Plasma Adrenomedullin in Various Diseases and Exercise-Induced Change in Adrenomedullin in Healthy Subjects

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Adrenomedullin is a novel hypotensive peptide recently discovered in human pheochromocytoma. In the present study, we measured the plasma immunoreactive adrenomedullin of healthy subjects and patients with various diseases. Immunoreactive adrenomedullin was found to circulate in blood of the healthy subjects at a considerable concentration (3.3±0.3 fmol/ml). Plasma adrenomedullin was significantly increased in the patients with congestive heart failure (5.4±0.3 fmol/ml), essential hypertension (5.3±0.4 fmol/ml) and renal disease (4.9±0.4 fmol/ml). In healthy volunteers physical exercise significantly increased the plasma adrenomedullin concentration. The increase of adrenomedullin was inversely related to systolic blood pressure. These findings indicate that adrenomedullin participates in the circulation control in both physiological and diseased conditions. Although the exact origin of circulating adrenomedullin is still unknown, it is thought to be released rapidly by acute exercise, thereby regulating the cardiovascular system by its vasodilating activity.

Key words: human adrenomedullin, heart disease, renal disease, essential hypertension, physical exercise

Introduction

Adrenomedullin is a novel hypotensive peptide recently discovered by monitoring the elevating activity of platelet cyclic adenosin 3',5'-monophosphate (cAMP) in human pheochromocytoma (1). The peptide consisting of 52 amino acids has one intramolecular disulfide bond and is slightly homologous with the calcitonine gene-related peptide (CGRP). Adrenomedullin elicits a potent and long lasting hypotensive effect when injected intravenously in anesthetized rats (2). Sequence analysis of cloned human adrenomedullin cDNA showed that the human adrenomedullin precursor consist of 185 amino acids with a putative signal peptide (3). Studies on regional distribution in human tissues revealed that immunoreactive adrenomedullin is abundant in the normal adrenal medulla as well as in pheochromocytoma tissue (1, 4). A considerable concentration of immunoreactive adrenomedullin circulates in human bloods (4). These findings indicate that adrenomedullin may be a new hormone which participates in circulation control.

In the present study, to investigate the physiological and clinical roles of adrenomedullin, we measured the plasma immunoreactive adrenomedullin level in healthy subjects and patients with various diseases using a recently developed sensitive and specific radioimmunoassay (RIA) for adrenomedullin (5). We asked healthy subjects to perform an exercise and examined the change of plasma adrenomedullin. Moreover, we studied the molecular form of immunoreactive adrenomedullin in the plasma of healthy subjects.

Methods

Subjects and study protocol
Study 1: Plasma level of adrenomedullin in healthy subjects and patients with various diseases.

Eleven healthy subjects and 117 patients with various disease were studied. Seven healthy subjects undertook an ergometer exercise test. We obtained informed consent from all the subjects studied. Among the 117 patients, 50 had congestive heart failure in various stages; 39 were in New York Heart Association (NYHA) Classification I or II, and 11 in NYHA III or IV. The remaining patients consisted of 19 with essential hypertension (EHT), and 11 with renal diseases including 6 with chronic glomerulonephritis and 5 with nephrotic syn-
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Procedure of human adrenomedullin for RIA

The antiserum to adrenomedullin recognized the peptide with high affinity at a final dilution of 1:24,000. Half-maximum inhibition of the radioiodinated ligand binding by adrenomedullin was observed at 4 fmol/tube. Adrenomedullin was measured from 0.5 to 32 fmol/tube by this RIA system. The intra- and inter-assay coefficients of variance were 5% and 8%, respectively.

The incubation buffer for RIA was 0.05 M sodium phosphate buffer (pH 7.4), containing 0.5% BSA, 0.5% Triton X-100, 0.08M NaCl, 0.025M EDTA 2Na, 0.05% NaN₃ and Trasylol 500 KIU/ml. A disposable plastic tube (10×75 mm) was used for the assay. All assay procedures were performed at 4°C. Either the standard human adrenomedullin or the unknown sample (100 µl) was incubated with the anti-human adrenomedullin antiserum diluent (200 µl) for 12 hours, then the tracer solution (18,000–20,000 cpm in 100 µl) was added. After the incubation for 36 hours, an anti-rabbit IgG goat serum diluent (100 µl) was added. After standing for 24 hours, the tubes were centrifuged at 2,000xg for 30 minutes at 4°C and radioactivity of the precipitate was measured in Aloka ARC-600 gamma counter.

Statistical analysis

All data represent as the mean ± SEM. Multiple comparison was evaluated by one-way analysis of variance (ANOVA) followed by Fisher’s t-test. The relationship between variables was assessed by linear simple regression analysis.

Results

Study 1: Plasma levels of adrenomedullin in healthy subjects and patients with various diseases.

Figure 1 shows the plasma adrenomedullin levels in the patients with various diseases as well as the healthy subjects. The plasma levels of adrenomedullin averaged 3.3 ± 0.3 fmol/ml (mean ± SE) in healthy subjects. The mean value of plasma adrenomedullin in the patients with congestive heart failure was 5.4 ± 0.3 fmol/ml, which was significantly higher than that in the healthy subjects (p<0.05). Furthermore, the patients with severe heart failure (NYHA III, IV) showed a significantly higher level of adrenomedullin (8.3 ± 0.6 fmol/ml), when compared with those with less severe heart disease (NYHA I, II, 4.6 ± 0.2 fmol/ml, p<0.05). The plasma adrenomedullin was also significantly increased in both EHT (5.3 ± 0.4 fmol/ml) and renal disease (4.9 ± 0.4 fmol/ml). In particular, the adrenomedullin level in ESRD on HD was comparable to that of patients with severe heart failure. The details concerning either chronic renal failure of digestive disease will be reported elsewhere.

Study 2: Change in plasma level of adrenomedullin during exercise in healthy subjects.

Seven healthy males (mean age 24.4 ± 0.6 years) were studied. The subjects were not obese (body mass index was 21.00) and normotensive (mean values of systolic/diastolic blood pressure were 114±1.1/71±1.7 mmHg), and their blood...
Figure 1. Plasma immunoreactive adrenomedullin in healthy controls and in patients with various diseases. EHT: essential hypertension, ESRD on HD: end stage renal disease on maintenance hemodialysis, GI disease: gastro-intestinal disease, ir-AM: immunoreactive adrenomedullin, NYHA: New York Heart Association Classification, (mean ± SE, *p<0.05, vs control).

Figure 2. Changes in blood pressure and heart rate during exercise. DBP: diastolic blood pressure, HR: heart rate, MAX: at maximal exercise, MBP: mean blood pressure, REC: at recovery, REST: at rest, SBP: systolic blood pressure, *p<0.05, **p<0.01, vs at rest.
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Figure 3. Changes in the plasma adrenomedullin and catecholamine during exercise. AM: adrenomedullin, DOPA: dopamine, Epi: epinephrine, MAX: at maximal exercise, NE: norepinephrine, REC: at recovery, REST: at rest, *p<0.05, **p<0.01, vs at rest.

Table 1. Correlation Coefficients between Changes in Plasma Adrenomedullin (Δ AM) and Those in Other Parameters

<table>
<thead>
<tr>
<th>Δ AM</th>
<th>vs. Δ SBP</th>
<th>r  = -0.86*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ DBP</td>
<td>r         = -0.49</td>
<td></td>
</tr>
<tr>
<td>Δ HR</td>
<td>r         = -0.11</td>
<td></td>
</tr>
<tr>
<td>Δ NE</td>
<td>r         = 0.17</td>
<td></td>
</tr>
<tr>
<td>Δ Epi</td>
<td>r         = -0.24</td>
<td></td>
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</tbody>
</table>

SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, NE: plasma norepinephrine, Epi: plasma epinephrine, *p<0.05.

chemical data were within the normal range. Figure 2 shows the changes of blood pressure and heart rate during the exercise. Both systolic blood pressure and heart rate were significantly increased by the exercise (p<0.05). The change of diastolic blood pressure was not significant. The plasma responses of adrenomedullin and catecholamines to the exercise are shown in Figure 3. Plasma norepinephrine and dopamine were significantly increased during the exercise (p<0.05), but the plasma epinephrine was not. The plasma level of adrenomedullin was found to be significantly increased.

We examined the relationship between the increments of adrenomedullin and those of other parameters during the exercise test (Table 1). There was a significant negative correlation between the absolute increase of adrenomedullin and that of systolic blood pressure (r=-0.849, p<0.05) (Fig. 4). Moreover, before the exercise, the level of adrenomedullin was not correlated with that of norepinephrine. However, the maximum values during the exercise were significantly correlated with each other (Fig. 5).

Study 3: Characterization of adrenomedullin in human plasma.

We examined the molecular form of immunoreactive adrenomedullin in human plasma from healthy subjects. The sample was applied to HPLC on a reverse phase column that was also monitored by RIA for human adrenomedullin. As shown in Fig. 6, one major peak of immunoreactive adrenomedullin that was more than 90% of the total immuno-reactivity emerged at the position almost identical with that of
Figure 4. Relationship between the increments of adrenomedullin (ΔAM) and that of systolic blood pressure (ΔSBP) during exercise.

Figure 5. Relationship between the plasma adrenomedullin and the plasma norepinephrine at maximal exercise. ir-AM: immunoreactive adrenomedullin, NE: plasma norepinephrine.

Figure 6. Reverse-phase HPLC of plasma sample monitored by RIA for adrenomedullin. Sample obtained in gel filtration; Column, TSK ODS SIL 120 A (4.6x15 cm, Tosoh); solvent system, H₂O/CH₃CN/10%TFA=(1) 90: 10: 1, (2) 40:60:1 (v/v). Linear gradient for 60 minutes. Flow rate:1 ml/min. The arrow indicates the elution position of authentic AM. ir-AM: immunoreactive adrenomedullin.

Discussion

The present study clearly demonstrated that plasma adrenomedullin was significantly higher in cardiovascular diseases including heart failure, essential hypertension and renal disease. Particularly, the patients with severe congestive heart failure showed a higher level of adrenomedullin. We have already shown that the adrenomedullin elicits a strong and long-lasting hypotensive effect when injected in anesthetized rats (1). Furthermore, the hypotensive effect of adrenomedullin is caused by vasodilation, followed by a decrease in total peripheral resistance (3). It is well known that the sympathetic and renin-angiotensin systems are often exaggerated in congestive heart failure and essential hypertension. Therefore, the increase of plasma adrenomedullin in these cardiovascular disease is thought to occur as a compensatory or a counteracting mechanism for such pressor systems, thus modifying the pathophysiological conditions of the diseases.

We have shown that adrenomedullin is slightly sequentially homology with CGRP (1). The hypotensive or vasodilation effect as well as the CAMP increasing effect of adrenomedullin in the vascular smooth muscle cell is very similar to that of CGRP (6). Furthermore, it has been reported that plasma CGRP increases in cardiovascular diseases including congestive heart failure, essential hypertension and renal failure. Considering the characteristics the adrenomedullin is widely distributed in peripheral tissues, this peptide may share an important role in the circulation control with CGRP in the peripheral tissues.

In the latter part of this study, we showed for the first time that the plasma adrenomedullin increased by exercise in the healthy subjects. In addition, at maximum exercise, the plasma adrenomedullin level was significantly correlated with the norepinephrine level. These data suggest that plasma adrenomedullin has an apparent correlation with sympathetic activity, indicating that the sympathetic stimulation, at least plasma norepinephrine, may contribute to the adrenomedullin secretion. However, since there was no correlation between the increments of epinephrine and adrenomedullin, further studies are required to elucidate the exact mechanism of exercise-
induced adrenomedullin secretion. On the other hand, it should be emphasized that the increments of plasma adrenomedullin are inversely related with systolic blood pressure. The finding suggests that the increase plasma adrenomedullin exerts a fall of systolic blood pressure by its vasodilatory effect. Moreover, it is implicated that the elevated blood pressure during exercise may directly or indirectly stimulate the adrenomedullin secretion, which may act to lower the elevated blood pressure toward the baseline. However, it seems unlikely that the increased plasma concentration of adrenomedullin during exercise could directly affect the blood pressure, because the depressor level of plasma adrenomedullin, which is estimated from rat experiments receiving intravenous injections of this peptide, is far higher than the increased levels due to exercise. In contrast, since the majority of plasma adrenomedullin is considered to spill over from the vascular endothelial cells (7), the exercise-induced release of adrenomedullin may reflect the increased activity as an autocrine or a paracrine regulator. Thus, it could be considered that some factors other than the sympathetic stimulation may also participate in adrenomedullin secretion. These data, therefore, suggest possibilities that adrenomedullin is released by mechanical stimuli as well as by other humoral factors regulating circulation system.

We reproducibly demonstrated that immunoreactive adrenomedullin circulating in human plasma is identical or very similar to the authentic adrenomedullin by analysis using a reverse-phase HPLC combined with RIA for adrenomedullin. However, we have not yet clarified the origin of plasma adrenomedullin. In our recent report high levels of adrenomedullin mRNA were also found in lung, cardiac ventricle and kidney as well as in pheochromocytoma or adrenal medulla, indicating that adrenomedullin is biosynthesized in these tissues (2, 4). Adrenomedullin directly stimulates the cAMP production in the vascular smooth muscle cells and the increased cAMP may participate in vasodilation (3, 5). Furthermore, it has very recently been reported that endothelial cells actively synthesize and secrete adrenomedullin (7). It is, therefore, suggested that adrenomedullin secreted from the endothelial cell may activate the specific receptor on the vascular smooth muscle cell, regulating the vascular tone. The present data indicated that the increments of epinephrine and those of adrenomedullin were not correlated with each other. Furthermore, there was no correlation between plasma norepinephrine and adrenomedullin at baseline, but at maximum exercise a significant correlation was found between these two. These findings suggest that the adrenal glands, major sources of epinephrine, are not the main sources of the circulating adrenomedullin. In addition to the adrenal medulla, plasma adrenomedullin might in part originate from endothelial cells.

In summary, we have measured the plasma adrenomedullin in healthy subjects and in patients with various diseases. The plasma adrenomedullin was significantly higher in heart disease, essential hypertension, renal disease and pulmonary disease. Particularly, the patients with severe congestive heart failure showed a higher plasma level of adrenomedullin. These findings indicate that adrenomedullin may play some role in diseased conditions via its vasoactive function. In the healthy volunteers the plasma adrenomedullin was increased due to exercise with a concomitant increase in the systolic blood pressure and plasma norepinephrine. The increment of plasma adrenomedullin had an inverse relation with that of the systolic blood pressure. Moreover, there was a significant correlation between the plasma levels of adrenomedullin and those of norepinephrine at the maximum exercise. These results indicate that adrenomedullin is secreted in response to physiological exercise, which may be mediated by factors such as the plasma norepinephrine and the blood pressure. The present study as well as our previous reports (4, 5, 7) suggest that adrenomedullin may play some role in the circulation control in the physical state as well as in diseased conditions. Further studies on the source of plasma adrenomedullin, its releasing mechanism and pathophysiological roles of this substance are necessary.

References