Adrenomedullin (AM) is a potent vasodilator peptide that was first detected in a human pheochromocytoma while monitoring cyclic AMP (cAMP) in rat platelets (1). The development of a specific radioimmunoassay (RIA) revealed that AM is present not only in the normal human adrenal medulla but also in tissues such as aorta, kidney, lung, and cardiac atrium and ventricle, where AM mRNA is abundantly expressed (2, 3). High concentrations of AM were also found to circulate in the blood of humans (4). When injected intravenously, synthetic AM exerts a potent hypotensive action, accompanied by a marked reduction of total peripheral resistance in rats (5). Ebara et al. observed increases in urinary volume and sodium excretion after an intrarenal arterial injection of AM in dogs (6). Atrial and brain natriuretic peptides (ANP and BNP) are cardiac hormones thought to act against elevation of blood pressure and retention of body fluid in cardiovascular diseases such as hypertension and heart failure through their vasodilator and natriuretic effects (7). ANP and BNP exert their biological actions via an accumulation of intracellular cyclic GMP (cGMP) (7), whereas many of the actions of AM are mediated by cAMP (8). Since the AM actions are similar to those of ANP and BNP despite differences in their intracellular signaling systems, we hypothesized that AM functions along with ANP and BNP to act against further elevation of blood pressure in hypertensive patients.

The aim of the present study was to investigate the pathophysiological role of AM in essential and malignant hypertension (EHT and MHT). We measured plasma AM concentrations in patients with EHT or MHT, and compared them with those in normotensive subjects and with plasma levels of ANP and BNP. Additionally, we examined changes in the plasma levels of these bioactive peptides after antihypertensive treatment in patients with MHT.

**Methods**

**Subjects and Blood Sampling**

Forty-two patients with EHT of WHO stage I or II (25 to 82 yr of age) and 9 patients with MHT (37 to 64 yr of age) were enrolled in this study. Hyperten-
Hypertension was defined as a systolic blood pressure (SBP) of 140 mmHg or greater, a diastolic blood pressure (DBP) of 90 mmHg or greater, or the use of anti-hypertensive agents. Secondary hypertension was ruled out by a comprehensive medical examination in the EHT group. The MHT group consisted of 9 patients who were referred to our hospital for urgent antihypertensive treatment. Their DBPs were 120 mmHg or greater, except for 2 patients who had been given a calcium channel blocker or adrenergic inhibitors before admission. A fundoscopic examination revealed hypertensive retinopathy of grade IV in 5 patients and grade III in 4, and their plasma renin activity (PRA) was 12.4 ± 3.3 ng/h/ml. After admission, either oral or intravenous antihypertensive agents were used to urgently lower their blood pressure, and blood was collected before and after treatment for 1 to 3 wk (average, 13.9 d). The controls were 46 normotensive subjects 31 to 81 yr of age who had no abnormality on a regular health checkup. Informed consent for the collection of blood was obtained from all subjects. Blood samples were drawn from an antecubital vein early in the morning after an overnight fast and were transferred to tubes with 1 mg/ml of EDTA-2Na and 500 kallikrein inhibitory units (KIU)/ml of aprotinin. Plasma was obtained by centrifugation at 3,000 rpm for 10 min at 4°C and stored at -40°C until assay.

**Assay Procedure**

The plasma AM concentrations were measured with a specific RIA after the extraction of plasma as described previously (4). In brief, 2 ml of plasma was loaded onto a Sep-Pak C18 cartridge (Millipore-Waters, Milford, MA, USA) equilibrated with 5 ml of saline. After the cartridge was washed with 5 ml of saline and 10% of acetonitrile in 0.1% trifluoroacetic acid (TFA), the absorbed material was eluted with 4 ml of 60% acetonitrile in 0.1% TFA, lyophilized, and stored at -40°C until assay. The plasma extract was dissolved in 250 μl of RIA buffer, 50 mmol/l sodium phosphate buffer (pH 7.4) containing 0.5% BSA, 0.5% Triton X-100, 80 mmol/l NaCl, 25 mmol/l EDTA-2Na, 0.05% NaN₃, and 500 KIU/ml aprotinin. One hundred milliliters of the dissolved plasma extract was subjected to a specific RIA for human AM (hAM) as reported previously (4). The cross-reactivities of the anti-hAM antiserum used in this RIA were 100% with hAM with a C-terminal carboxyl structure (hAM-COOH), 100% with hAM(1-51)-COOH, and less than 0.5% with hAM(13-52). The intra- and inter-assay coefficients of variation for this assay were 5.0% and 4.8%, respectively.

The plasma ANP concentration was measured with a specific immunoradiometric assay for human ANP (ShionoRIA ANP kit, Shionogi & Co., Ltd., Osaka, Japan). The plasma BNP concentration was measured by a method similar to that for ANP, developed by the same company (ShionoRIA BNP kit). The accuracies as well as the detailed methods of these assays have been described previously (9).

**Statistical Methods**

All data are expressed as means ± SEM. Multiple comparisons were evaluated with either one-way analysis of variance or the chi-square test. Comparison between two variables was done by unpaired t-test. Paired t-test was used to compare the variables before and after antihypertensive treatment in the MHT group. A linear regression analysis was used to assess correlations, and the significance of correlations was further confirmed by the non-paramet-
ric test of Kendall’s method. P value less than 0.05 was considered to indicate statistical significance.

Results
The clinical profiles of the study subjects are shown in Table 1. There was no significant difference in sex or body mass index (BMI) among the three groups, while the MHT group was significantly younger than the EHT group. The SBP and DBP in the EHT patients were both significantly higher than those of the controls, and the values in the MHT patients were higher still. The serum creatinine level in the MHT patients was significantly elevated as compared with the controls and EHT patients.

Table 2 shows the plasma concentrations of AM, ANP, and BNP in the three groups. The plasma AM concentration of the EHT patients was significantly ($p < 0.01$) higher than that of the controls, and similar elevations were observed in the plasma ANP and BNP concentrations ($p < 0.05$). The plasma concentrations of these bioactive peptides were further elevated in the MHT patients, and the greatest degree of increase was seen in plasma BNP. Figure 1 shows the relation between the plasma AM concentration and blood pressure or the plasma concentrations of natriuretic peptides. The plasma AM levels significantly ($p < 0.01$) correlated with the SBP and DBP values (Fig. 1A and 1B). Interestingly, significant relations ($p < 0.01$) were also noted between the plasma concentrations of AM and those of the natriuretic peptides ANP and BNP (Fig. 1C and 1D).

In the patients with MHT, the SBP and DBP were reduced by antihypertensive treatment from $211 \pm 9$ to $163 \pm 5$ ($p < 0.01$) and from $133 \pm 7$ to $100 \pm 3$ mmHg ($p < 0.01$), respectively. As shown in Fig. 2, the elevated plasma AM levels in these patients significantly ($p < 0.05$) declined from $14.1 \pm 3.8$ to $10.4 \pm 2.8$ pmol/l. Similarly, the increased plasma levels of ANP and BNP decreased slightly after 1 to 3 wk of treatment in the MHT group. The increased plasma renin activity (PRA) in this group significantly ($p < 0.05$) declined from $12.4 \pm 3.3$ to $3.9 \pm 1.6$ ng/h/ml, while the serum creatinine level (μmol/l) after treatment ($203 \pm 68$) was slightly higher than the basal value ($191 \pm 50$).

Discussion
The present study showed increased plasma AM levels in patients with EHT, a finding consistent with previous reports on plasma AM in patients with essential or secondary hypertension (10–13). The plasma AM significantly correlated with the systolic and diastolic blood pressures, when analyzed by putting all data from the three groups together, in the present study. In contrast, Köhno et al. and Sumitomo et al. found no significant relation between the plasma AM level and blood pressure in patients with essential hypertension (12, 13). These discrepancies may have resulted from differences in subjects studied or in methods used for statistical analysis of the data. Our study also showed that the levels of plasma AM were closely related to those of ANP and BNP. Secreted from the heart, both ANP and BNP are reported to function as hormones acting against a further elevation of blood pressure in hypertensive patients, through their natriuretic and vasodilator effects (7). To date, AM has been shown to have a broad spectrum of bio-
logical actions (8), including potent vasodilatation, natriuresis, inhibition of renin and aldosterone secretion, and inhibition of vascular smooth muscle cell proliferation and migration (8). However, it is unclear whether these actions occur at physiological concentrations of plasma AM. We recently observed that a low dose of chronically infused synthetic AM significantly lowered blood pressure in conscious rats and elevated the plasma AM by 1 pmol/l, a level within the physiological range (14). Thus, at the plasma levels observed in the present study, AM may function, together with ANP and BNP, to counteract a further elevation of blood pressure in hypertensive patients.

Discovered in a human pheochromocytoma, AM has subsequently been shown to be produced in various human tissues and organs, such as normal adrenal medulla, lung, kidney, and cardiac atrium and ventricle (2, 3). In addition, AM has been shown to be synthesized and secreted from cultured cardiac myocytes, mesangial cells, and vascular endothelial and smooth muscle cells (15-18). It has been reported that both plasma ANP and BNP concentrations in hypertensive patients are higher than those in normotensive controls (19, 20), and consistent results were obtained in the present study. In hypertensive patients, ANP release from the cardiac atrium is thought to be increased by an elevated left atrial pressure resulting from reduced left ventricular compliance (7, 19). BNP secretion from the cardiac ventricle is also augmented in response to increased afterload to the heart in hypertensive patients (20). As for the source of plasma AM, many efforts have been made to specify the major organ or tissue responsible for AM production and secretion in humans; however, no study has clearly identified the site (8). Jougasaki et al. reported a significant step-up of the AM level in the coronary sinus, suggesting that AM is secreted from the human heart (21). On the other hand, we found that the plasma AM level in the femoral vein is significantly higher than that in the femoral artery, a finding that suggests secretion of AM from the vasculature of humans (unpublished data).

As mentioned above, a number of researchers showed increased plasma AM levels in patients with essential or secondary hypertension (10-13); however, there are few reports on plasma AM levels in malignant hypertension. In the present study, the plasma AM level in patients with MHT was higher than that in patients with EHT of WHO stage I or II and gradually, not sharply, decreased after antihypertensive treatment. Kohno et al. reported that the elevated plasma AM level in patients with essential hypertension was unchanged after 4 wk of antihypertensive treatment, whereas they found an intimate relation between plasma AM and serum creatinine levels (12). It is possible that reduced renal clearance of peptides accounts for the elevations of these bioactive peptides in patients with MHT, but their serum creatinine levels remained unchanged during treatment. The renin-angiotensin system is known to have an important role in the pathophysiology of MHT (22). Indeed, PRA in the patients with MHT was much higher than the upper limit of the normal range and was reduced by antihypertensive treatment. The results of in vitro experiments suggest that angiotensin II is an important factor stimulating the production and secretion of AM in cardiac myocytes and vascular smooth muscle cells (15, 18). In any case, further studies are necessary to identify the source and mechanism for the increased plasma AM levels in patients with MHT and EHT.

In summary, taken together with the biological effects of AM, our results suggest that AM may participate, along with ANP and BNP, in mechanisms counteracting a further elevation of blood pressure in patients with EHT and MHT.
References
