Increased plasma levels of adrenomedullin in patients with hypertrophic cardiomyopathy: its relation to endothelin-1, natriuretic peptides and noradrenaline

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1. The aim of this study was to elucidate the pathophysiological role of adrenomedullin and the relation between adrenomedullin and other hormones in patients with hypertrophic cardiomyopathy.

2. Fourteen patients with hypertrophic obstructive cardiomyopathy (HOCM), 26 patients with hypertrophic non-obstructive cardiomyopathy (HNCM) and 14 normal control subjects participated in this study. Radioimmunoassay for plasma adrenomedullin concentration was performed with adrenomedullin-M antibody. Plasma levels of endothelin-1, atrial and brain natriuretic peptides and noradrenaline were also measured.

3. Plasma levels of adrenomedullin were higher in patients with hypertrophic cardiomyopathy (8.43 ± 3.73 pmol/l) than in normal controls (5.24 ± 0.44 pmol/l, P < 0.005). There was no significant difference between HOCM and HNMC patients. There was a weak correlation between plasma levels of adrenomedullin and total 12-lead QRS voltage in patients with hypertrophic cardiomyopathy (r = 0.323, P < 0.05).

4. Plasma levels of endothelin-1, atrial and brain natriuretic peptides were higher in hypertrophic cardiomyopathy than in normal controls. Endothelin-1 showed no significant difference between HOCM and HNMC patients, but atrial and brain natriuretic peptides were higher in HOCM than in HNMC patients. There was a positive correlation between plasma levels of adrenomedullin and endothelin-1 (r = 0.575, P < 0.0001), but no correlation between plasma levels of adrenomedullin and atrial natriuretic peptide, brain natriuretic peptide and noradrenaline.

5. Our results indicate that adrenomedullin may play an important role to maintain haemodynamics in patients with hypertrophic cardiomyopathy, and its action may be related to endothelin-1 but independent of atrial natriuretic peptide, brain natriuretic peptide and noradrenaline.

INTRODUCTION

Adrenomedullin is a novel peptide recently isolated from phaeochromocytoma, eliciting vasorelaxant activity, which is the strongest among all known peptides [1]. Adrenomedullin is shown to circulate in the blood, and the plasma levels are higher in patients with hypertension [2], renal failure [2] and heart failure [3–5] than in normotensive controls. In addition, previous reports indicated that plasma levels of adrenomedullin correlated with plasma levels of noradrenaline, atrial natriuretic peptide (ANP) or brain natriuretic peptide (BNP) in patients with heart failure [3, 5]. Thus, adrenomedullin seems to act against the elevation of blood pressure and volume expansion in these conditions. However, as pointed out in a review by Richards et al. [6], it remains to be determined whether there are complementary interactions between adrenomedullin and other vasoactive peptides or hormones such as endothelin-1 (ET-1), natriuretic peptides and so on in various diseases.

The source of circulating adrenomedullin in humans is still unknown [7]. Recently, Sugo et al. [8] reported that adrenomedullin was produced from cultured vascular endothelial cells as well as vascular smooth muscle cells [9], and the secretion rate of adrenomedullin from endothelial cells was almost comparable to that of ET-1 [8]. The production of adrenomedullin is markedly augmented by various cytokines and hormones [9–11]. In addition, the existence of an adrenomedullin-specific receptor was confirmed in vascular smooth muscle cells [12] and aortic endothelial cells [13]. These experimental
data suggest that adrenomedullin functions as an autocrine and/or paracrine regulator.

Hypertrophic cardiomyopathy is known to have not only a marked hypertrophy of the left ventricle, but also structurally altered intramural coronary arteries [14, 15]. An association between marked left ventricular hypertrophy and disorder of intramural coronary arteries in hypertrophic cardiomyopathy may cause severe prolonged myocardial ischaemia [16, 17], and contribute to the formation of marked myocardial fibrosis [14]. Under these conditions, adrenomedullin may act as a regulator in the vascular wall of hypertrophic cardiomyopathy.

To confirm this hypothesis, we measured plasma adrenomedullin in patients with hypertrophic cardiomyopathy. In addition, we examined whether plasma levels of adrenomedullin were related to plasma levels of ET-1, ANP, BNP and noradrenaline in hypertrophic cardiomyopathy.

PATIENTS AND METHODS

Study patients

Forty patients with hypertrophic cardiomyopathy and 14 normal control subjects participated in this study after giving informed consent. The study was approved by the ethics committee of our institution. The diagnosis of hypertrophic cardiomyopathy was made as reported previously using echocardiography and cardiac catheterization [18]. Patients who had associated cardiac diseases, such as coronary artery disease, valvular heart disease and hypertension, and patients with musculoskeletal injury or renal dysfunction, were excluded from this study. Patients with hypertrophic cardiomyopathy were subdivided into two groups; 14 had hypertrophic obstructive cardiomyopathy (HOCM) and 26 had hypertrophic non-obstructive cardiomyopathy (HNCM). The obstructive type was diagnosed when a patient had a pressure gradient above 20 mmHg without provocation in the left ventricular outflow tract. The mean of left ventricular pressure gradient in the 14 patients with HOCM was 97.3 ± 59.8 mmHg (range 36–213 mmHg).

Fourteen normal control subjects consisted of eight normal volunteers and six subjects who underwent cardiac catheterization due to chest pain of unknown aetiology and whose coronary arteriograms were normal.

Estimation of left ventricular hypertrophy

Left ventricular wall thickness in patients with hypertrophic cardiomyopathy is not symmetrical. In addition, the shape of the left ventricular cavity is not spheroidal in almost all the patients with hypertrophic cardiomyopathy. Thus, left ventricular mass cannot be accurately determined by echocardiography. Therefore, in this study we measured total 12-lead QRS voltage, which was considered to be the most sensitive of various electrocardiographic indicators [19] for left ventricular hypertrophy.

M-mode echocardiography

Echocardiographic measurements were carried out using an SSD-9000 echocardiograph with a 3.5 MHz transducer (ALOKA Inc, Tokyo, Japan). M-mode echocardiographic recording was carried out after the cardiac anatomy was visualized by two-dimensional echocardiography. Interventricular septal thickness and left ventricular posterior wall thickness, left atrial dimension, left ventricular internal dimensions at end-diastole and end-systole and left ventricular fractional shortening were measured according to the criteria of the American Society of Echocardiography [20]. Cardiac output was determined echocardiographically as reported previously [21] using the method of Teichholz et al. [22], and total peripheral resistance was calculated from the following formula: total peripheral resistance (dyne s cm \(^{-5}\)) = mean blood pressure × 1332 × 60/cardiact index.

Blood sampling

Fifteen millilitres of blood were withdrawn through the antecubital vein in the morning after 30 min of supine rest to measure adrenomedullin, ET-1, ANP, BNP, adrenaline and noradrenaline. Blood was immediately transferred into chilled glass tubes, containing disodium-EDTA (1 mg/ml) and aprotinin (500 units/ml), and was centrifuged immediately at 4°C. The plasma was frozen and stored at −80°C until assay.

Assay for plasma adrenomedullin concentration

The radioimmunoassay for adrenomedullin was performed with adrenomedullin-M antibody, using the method reported previously [3, 23, 24]. Briefly, the radioimmunoassay buffer consisted of 0.05 mol/l sodium phosphate buffer (pH 7.4) containing 0.5% BSA, 0.5% Triton X-100, 0.08 mol/l sodium chloride, 0.025 mol/l disodium-EDTA, 0.05% sodium azide and 500 kallikrein inhibiting units/ml aprotinin. One hundred microlitres of the dissolved plasma extract were subjected to a specific radioimmunoassay for human adrenomedullin, as reported previously [24]. The cross-reactivities of an anti-human adrenomedullin antiserum used in this radioimmunoassay were 100% with human adrenomedullin and a C-terminal carboxyl structure (human adrenomedullin-COOH), 100% with human adrenomedullin-(1-51)-COOH, and less than 0.5% with human adrenomedullin-(13-52). The intra- and interassay coefficients of variation for this assay were 5.0% and 4.8%, respectively.
Adrenomedullin in hypertrophic cardiomyopathy

Assays for other hormones

Radioimmunoassay was performed to measure plasma levels of ET-1 (using rabbit anti-ET-1 antiseraum: Peninsula Laboratories Inc., Belmont, CA, U.S.A.), ANP (Shiono RIA assay kit; Shionogi Co., Ltd., Osaka, Japan) and BNP (S-1215, Shionogi Co., Ltd.). Plasma levels of adrenaline and noradrenaline were determined by HPLC.

Statistical analysis

Data are expressed as means ± SD. The statistical evaluation was performed by one-way analysis of variance with subsequent Scheffe’s multiple range tests. Correlation coefficients were calculated by linear regression analysis. A P value <0.05 was considered statistically significant.

RESULTS

Clinical profiles of the subjects

Table 1 shows the clinical profiles of normal control subjects and patients with hypertrophic cardiomyopathy. There were no significant differences in age, heart rate, mean blood pressure and serum creatinine among normal control subjects and patients with HOCM and HNCM. Two patients with HOCM and three with HNCM were at class III of functional status estimated by New York Heart Association class.

Haemodynamic parameters determined electrocardiographically and echocardiographically

As shown in Table 1, there was no significant difference in total 12-lead QRS voltage between HOCM and HNCM patients. Table 2 indicates that both interventricular septal thickness and left ventricular posterior wall thickness were greater in HOCM and HNCM patients than in normal control subjects. Left ventricular dimensions were smaller in patients with HOCM than in normal control subjects and patients with HNCM. Left atrial dimension was greater in HOCM and HNCM patients than in normal control subjects. Fractional shortening was greater in patients with HOCM than in HNCM patients and normal control subjects. Stroke index and cardiac index were smaller in HOCM and HNCM patients than in normal control subjects, whereas total peripheral resistance was greater.

Plasma levels of adrenomedullin

As shown in Fig. 1, the plasma level of adrenomedullin was greater in patients with hypertrophic cardiomyopathy than in normal control subjects. However, there was no significant difference between HOCM and HNCM patients (Table 3). In addition, there was no significant difference in the plasma level of adrenomedullin between patients with hypertrophic cardiomyopathy in NYHA class III (8.37 ± 1.07 pmol/l) and in NYHA class I or II (8.44 ± 3.99 pmol/l). As shown in Fig. 2, there was a significant correlation between the plasma level of adrenomedullin in patients with hypertrophic cardiomyopathy and the NYHA class. Patients in NYHA class III had significantly higher plasma levels of adrenomedullin than patients in NYHA class I or II.

Table 1. Baseline characteristics of normal control subjects, and patients with HOCM and HNCM. Values are means ± SD. Statistical significance: *P<0.001 compared with control subject. Abbreviation: NYHA, New York Heart Association.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age (years)</th>
<th>Heart rate (beats/min)</th>
<th>Mean blood pressure (mmHg)</th>
<th>Serum creatinine (mg/dl)</th>
<th>NYHA class I or II/III</th>
<th>Total 12-lead QRS voltage (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>52.8 ± 15.4</td>
<td>65 ± 8</td>
<td>95 ± 5</td>
<td>0.9 ± 0.3</td>
<td>—</td>
<td>158.6 ± 33.1</td>
</tr>
<tr>
<td>HOCM</td>
<td>14</td>
<td>56.6 ± 13.9</td>
<td>69 ± 7</td>
<td>94 ± 7</td>
<td>1.0 ± 0.2</td>
<td>12/2</td>
<td>272.3 ± 93.7*</td>
</tr>
<tr>
<td>HNCM</td>
<td>26</td>
<td>58.9 ± 13.1</td>
<td>63 ± 6</td>
<td>96 ± 11</td>
<td>1.0 ± 0.3</td>
<td>23/3</td>
<td>242.3 ± 75.9*</td>
</tr>
</tbody>
</table>

Table 2. Echocardiographic data of normal control subjects, and patients with HOCM and HNCM. Values are means ± SD. Statistical significance: *P<0.05, †P<0.01, ‡P<0.001 compared with control subjects; §P<0.01 compared with HOCM. Abbreviations: IVST, interventricular septal thickness; PWT, posterior wall thickness; LVDd, left ventricular dimension at end-diastole; LVDs, left ventricular dimension at end-systole; LAD, left atrial dimension; FS, fractional shortening; SI, stroke index; CI, cardiac index; TPR, total peripheral resistance.

<table>
<thead>
<tr>
<th></th>
<th>IVST (mm)</th>
<th>PWT (mm)</th>
<th>LVDd (mm)</th>
<th>LVDs (mm)</th>
<th>LAD (mm)</th>
<th>FS (%)</th>
<th>SI (ml beat⁻¹ m⁻²)</th>
<th>CI (l min⁻¹ m⁻²)</th>
<th>TPR (dyne s cm⁻⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.7 ± 0.9</td>
<td>7.8 ± 0.9</td>
<td>50.4 ± 2.9</td>
<td>31.6 ± 3.0</td>
<td>33.8 ± 4.5</td>
<td>37.1 ± 6.8</td>
<td>52.1 ± 12.5</td>
<td>3.29 ± 0.62</td>
<td>2384 ± 492</td>
</tr>
<tr>
<td>HOCM</td>
<td>21.0 ± 3.5†</td>
<td>13.6 ± 3.5‡</td>
<td>41.9 ± 5.18</td>
<td>22.6 ± 4.6‡</td>
<td>44.5 ± 5.7‡</td>
<td>46.3 ± 6.0‡</td>
<td>37.6 ± 5.7‡</td>
<td>2.60 ± 0.43†</td>
<td>2990 ± 698†</td>
</tr>
<tr>
<td>HNCM</td>
<td>18.6 ± 4.6‡</td>
<td>12.2 ± 2.9‡</td>
<td>47.5 ± 5.15</td>
<td>30.6 ± 7.25</td>
<td>43.5 ± 6.6‡</td>
<td>36.4 ± 8.7‡</td>
<td>39.4 ± 9.9*</td>
<td>2.66 ± 0.53†</td>
<td>2969 ± 532</td>
</tr>
</tbody>
</table>

Download the data table as: **CSV**
adrenomedullin and total 12-lead QRS voltage in patients with hypertrophic cardiomyopathy \((P<0.05, r = 0.323)\).

**Plasma levels of ET-1, ANP, BNP, adrenaline and noradrenaline**

As shown in Fig. 3 and Table 3, plasma levels of ET-1, ANP and BNP were higher in hypertrophic cardiomyopathy than in normal control subjects. Of these peptides, there was no significant difference in the level of ET-1 between HOCM and HNCM patients, but ANP and BNP levels were greater in HOCM than in HNCM patients. There were no significant differences in plasma levels of adrenaline and noradrenaline between patients with hypertrophic cardiomyopathy and normal control subjects.

**Relation between plasma levels of adrenomedullin and other humoral factors**

Figure 4 shows the relation between plasma levels of adrenomedullin and plasma levels of ET-1. There was a significant correlation between the two variables \((P<0.0001, r = 0.575)\). However, as shown in Fig. 5, there were no significant correlations between plasma levels of adrenomedullin and plasma levels of noradrenaline, ANP or BNP. There were also no significant correlations between ET-1 and ANP or BNP. There was a good relationship between plasma levels of ANP and BNP in patients with hypertrophic cardiomyopathy \((P<0.0001, r = 0.881)\).

**DISCUSSION**

The present study documents for the first time that the plasma levels of adrenomedullin are markedly elevated in patients with hypertrophic cardiomyopathy. In addition, there is no significant difference in plasma levels of adrenomedullin between HOCM and HNCM. Furthermore, plasma levels of adrenomedullin correlate well with those of ET-1, but there are no significant correlations between plasma levels of adrenomedullin and ANP, BNP, adrenaline or noradrenaline in patients with hypertrophic cardiomyopathy.

**Source of circulating adrenomedullin**

The highest concentration of immunoreactive adrenomedullin in humans was present in adrenal medulla, and relatively high concentrations were present in atrium, lung, pancreas and small intestine, and smaller amounts in brain and ventricle [24]. On the other hand, high levels of adrenomedullin mRNA were found in ventricle, kidney and lung as well as in adrenal medulla [25]. Immunohistochemi-

<p>| Table 3. Neurohumoral factors in normal control subjects, and patients with HOCM and HNCM. Values are means ± SD. Statistical significance: *P&lt;0.05, **P&lt;0.02, ***P&lt;0.001 compared with control subjects; $P&lt;0.0002$ compared with HOCM. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Adrenomedullin (pmol/L)</th>
<th>ET-1 (pg/ml)</th>
<th>ANP (pg/ml)</th>
<th>BNP (pg/ml)</th>
<th>Plasma adrenaline (pg/ml)</th>
<th>Plasma noradrenaline (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.2±0.4</td>
<td>1.47±0.28</td>
<td>10.7±6.3</td>
<td>6.3±2.7</td>
<td>37.3±25.6</td>
</tr>
<tr>
<td>HOCM</td>
<td>9.7±5.6†</td>
<td>2.68±0.92‡</td>
<td>99.3±74.7‡</td>
<td>430.6±295.7§</td>
<td>40.7±34.7</td>
</tr>
<tr>
<td>HNCM</td>
<td>7.8±1.9†</td>
<td>2.31±0.55†</td>
<td>39.9±28.2‡§</td>
<td>211.9±299.5%§</td>
<td>35.4±48.1</td>
</tr>
</tbody>
</table>
Adrenomedullin in hypertrophic cardiomyopathy

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

NC
(n=7)
HCM
(n=35)

Fig. 3. Comparison of plasma level of ET-1 in normal control subjects (NC) and patients with hypertrophic cardiomyopathy (HCM)

Pathophysiological role of adrenomedullin in hypertrophic cardiomyopathy

Plasma levels of adrenomedullin have been reported to be elevated in patients with heart failure in a dependent NYHA manner [5]. In our study, however, the elevation in plasma levels of adrenomedullin may not be related to the functional status. Thus, the elevated plasma levels of adrenomedullin may be related to hypertrophic cardiomyopathy itself.

The exact mechanism of elevation of plasma levels of adrenomedullin in hypertrophic cardiomyopathy remains unknown. Our results indicate that the extent of left ventricular hypertrophy reflected by total 12-lead QRS voltage is weakly
related to plasma adrenomedullin levels. It is recognized that electrocardiographic indices of left ventricular hypertrophy are poorer than echocardiographic indices. Therefore, the correlation between plasma levels of adrenomedullin and total 12-lead QRS voltage might be weak. A recent study indicates that plasma levels of ANP and BNP may be a marker of left ventricular hypertrophy [26]. In the present study, despite the marked difference in plasma levels of ANP and BNP between HOCM and HNCM patients, there was no significant difference in plasma levels of adrenomedullin between HOCM and HNCM patients. These findings suggest that the severity of left ventricular hypertrophy is not necessarily essential for high plasma levels of adrenomedullin in hypertrophic cardiomyopathy.

Several clinical and experimental studies indicate that the plasma level of ET-1 is increased in heart failure [27–30]. ET-1 may function to maintain vascular tone in heart failure. In addition, it is reported that ET-1 and its receptor are present in hypertrophic cardiomyopathy, and the plasma level of ET-1 is higher in hypertrophic cardiomyopathy than in normal controls [31]. In our study, total peripheral resistance was significantly higher in both HOCM and HNCM than in normal controls, and a considerably good correlation was observed between plasma levels of adrenomedullin and ET-1. The increase in plasma levels of ET-1 in hypertrophic cardiomyopathy may be closely related to this high total peripheral resistance. Taking into consideration that the secretion rate of adrenomedullin from endothelial cells is almost comparable to that of ET-1 [8], the high plasma level of adrenomedullin may counteract the haemodynamic effects of ET-1.

Coronary arteries in hypertrophic cardiomyopathy are characterized by a thick wall and a small lumen [14, 15]. Coronary vasodilator reserve is disturbed not only in the hypertrophied interventricular septum, but also in the non-hypertrophied free wall of the left ventricle [16]. Transient myocardial ischaemia is frequently related to sudden cardiac arrest or syncope in patients with hypertrophic cardiomyopathy [17]. Experimental studies indicate that the coronary bed is susceptible to extreme ET-1-induced vasoconstriction [32–34], especially when the native coronary endothelium has been altered or damaged [32]. In addition, ET-1 induces cardiac cell hypertrophy [35, 36]. Thus, ET-1 may be related to myocardial ischaemia in hypertrophic cardiomyopathy. Adrenomedullin may participate to counteract the effect of ET-1 and to maintain the local control of coronary vascular tone in hypertrophic cardiomyopathy.

**Relation of adrenomedullin to other vasoactive substances in hypertrophic cardiomyopathy**

Previous reports indicate that plasma levels of adrenomedullin correlate with plasma levels of noradrenaline, ANP or BNP in patients with heart failure [3, 5]. In our study, however, there were no significant correlations between plasma levels of adrenomedullin and noradrenaline, ANP or BNP. There were also no significant differences between
ET-1 and ANP or BNP in our study. McMurray et al. [29] also showed that there was no significant correlation between ET-1 and ANP. The different results in our study from previous studies may be due to the difference in disease. Increased plasma volume may be one of the stimulators of elevated levels of plasma adrenomedullin in renal failure or heart failure [2, 3, 37]. However, plasma volume in hypertrophic cardiomyopathy is rather less than that in normal control subjects.

The present study also indicates that the plasma levels of vasoactive substances are different in hypertrophic cardiomyopathy. Plasma levels of adrenomedullin and ET-1 are high and are identical in HOCM and HNCM. Plasma levels of ANP and BNP are also high, but are markedly different between HOCM and HNCM. Adrenaline and noradrenaline show no difference in plasma level between normal control subjects and patients with hypertrophic cardiomyopathy. These findings indicate that the pathophysiological role of these substances may be different in hypertrophic cardiomyopathy. Plasma levels of ANP and BNP may mainly reflect the change of left ventricular haemodynamics, because of their marked difference between HOCM and HNCM. To the contrary, plasma levels of adrenomedullin and ET-1 show no significant difference between HOCM and HNCM. This finding may indicate that adrenomedullin and ET-1 mainly function as autocrine and/or paracrine regulators to accommodate vascular tone to the new pathophysiological condition in hypertrophic cardiomyopathy.

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REFERENCES


