myocytes and fibrosis) rather than as an adaptive response.3 There might be unknown causes of the high plasma concentrations of ANP and BNP in some HCM patients and further studies are needed.

Study Limitations
First, we did not perform myocardial biopsy or genetic analysis to diagnose HCM. However, neither myocardial biopsy nor genetic analysis is necessary for a differential diagnosis of HCM18,34 and we carefully reviewed the family history, history of hypertension, echocardiographic findings and results of cardiac catheterization to establish the differential diagnosis. We believe, therefore, that our diagnosis of HCM and HHD was accurate. Second, we evaluated diastolic function using only echocardiographic parameters, although echocardiography is widely used to evaluate diastolic function. Instead, other parameters, such as LV end-diastolic pressure and minimum LV dp/dt, determined by cardiac catheterization, are used, but these methods are invasive and are generally difficult to perform routinely. In this study, diagnostic cardiac catheterization had been performed before the study and blood sampling was not done at the that time. Thus, we were not able to use LV end-diastolic pressure and minimum LV dp/dt in this study. However, other recent studies of diastolic function have used echocardiographic parameters as the standard parameters of diastolic function.24,35 Finally, the incorporation of plasma ANP and BNP would be necessary to determine the differences in the neurohumoral profiles of HCM and HHD without LV systolic dysfunction. We excluded patients with low LVEF because LV systolic dysfunction per se has an effect on neurohumoral activation.

Conclusion
We demonstrated that the plasma concentrations of norepinephrine, ANP and BNP were significantly increased in HCM patients compared with HHD patients or controls, and that the increases in plasma ANP concentration might reflect LV diastolic dysfunction whereas that of BNP might reflect both the intraventricular pressure gradient and LV diastolic dysfunction. Our findings suggest the importance of measuring the plasma concentrations of natriuretic peptides, especially BNP, in the clinical evaluation of patients with HCM.

References


