Original Article

Relationship between the Contents of Adrenomedullin and Distributions of Neutral Endopeptidase in Blood and Tissues of Spontaneously Hypertensive Rats

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Adrenomedullin (ADM) is a multifunctional peptide with important roles in the cardiovascular system, especially in the adjustment of cardiovascular and renal homeostasis. ADM is present in plasma, organs and tissues, and its activity increases during hypertension. It remains unknown whether the clearance of this peptide is altered during hypertension. Neutral endopeptidase (NEP) is the major enzyme in ADM’s degradation. We observed the activity and distribution of NEP and the expression of its mRNA in the plasma, cardiac ventricle, aorta, jejunum and kidney of spontaneously hypertensive rats (SHRs) in order to study the possible role of NEP in elevating tissue ADM concentrations during hypertension. ADM and NEP were diffuse in all tissues studied. The level of tissue ADM was generally higher in SHR tissues than in control tissues, except in the renal medulla, and its mRNA expression was higher in all tissues. Plasma NEP activity, general NEP activity and the expression of NEP mRNA in the left ventricle, aorta and jejunum in SHRs was lower than that of controls, and the level of ADM was inversely correlated with NEP activity. NEP activity and mRNA and protein expression in SHR kidneys were higher than in control kidneys; moreover, the ADM content was positively correlated with NEP activity in the renal cortex. NEP activity in the lung of SHRs did not differ from that of controls. Thus, in SHRs, the local concentration and action of ADM in the tissues may be differentially regulated by NEP. (Hypertens Res 2004; 27: 109–117)

Key Words: spontaneously hypertensive rat, adrenomedullin, neutral endopeptidase

Introduction

It is well known that cardiovascular tissues synthesize and secrete miscellaneous bioactive substances that regulate circulatory homeostasis by paracrine, autocrine and endocrine mechanisms. Growing evidence indicates that these bioactive substances play an important role in the pathogenesis of hypertension and that the autocrine-paracrine systems are important targets for hypertension therapy (1). Adrenomedullin (ADM) is a potent vasodilatory peptide and is expressed in a variety of tissues or cell populations such as the heart, lung, kidney, intestine and vessels. ADM consists of 52 amino acids and has a ring cycle structure formed by an intramolecular disulfide bridge and a carboxy-terminal amidated residue (2). Imai and associates have established a line of transgenic mice that overexpress ADM in their vasculature; these mice have lower blood pressure and higher nitric oxide (NO) production than their wild-type littermates (3). Shindo and colleagues established an ADM gene knockout

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model and found that the blood pressure and NO production of heterozygote mice decreased, and homozygous mice died in utero of poorly developed vitelline vessels and abnormally constricted umbilical arteries (4). ADM is not only an indispensable factor for vascular integrity during embryonic development, but also it is a physiologically relevant regulator of blood pressure. Endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) both actively secrete ADM and express its receptors (2). In cardiovascular diseases such as simple hypertension or hypertension with complications, ADM expression in cardiovascular tissues is known to increase in proportion to the severity of the disease, which is considered a compensatory mechanism to reduce tissue damage (5). Recently, either the administration of the ADM peptide or ADM gene delivery resulted in a marked reduction in blood pressure, significant attenuation of cardiac hypertrophy, fibrosis and renal injury, and improvement of renal function in spontaneous or volume-overloaded hypertensive rats (6). Furthermore, these studies showed that in rat models of hypertension, the upregulation of ADM is an important biological attempt to compensate for cardiac and renal damage, and thus increased levels of ADM may provide a new therapeutic strategy for patients with hypertension (5).

There is growing interest in applying proteinase inhibitors for the treatment of hypertension. Such proteinase inhibitors can potentiate the availability of vasoactive peptides, which usually are small molecules with short half-lives (7). Neutral endopeptidase (E.C.3.4.24.11, NEP) is the major enzyme for the degradation of ADM (8). Rademaker and coworkers (9) administered ADM together with the NEP inhibitor SCH32615 to sheep with heart failure and showed better therapeutic effects than those obtained with either agent alone. Combining ADM with NEP inhibitors would thus increase the effectiveness of current anti-hypertension therapies (7). However, little is known about the changes in activity, expression and distribution of NEP during hypertension, and it also remains unknown whether or not such changes are relevant to alterations in ADM content.

We studied the activity and expression of NEP and the concentration of ADM in the plasma, as well as the levels of ADM and ADM mRNA expression in the heart, thoracic and abdominal aorta, lung, jejunum and kidney tissue of spontaneously hypertensive rats (SHRs) in order to gain a better understanding of the possible role of NEP in changing the level of ADM in cases of hypertension.

### Methods

**Materials**

Radioimmunoassay kits for rat ADM were purchased from Phoenix Pharmaceutical (Belmont, USA). Aprotinin, N-dansyl-Ala-Gly-τ-nitro-Phe-Gly (DAGNPG), dansyl-τ-Ala-Gly (DAG) and thiophan were purchased from Sigma (St. Louis, USA); trizol was obtained from GIBCO BRL (Gaithersburg, USA); deoxyribonucleoside triphosphate (dNTP) from Clontech Laboratories (Palo Alto, USA); and Moloney murine leukemia virus transcriptase (MMLV), Taq, RNAsin and Oligo(dT)15 primer from Promega (Madison, USA). Oligonucleotides were synthesized by Sai Baisheng Biotechnology (Beijing, China). The sequences of the oligonucleotide primers were as follows: ADM-S, 5’-CTC GAC ACT TCC TCG CAG TT-3’ and ADM-A, 5’-GCT GGA GAC GCT GAG GTG TGT GTC-3’. We studied the activity and expression of NEP and the degradation of ADM (8). Rademaker and coworkers (9) administered ADM together with the NEP inhibitor SCH32615 to sheep with heart failure and showed better therapeutic effects than those obtained with either agent alone. Combining ADM with NEP inhibitors would thus increase the effectiveness of current anti-hypertension therapies (7). However, little is known about the changes in activity, expression and distribution of NEP during hypertension, and it also remains unknown whether or not such changes are relevant to alterations in ADM content.

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**Determination of the Level of Immunoreactive ADM (ir-ADM) in Plasma and Tissues and the Characteristics of Secreted ADM**

Blood samples of each rat were taken with the use of EDTA-Na2: (1 mg/ml), aprotinin (500 KIU) and heparin. The plasma was separated by centrifugation (1,600 × g for 15 min) and stored at -70 °C for assayng. Chopped tissues were boiled for 10 min in 1 mol/l acetic acid and homogenized with a Polytron at 4°C. The extract solution was centrifuged at 24,000 × g for 30 min. The plasma and tissue extract solution were loaded onto a Sep-Pak C18 cartridge and were pre-equilibrated with 0.5 mmol/l acetic acid, and the adsorbed mater-
The recovery of isolated total tissue RNA was then quantified by the use of fractions of tissues was determined by the Coomassie Brilliant Blue method. 

Assay of NEP Activity in Plasma and Tissues

NEP activity in the plasma and in the particulate fractions of the tissue homogenates was measured by spectrofluorometric assay using the synthetic peptide DAGPNG, as previously described (11). The reaction was carried out in the presence or absence of thiorphan (20 nmol/l), a specific inhibitor of NEP, and only the activity inhibited by thiorphan equaled to the NEP activity. Blood samples of each rat were mixed with heparin, then centrifuged (1,600 × g for 15 min, 4 ºC), and the plasma was separated for the NEP assay. The particulate fractions of the tissues were prepared according to a method described by Wolff and colleagues (12), with a few modifications. Tissues were washed extensively with cold saline solution to remove all of the blood, and were homogenized three times (5 s each) in a 10 vol solution of iced cold Tris-HCl buffer (0.05 mol/l, pH 7.4) by a Polytron set at its maximum speed. Then, the samples were homogenized in a motor-driven glass-Teflon homogenizer (medium speed, 10 strokes). The homogenate was then filtrated through a four-layer cheesecloth, and the filtrate was centrifuged at 1,000 × g at 4 ºC for 20 min. The pellet was resuspended in fresh iced Tris-HCl buffer and centrifuged at 1,000 × g (4 ºC, 20 min) at least twice. The final pellet was resuspended in fresh iced Tris-HCl buffer with a protein concentration of about 4 mg/ml, and the protein concentration of the particulate fractions of tissues was determined by the Coomassie Brilliant Blue method.

Reverse Transcription (RT)–Polymerase Chain Reaction (PCR) Studies

The expression of ADM and NEP mRNA was assessed by semi-quantitative RT–PCR as previously described (13). Isolated total tissue RNA was then quantified by the use of an ultraviolet (UV) spectrophotometer (DU-68, Beckman, Fullerton, USA). RT to cDNA was accomplished by priming 2 μg of total RNA samples with MMLV and oligo (dT) 15 primer. The products were then used for the following PCR amplification: the PCR reaction mixture was in a 25 μl volume containing 2.5 mmol/l dNTP 1 μl, 10 μl PCR buffer (20 mmol/l MgCl2, 500 mmol/l KCl, 1.5 mol/l Tris-HCl, pH 8.7), 2.5 μl cDNA, 200 nmol/l of the appropriate ADM or NEP paired primers and 1.25 U of Taq DNA polymerase. The following PCR cycles were used: 94 ºC for 30 s, 57 ºC for 30 s, and then 74 ºC for 5 min. As an internal control for each PCR reaction, β-actin mRNA was also amplified with each sample. A total of 200 nmol/l of actin primers and cDNA 2 μl were amplified under the same reaction conditions. All PCR products were loaded onto a 1.5% agarose-Tris-acetate-EDTA gel before electrophoresis, and then the products were visualized by ethidium bromide staining. The UV illumination photos then underwent computerized densitometric analysis. The final results are expressed as the ratios of ADM PCR product (446 bp) or NEP PCR product (440 bp) to the β-actin PCR product (291 bp) for each sample. The amplified ADM cDNA, NEP cDNA and β-actin were confirmed by digestion of the PCR products with the restriction enzyme MSP I. All experiments were repeated three times.

NEP Immunohistochemistry

The immunohistochemical localization of NEP was investigated according to the method described by Gurcharan and colleagues (14), with some modifications. After being anesthetized with pentobarbital sodium (45 mg/kg, intraperitoneal administration), SHRs and WKY rats (three in each group) were perfused with 100 ml of saline, followed by 200 ml 4% paraformaldehyde in 0.1 mol/l phosphate buffer by use of an aortic catheter (at approximately 100 mmHg). Tissues were kept in the same fixative solution overnight at 4 ºC and then were cryopreserved in 30% sucrose in phosphate buffer for 24 h at 4 ºC. Tissue sections that were approximately 10 μm thick were cut by a cryostat microtome and mounted onto poly-l-lysine coated glass slides before being incubated with Tris-buffered saline (TBS) containing 0.1% Triton X-100, 3% normal rabbit serum, and 1% dry milk for 1 h. Endogenous peroxidase activity was quenched by incubating the sections with 3% (vol/vol) H2O2 in 70% methanol for 30 min at room temperature. Indirect immunohistochemical detection of NEP was carried out with an avidin-biotin peroxidase (ABC-HRP) immunostaining kit (Vector, Burlingame, USA). Goat polyclonal antibody against rat NEP (1:180) was applied at room temperature and 4 ºC in a humidified chamber for 1 h and overnight before staining. Immunoreactive sites were revealed by incubation with 0.05% (wt/vol) diaminobenzidine (DAB) and 0.003% (vol/vol) H2O2 in 50 mmol/l Tris-HCl (pH 7.6) for 5 min. Sections were then counterstained with hematoxylin before
being dehydrated in ethanol. Light microscopy evaluations of the sections were then performed by a blinded judge. The presence of NEP was indicated by a brown stain on the cytomembrane or in the cytoplasm. A Leica Q550IW system (Leica, Heerbrugg, Germany) was used to analyze the mean optical density and the area of NEP staining. For the negative controls, nonimmunized goat serum was substituted for the primary antibody.

**Statistical Analysis**

Results are shown as the mean \( \pm \) SD. Comparisons between the two groups were performed with Student’s \( t \)-test. A \( p \) value of \( \leq 0.05 \) was considered to be statistically significant.

**Results**

**General Characteristics of Hypertension in SHRs**

As shown in Table 1, the systolic pressure of the caudal artery and the ratio of heart weight to body weight in SHRs were 60% \( (p<0.01) \) and 41% \( (p<0.01) \), higher than those of WKY rats respectively (Table 1). The ratio of the left kidney weight to body weight was 8.2% \( (p<0.05) \) lower in SHRs than in WKY rats, and no significant difference was observed between the heart rates of these two types of rat.

**ir-ADM Content**

The concentration of plasma ir-ADM in WKY rats was 11.15 \( \pm \) 1.59 pmol/l. As shown in Fig. 1, the assayed tissues were rich in ADM. Given in descending order from the highest to the lowest concentration, tissues containing ir-ADM were as follows: lung, renal cortex, renal medulla, jejunum, thoracic and abdominal aorta, and cardiac ventricle.

Compared with WKY rats, SHRs had a 40% higher plasma ir-ADM content (15.63 \( \pm \) 1.55 pmol/l, \( p<0.01) \). The tissue ir-ADM content in SHRs was higher than in WKY rats within a range of +22% (lung) to +79% (aorta) \( (p<0.05 \text{ or } p<0.01, \text{ respectively}) \), but this relative increase was not observed in the renal medulla, which showed no significant change. Given in descending order from the highest to the lowest concentration, tissues containing ir-ADM were as follows: lung, renal cortex, jejenum, thoracic and abdominal aorta, renal medulla and cardiac ventricle.

Molecular forms of ADM produced by the tissues were characterized by the use of reverse-phase HPLC (Fig. 2). ir-ADM production consisted of one major and one minor peak, whereby the major peak appeared at an elution position identical to that of synthetic rat ADM (1–50)-NH\(_2\), the whole active molecule of the rat ADM peptide.

**PreproADM Gene Expression**

RT-PCR results are shown in Fig. 3. Compared with WKY rats, SHRs had higher levels of preproADM mRNA in the
cardiac ventricle, thoracic aorta, jejunum and kidney (p < 0.01) by 13.6%, 64.1%, 55.2% and 37.6%, respectively.

NEP Activity

The activity of plasma NEP in WKY rats was 2.81 ± 0.63 U/ml. As shown in Fig. 4, the assayed tissues were rich in NEP. In descending order from highest to lowest, the tissues showing NEP activity were as follows: renal cortex, renal medulla, lung, jejunum, thoracic and abdominal aorta and cardiac ventricle. The plasma NEP activity (0.47 ± 0.092 U/ml) in SHRs was 83% (p < 0.01) lower than in WKY rats, but the changes in NEP activity in SHRs were tissue-specific. NEP activity was much lower in the myocardium, aorta and jejunum of SHRs than in WKY rats, by - 83%, - 60% and - 46%, respectively (all p < 0.01), and NEP activity was higher in the renal cortex and medulla of SHR rats than in those of WKY rats by + 56% and + 108%, respectively (both p < 0.01), but no significant differences (p > 0.05) were observed in the lung tissue. Orders of tissue NEP activity from the highest to the lowest were the same as for the WKY rats.

Fig. 3. Gene expression of adrenomedullin (ADM) and β-actin gene in the left ventricle, aorta, jejunum and kidney of Wistar Kyoto (WKY) rats (Control) and spontaneously hypertensive rats (SHRs) (n = 3/group). A: Lanes 1 and 2, left ventricle in WKY rats and SHRs, respectively; Lanes 3 and 4, aorta in WKY rats and SHRs, respectively; Lanes 5 and 6, jejunum in WKY rats and SHRs, respectively; Lanes 7 and 8, kidney in WKY rats and SHRs, respectively. B: Changes in adrenomedullin (ADM) gene expression in the tissues of SHRs and WKY rats. The data (expressed as the ratio of target gene ADM to the β-actin gene) are presented as the mean ± SD (n = 3/group) and the results were compared by unpaired Student’s t-test. ** p < 0.01.

Fig. 4. Changes in neutral endopeptidase (NEP) activity in the tissues of spontaneously hypertensive rats (SHRs) and Wistar Kyoto rats (Control). The data (expressed as U/mg protein) are presented as the mean ± SD (n = 8/group) and are compared by unpaired Student’s t-test. 1 U = 1 nmol/min; * p < 0.05 and ** p < 0.01.

NEP Immunohistochemistry

All myocytes of WKY rats (Fig. 5A) showed intense, positive NEP staining along the plasma membrane and in the cytoplasm. Compared with WKY rats, SHRs showed weaker myocardial NEP staining (Fig. 5B); the mean optical density and area of staining were 13% (p < 0.05) and 52% (p < 0.01) lower, respectively (Table 2).

In the thoracic aortas of WKY rats (Fig. 5C), intense NEP staining was observed along the plasma membrane of ECs and adventitial fibroblasts, but this staining was less intense in the VSMCs. In the SHRs, NEP staining in the ECs was attenuated; the mean optical density and area of NEP staining were 9% (p < 0.05) and 29% (p < 0.01) lower than that in WKY rats. NEP staining of VSMCs in SHRs was also less intense than that in WKY rats, with a mean optical density and area of NEP staining at 28% and 51% (p < 0.01) lower in SHRs. The staining in the adventitial fibroblasts in SHRs (Fig. 5D) was more intense than in those in WKY rats, and the mean optical density and area of NEP staining were 21% (p < 0.01) and 17% (p < 0.05) higher in the SHRs than those in WKY rats (Table 2).

In the kidney of WKY rats (Fig. 5E), intensive NEP staining was detected in the distal and collecting tubule epithelia (along the membrane, especially the brush border, and in the cytoplasm) as well as in the glomerular capillary endothelium and podocytes (along the membrane). In the kidney of SHRs, NEP staining was more intense (Fig. 5F) than in the
Fig. 5. The immunoreactivity of neutral endopeptidase (NEP) in the ventricles, aortas, kidneys of Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs) (n = 3/group; all sections: ×400). A,B, ventricle of WKY rats and SHRs, respectively; C,D, aorta of WKY rats and SHRs, respectively; E,F, kidney of WKY rats and SHRs, respectively.

Table 2. The Mean Optical Density (OD) and Staining Area (μm²) of Neutral Endopeptidase Immunoactivity in Tissues of Wistar Kyoto Rats (Control) and Spontaneously Hypertensive Rats (SHRs)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>SHR</th>
<th>Staining area (μm²)</th>
<th>Control</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Left ventricle</td>
<td>0.59 ± 0.04</td>
<td>0.52 ± 0.05 *</td>
<td>4,452.45 ± 252.47</td>
<td>2,152.21 ± 349.57 **</td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td></td>
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<tr>
<td>Endometrium</td>
<td>0.65 ± 0.06</td>
<td>0.59 ± 0.06 *</td>
<td>366.31 ± 34.64</td>
<td>258.68 ± 38.07 **</td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>0.61 ± 0.07</td>
<td>0.44 ± 0.06 **</td>
<td>239.16 ± 39.57</td>
<td>116.99 ± 25.01 **</td>
<td></td>
</tr>
<tr>
<td>Adventitia</td>
<td>0.52 ± 0.07</td>
<td>0.63 ± 0.07 **</td>
<td>1,215.74 ± 129.32</td>
<td>1,419.99 ± 208.96 *</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.47 ± 0.04</td>
<td>0.58 ± 0.04 **</td>
<td>8,832.52 ± 1,375.31</td>
<td>15,233.50 ± 2,253.30 **</td>
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</tr>
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Mean ± SD (n = 8/group). * p < 0.05, ** p < 0.01.
and are compared by unpaired Student's t-test. The linear regression analysis indicated a strong positive correlation between ADM content and NEP activity in the renal cortex (\(r = 0.681, p < 0.01, n = 16\)). However, a negative correlation between ADM content and NEP activity was observed among the plasma, cardiac ventricle, aorta and jejunum (\(r = -0.775, p < 0.01\) for the plasma; \(r = -0.557, p < 0.05\) for the cardiac ventricle; \(r = -0.740, p < 0.05\) for the aorta; \(r = -0.720, p < 0.01\) for the jejunum; total, \(n = 16\)). No significant correlation was observed between ADM content and NEP activity in the lung and renal medulla (\(r = -0.081\) and 0.444, respectively; both \(p > 0.05\), \(n = 16\)).

**Discussion**

ADM is a peptide that carries out a number of functions and which plays an important regulatory role in the cardiovascular system in that it induces vasodilation, hypotension and natriuresis (2). Although ADM was originally found in human pheochromocytoma, preproADM gene expression and its immunoreactivity are widely distributed in cardiovascular system, kidney, digestive organ, and nervous system tissues (2). Endogenous plasma ADM is mainly synthesized in and secreted by the vascular endothelium and VSMCs, and it plays an important role in cardiovascular and renal homeostasis (15). During hypertension, heart failure and renal dysfunction, increased ADM activity has been detected in the plasma and in many organ tissues (2). Chao and associates (6) reported that human ADM gene delivery maintained significantly lower blood pressure for more than 20 days, reduced cardiac hypertrophy and fibrosis, and attenuated volume- or pressure-induced renal damage and impairment of renal hemodynamics in genetic and experimental hypertensive rats; these effects were found to be mediated by the activation of the cAMP and/or NO-cGMP signaling pathways of ADM. ADM gene delivery is also known to protect against myocardial infarction, ventricular arrhythmias and apoptosis in ischemia/reperfusion injury and it reduces superoxide production as well (6). ADM is a paracrine antiapoptotic agent with a cytoprotective effect, which can be neutralized by a monoclonal antibody to ADM (16). These studies indicate that endogenous ADM contributes to a cytoprotective effect against organ damage (5).

As a short peptide with low molecular weight (about 6 kDa), the circulating half-life of ADM is only 22 \(\pm\) 1.6 min in humans (17). ADM may act as a local or an endocrine factor. Usually, it acts through an autocrine or paracrine mechanism, and its plasma concentration in healthy individuals is quite low (pmol/l range) (2). Two clearance pathways of tissue ADM have been recognized: protease hydrolysis and binding to ADM clearance receptors (2). Increased plasma ADM has been observed during systemic NEP inhibition, which confirms that ADM is a substrate of NEP (18). It was later confirmed that NEP is the major degradation enzyme of ADM and natriuretic peptides (8).

NEP is an 87–96-kDa membrane-bound metalloprotease,
with an optimal action pH of about 7. Its zinc binding action center contributes to the cleavage of ADM at hydrophobic residues. The human NEP gene is located at chromosome 3, which spans more than 80 kilobases and contains 24 minigenes (19). Currently, ADM can be hydrolyzed from hydrophobic residues by metalloprotease in vitro to generate ADM (8–52), (26–52) and (33–52), and further aminopeptidase activity will produce ADM (2–52), (27–52) and (28–52); these degradation products are thought to possess different biological activities (20). Gonzalez and colleagues reported that the local concentration of vasoactive peptides in a vessel wall was regulated by the NEP pathway in the immediate vicinity of their target cells (21). However, it remains unclear how the NEP activity and distribution change in tissues under conditions of hypertension and in the context of other cardiovascular diseases. It is also unclear whether or not alterations in NEP play an important role in regulating ADM content and activity in the context of these diseases.

In the current study, as has also previously been reported, we observed diffuse distribution and mRNA expression of ADM in the plasma and cardiac ventricle, aorta, jejunum and kidney tissues, but ADM mRNA expression was not specially observed in the renal medulla tissues of SHRs (2). In addition, we observed the diffuse distribution of NEP in plasma and tissues. However, unlike changes in ADM, changes in NEP in SHRs were tissue-specific. Compared with WKY rats, SHRs showed lower NEP activity in the plasma and in myocardium, aorta and jejunum tissues, and higher activity in the renal cortex and medulla tissues; however, no significant difference between WKY and SHR rats was found in the pulmonary tissue. Immunohistochemical assays indicated that NEP expression was lower in the heart, aortic intima and media tissue, but was higher in the aortic adventitia and kidney in SHRs than in WKY rats. RT-PCR analysis revealed that changes in the NEP mRNA levels in SHRs were similar to the changes in protein expression. Compared with WKY rats, SHRs showed 34%, 24% and 23% lower NEP gene expression in the myocardium, thoracic aorta and jejunum, respectively, and 86% higher expression in the kidney (Fig. 6).

Moreover, the same tissues showed a significant discrepancy between the types of rat as regards changes in ADM content and NEP activity. ADM content and NEP activity were positively correlated in the renal cortex and were negatively correlated in the plasma and the myocardium, aorta and jejunum tissues.

The content of ADM in blood and local tissues may be regulated by the balance between its production and degradation. It is well known that ADM mRNA expression and its synthesis and secretion are upregulated by many factors such as angiotensin II, endothelin-1, norepinephrine, and physical stimuli (shear stress and/or stretching), which participate in the pathogenesis of hypertension (2). However, little is known about alterations in NEP during hypertension (19). The overexpression of angiotensin converting enzyme (ACE) significantly decreased NEP activity; this possible mechanism could be related to reduced Ang-(1–7) and bradykinin levels or to increased Ang II levels through a receptor-mediated mechanism (22). Phorbol 12-myristate 13-acetate (PAM) and diacyl-glycerol (DAG), the activators of protein kinase C (PKC), induced internalization of NEP and decreased NEP activity (19). Interferon (IFN)-γ and PMA were also shown to reduce NEP mRNA expression (19). Chronic salt loading was associated with increased NEP activity in the urine and isolated proximal tubules, which suggests that this enzyme may be regulated by salt balance (23). The activators of adenylyl cyclase or protein kinase A (PKA), glucocorticoid, thrombin, calcitonin and cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α and granulocyte-macrophage colony stimulating factor, can increase NEP mRNA expression and activity (19). Analysis of clones differing from one another in the 5′ flanking region of NEP suggests that its gene transcription could be controlled by alternative splicing of common or distinct pre-mRNAs, leading to differentially controlled gene expression in a tissue-specific and/or developmentally regulated manner (19). In addition, NEP expression may differ between tissues due to differences in physiological and metabolic characteristics among tissues (21). In the current study, decreased NEP expression and activity was observed in the heart, aorta and jejunum of SHRs, which indicates that local, downregulatory factors for NEP expression may be predominant in these tissues, whereas increased NEP expression activity in the kidney suggests that local, upregulatory factors may predominate.

Vasopeptidase inhibition represents a novel approach for the treatment of hypertension and may decrease the morbidity and mortality of associated cardiovascular diseases such as heart failure (8). The primary consequence of NEP inhibition is the potentiation of endogenous vasodilatory and natriuretic systems such as ADM and natriuretic peptides, which results in natriuresis, diuresis and antihypertensive effects. Clinical experiments investigating hypertension treatments have shown that inhibitors of NEP (NEPIs) enhanced the effects of endogenous ADM and atrial natriuretic polypeptide (ANP), and increased the urinary excretion of sodium and cyclic GMP, but NEPIs had no effect on the potassium excretion in urine (8). In our study, plasma and tissue ADM were elevated to compensate for hypertension, in part through the upregulation of ADM mRNA, and tissue-specific changes of NEP expression and activity were also observed. These findings indicate that in SHRs, ADM induces a variety of protective effects in the tissues, as NEP might mediate a unique response to increased ADM in different tissues. Therefore, tissue-specific changes in NEP should be taken into account with NEPI treatment of hypertension.

In this study, we determined the ADM contents and the levels of ADM mRNA expression, as well as the distribution of NEP in the blood and tissue of SHRs. We also observed how tissue ADM and NEP changed in such rat model of hypertension. Compared with WKY rats, SHRs showed in-
creased ADM content and mRNA expression in the plasma and most of the tissues studied, except in the renal medulla. NEP changes in tissues were found to be tissue-specific, except in the kidney, where NEP activity increased, and in the lung, where it no significant change was observed. The downregulation of NEP in the plasma and other tissues, as well as the upregulation of ADM mRNA expression might contribute to elevated ADM content. In cases of hypertension, further identification of tissue changes in NEP will remain important in order to gain a better understanding of the pathophysiological role of NEP.

References