Clinical Studies on the Sites of Production and Clearance of Circulating Adrenomedullin in Human Subjects

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Abstract Adrenomedullin is a novel hypotensive peptide, newly discovered in pheochromocytoma. Because immunoreactive adrenomedullin is present in human plasma, adrenomedullin may play a role in regulating blood pressure. A recent report showed that human adrenomedullin mRNA is expressed not only in pheochromocytoma but also in the normal adrenal medulla, kidney, lung, and ventricle. However, whether or not these organs actually release adrenomedullin into the circulation remains unknown. To investigate the sites of production and degradation of adrenomedullin in human subjects, we obtained blood samples from various sites and measured immunoreactive adrenomedullin concentrations. In study 1, blood samples were obtained from the infrarenal inferior vena cava, suprarenal inferior vena cava, superior vena cava, right atrium, right ventricle, pulmonary artery, pulmonary capillary, left ventricle, and aorta during cardiac catheterization in 15 patients with ischemic heart disease (67±10 years). In study 2, blood samples were taken from the infrarenal inferior vena cava, suprarenal inferior vena cava, superior vena cava, right and left renal veins, and left adrenal vein in 5 hypertensive patients (42±14 years) suspected of having renovascular hypertension. In study 3, peripheral venous blood samples were obtained in 2 patients (males, 45 and 36 years old) with pheochromocytoma at rest and during hypertensive attacks. Plasma adrenomedullin concentrations were measured by a newly developed radioimmunoassay. In study 1, there were no significant differences in plasma adrenomedullin concentrations in various sites of the right-side circulation. There was no step-up of plasma adrenomedullin levels in the coronary sinus. However, the plasma concentration of adrenomedullin in aorta was slightly but significantly lower than in pulmonary artery. In study 2, there was no difference of plasma adrenomedullin levels between right and left renal veins and aorta. The plasma concentration of adrenomedullin in left adrenal vein tended to be higher than in aorta, but not significantly. In study 3, the plasma adrenomedullin concentration did not increase at rest or even during a hypertensive attack in patients with pheochromocytoma, even though epinephrine and norepinephrine rose markedly. These results suggest that the adrenal glands, lungs, kidneys, and heart may not be the main sources of circulating adrenomedullin, despite the fact that these organs express adrenomedullin mRNA. The pulmonary circulation, however, may be one of the sites of adrenomedullin clearance.

Key Words • adrenal medulla • pulmonary circulation • adrenal glands • pheochromocytoma

Methods

Study 1

We studied 15 patients with ischemic heart disease (12 men, 3 women) aged 47 to 79 years (67±10). Of these, 12 patients had old myocardial infarctions and 3 had angina pectoris. Percutaneous transluminal coronary angioplasty had been done in 9 of the 12 patients with old myocardial infarctions and in 2 of the 3 with angina pectoris. Cardiac catheterization was performed the morning after an overnight fast. Medications were stopped 12 hours before the study. A Swan-Ganz catheter, inserted in the right internal carotid vein, measured pulmonary capillary wedge (PC), pulmonary arterial (PA), right ventricular (RV), right atrial (RA), and suprarenal vena caval (SVC) pressures. Blood samples were taken simulta-
neously from the same positions. Cardiac output was measured by the thermodilution method. Under fluoroscopic control, another catheter was positioned first in the proximal coronary sinus and then in the infrarenal and suprarenal inferior vena cavae (I-IVC, S-IVC) for blood sampling. A pigtail catheter was inserted into the brachial artery to measure aortic and left ventricular (LV) pressures with simultaneous blood sampling. After pressure recordings and blood samples were obtained, left ventriculography and coronary angiography were performed routinely. LV ejection fraction was calculated by the area-length method.

**Study 2**

Five hypertensive men, aged 21 to 50 years (42±14), suspected of having renovascular hypertension were studied. Two patients were ultimately diagnosed as having renovascular hypertension and the remaining three as having essential hypertension. Angiographic examination was performed in the morning after an overnight fast. Arterial and venous catheters were inserted with the femoral approach. Blood samples were obtained from the I-IVC, S-IVC, and right and left renal veins and aorta for measurement of plasma renin activity and adrenomedullin. A catheter was also positioned in the left adrenal vein under fluoroscopic control, and blood samples were obtained. The position of the catheter tip was confirmed by digital subtraction angiography by using a small amount of contrast medium. After blood sampling, angiography or digital subtraction angiography of the aorta was routinely performed.

**Study 3**

Two patients with pheochromocytoma were studied. In case 1, a 45-year-old man was admitted to the National Cardiovascular Center with a chief complaint of palpitations. Pheochromocytoma was diagnosed on the basis of a history, computed tomography, ultrasonography, MIBG scintigram, and increased plasma concentrations of epinephrine and norepinephrine. In case 2, a 36-year-old man was admitted to the National Cardiovascular Center with a chief complaint of paroxysmal hypertension and cold sweating. Pheochromocytoma was diagnosed as described above. When both men were hospitalized in our medical department, the blood sampling for measurement of adrenomedullin, epinephrine, and norepinephrine was done in the early morning after an overnight fast. In addition, blood sampling for adrenomedullin and catecholamines was done during paroxysmal hypertensive attacks. Immediately after the blood pressure measurement and blood sampling, regitine (1 mg) was injected intravenously, and the patients' symptoms subsided with the reduction in blood pressure. Pathological examination after surgery confirmed the diagnosis of pheochromocytoma, and the patients were completely symptom free postoperatively.

Informed consent was obtained from each patient for blood sampling.

**Assay for Immunoreactive Adrenomedullin**

Blood samples (5 mL) were added to chilled tubes containing disodium EDTA (1 mg/mL) and aprotinin (500 kallikrein inhibiting units [KIU]/mL) and were centrifuged immediately at 4°C. The plasma samples were stored at −80°C until they were extracted before radioimmunoassay. Sep-Pak C18 cartridges (Millipore Corp) were prewashed with 5 mL chloroform, 5 mL methanol, 50% acetic acid containing 0.1% trifluoroacetic acid (TFA), and 5 mL saline. Plasma (1.5 mL) was acidified with 18 µL of 1 mol/L HCl, diluted with 1.5 mL saline, and then loaded onto a Sep-Pak C18 cartridge. After washing with 5 mL saline, 0.1% TFA, and 20% acetic acid containing 0.1% TFA, the absorbed materials were eluted with 4 mL of 50% acetic acid containing 0.1% TFA. The eluate was then lyophilized. When assayed, it was dissolved in radioimmunoassay buffer and the clear solution was submitted to radioimmunoassay. The radioimmunoassay for adrenomedullin was reported previously. Briefly, the radioimmunoassay buffer was 0.05 mol/L sodium phosphate buffer (pH 7.4), containing 0.5% bovine serum albumin, 0.5% Triton X-100, 0.02 mol/L NaCl, 0.025 mol/L disodium EDTA, 0.05% NaN₃, and 500 KIU/mL aprotinin. Assay procedures were performed at 4°C. Standard adrenomedullin or unknown samples (100 µL) were incubated with anti-adrenomedullin diluent (200 µL) for 12 hours; then the tracer solution (18 000 to 20 000 cpm in 100 µL) was added. After incubation for 36 hours, anti-rabbit IgG goat serum diluent (100 µL) was added. After standing for 24 hours, the tubes were centrifuged at 200g for 30 minutes at 4°C. Radioactivity of the precipitate was measured with a gamma counter. The half-maximal inhibition of radiiodinated ligand binding by adrenomedullin was observed at 4 fmol per tube. From 0.5 to 32 fmol per tube of adrenomedullin was measurable by this radioimmunoassay system. The intra-assay and interassay coefficients of variance were 5% and 8%, respectively. The radioimmunoassay had 100% cross-reactivity with the Met sulfoxide form of adrenomedullin but had only 2% and 0.5% cross-reactivity with adrenomedullin (13-52) and adrenomedullin (1-12). These data indicate that the antibody recognizes the entire adrenomedullin molecule.

**Statistics**

Data are expressed as mean±SD. Comparisons of plasma adrenomedullin concentrations between the aorta and the PA, left adrenal vein, or the renal vein were evaluated with a paired t test. A probability value less than .05 was considered statistically significant.

**Results**

**Study 1**

Patient characteristics and cardiac catheterization data for the 15 patients with ischemic heart disease are presented in the Table. Heart rate was 70±7 beats per minute and mean arterial pressure was 102±19 mm Hg. Intracardiac pressures were slightly elevated: mean pulmonary capillary pressure was 12±5 mm Hg and LV end-diastolic pressure was 14±5 mm Hg. No patients had uncompensated heart failure at the time of cardiac catheterization. The plasma concentrations of adrenomedullin in the 15 patients with ischemic heart disease are presented in Fig 1. There seemed to be no
Values are mean±SD. SVC indicates superior vena cava; I-IVC, infrarenal inferior vena cava; S-IVC, suprarenal inferior vena cava; RA, right atrium; CS, coronary sinus; RV, right ventricle; PA, pulmonary artery; PCW, pulmonary capillary wedge; LV, left ventricle; and AO, aorta.

Fig 1. Bar graph shows plasma adrenomedullin concentrations at various sites in 15 patients with ischemic heart disease. Values are mean±SD. SVC indicates superior vena cava; I-IVC, infrarenal inferior vena cava; S-IVC, suprarenal inferior vena cava; RA, right atrium; CS, coronary sinus; RV, right ventricle; PA, pulmonary artery; PCW, pulmonary capillary wedge; LV, left ventricle; and AO, aorta.

Plasma concentrations of adrenomedullin in the renal and adrenal veins are presented in Fig 2. Plasma adrenomedullin concentrations in right and left renal veins were 3.09±1.59 and 2.91±1.48, respectively. The difference in plasma adrenomedullin concentrations between the aorta (2.67±1.73) and the renal vein was not significant. Plasma adrenomedullin concentrations were also similar in the I-IVC (3.19±1.29) and S-IVC (3.36±1.43). Plasma adrenomedullin concentration in the left adrenal vein (4.14±1.43) tended to be higher than in the aorta, but the difference was not significant.

Study 2

Plasma adrenomedullin concentrations in the renal vein and adrenal veins are presented in Fig 2. Plasma adrenomedullin concentrations in right and left renal veins were 3.09±1.59 and 2.91±1.48, respectively. The difference in plasma adrenomedullin concentrations between the aorta (2.67±1.73) and the renal vein was not significant. Plasma adrenomedullin concentrations were also similar in the I-IVC (3.19±1.29) and S-IVC (3.36±1.43). Plasma adrenomedullin concentration in the left adrenal vein (4.14±1.43) tended to be higher than in the aorta, but the difference was not significant.

Study 3

In case 1 with pheochromocytoma, plasma adrenomedullin concentration before surgical treatment was 2.25 pmol/L and blood pressure was 126/76 mm Hg at rest. Our normal mean adrenomedullin level in healthy subjects is 2.44±0.82 pmol/L (n=12, 42±9 years). Thus, plasma adrenomedullin concentration was within a normal range in this pheochromocytoma patient at rest, although plasma epinephrine, which was 610 ng/L (normal, <50), and plasma norepinephrine, which was 970 ng/L (normal, <310), were markedly elevated. During paroxysmal hypertensive attacks, the patient complained of palpitations and cold sweating. Blood pressure rose to 200/100 mm Hg, and epinephrine and norepinephrine increased (2440 and 1800, respectively) (Fig 3, top). However, plasma adrenomedullin concentration did not increase (2.00). In case 2, the plasma adrenomedullin concentration before surgical treatment was also not elevated at rest (2.03). Blood pressure was 136/80 mm Hg; plasma epinephrine concentration was 2400 and norepinephrine concentration was 2740, both remarkably elevated. During a hypertensive attack, blood pressure rose to 180/100 mm Hg, with a concomitant elevation of epinephrine and norepinephrine (4760 and 4590, respectively); however, the plasma adrenomedullin concentration did not change (2.03) (Fig 3, bottom).

Discussion

In the present study, which investigated the sites of production and clearance of adrenomedullin in humans, we took blood samples from various sites and measured plasma adrenomedullin concentration using our newly developed radioimmunoassay. The high specificity and sensitivity of the radioimmunoassay for adrenomedullin enabled us to determine the plasma concentration of immunoreactive adrenomedullin. Because our previous reports showed that human adrenomedullin mRNA is highly expressed in the adrenal glands, kidneys, lungs, and heart, we investigated mRNA production sites specifically in these four organs. There was no step-up of plasma adrenomedullin concentration in the coronary sinus. There were no significant differences in plasma adrenomedullin concentrations among the various sites in the right-side circulation, including the I-IVC, S-IVC, SVC, RA, RV, and PA. Mean plasma adrenomedullin concentration in the aorta was slightly but significantly lower than in the PA. There was no step-up of plasma adrenomedullin concentrations in the renal vein. Plasma adrenomedullin concentrations in the left renal vein tended to be higher than in the aorta, but the differences did not reach statistical significance. These results suggest that the adrenal glands are not the main source of circulating adrenomedullin, although they may secrete some adrenomedullin into the circulation, and that the lungs may be one site of adrenomedullin clearance.

Adrenomedullin is a newly discovered peptide from human pheochromocytoma that was identified by monitoring cyclic AMP activity.1 Adrenomedullin consists of 52 amino acids and has little homology with CGRP and amylin. In rats, the hypotensive effect of adrenomedullin is as strong as CGRP, which is one of the strongest vasore-
laxant peptides. Because immunoreactive adrenomedullin is also present in human plasma, it may play a role in circulatory control. CGRP is known to be distributed throughout the peripheral and central nervous systems. However, tissue contents and mRNA expression of adrenomedullin in the brain are barely detectable. Thus, we speculated that adrenomedullin may have a different mechanism of circulatory control than CGRP.

It is important to know the sites of production and clearance of circulating adrenomedullin to better understand the factors influencing plasma adrenomedullin concentrations. Therefore, we investigated the sites of the clearance and production of adrenomedullin in the present study, specifically in the organs in which adrenomedullin mRNA is highly expressed, and we measured plasma adrenomedullin concentrations in veins and arteries across these organs. A recent in vitro study showed that carbachol caused a rapid and transient secretion of immunoreactive adrenomedullin and catecholamine in cultured bovine adrenal medullary cells. These findings suggest that the adrenal glands may be one source of circulating adrenomedullin. Furthermore, tissue adrenomedullin concentration is much higher in the adrenal gland than in other tissues, including the lungs, kidneys, and heart, in which adrenomedullin mRNA is also highly expressed. In the present study, however, plasma adrenomedullin concentration was not significantly elevated in patients with pheochromocytoma. Even during hypertensive attacks, the plasma concentration of adrenomedullin did not increase, although plasma norepinephrine and epinephrine rose markedly. These findings further support the hypothesis that the adrenal glands do not greatly contribute to circulating adrenomedullin levels in humans, despite adrenomedullin having been discovered in pheochromocytoma and named accordingly.

There was no significant difference in plasma adrenomedullin concentrations between the coronary sinus and the aorta, and there was no step-up of plasma adrenomedullin concentration in the RA or LV. In contrast, the heart secretes atrial natriuretic peptide (ANP) into both coronary sinus and the Thevesian vein so that plasma ANP levels are elevated in both the RA and the left side of the heart. The present data indicate that the heart does not release a significant amount of adrenomedullin into the circulation, although adrenomedullin mRNA is highly expressed in the heart like ANP. The kidney is another site in which adrenomedullin mRNA is highly expressed. However, there were no differences in plasma adrenomedullin concentrations between the aorta and either renal vein. In this study, two patients with renovascular hypertension had narrowing of the renal artery of more than 75%. There was no difference in plasma adrenomedullin concentrations between the right and left renal veins in renovascular hypertensive patients.

In the present study, there was a significant difference in plasma adrenomedullin concentrations between the pulmonary artery and the aorta. This evidence suggests that the pulmonary circulation may be an important site of endogenous plasma adrenomedullin clearance in humans, even though adrenomedullin mRNA is highly expressed and tissue concentration of adrenomedullin is
relatively high in the lungs. The mechanisms by which plasma adrenomedullin is removed during passage through the lungs remain to be elucidated. Possible mechanisms for adrenomedullin removal include receptor binding, diffusion into lymphatics, and/or tissue uptake and degradation. To date, there are no studies of adrenomedullin clearance.

In summary, we measured plasma adrenomedullin concentrations in various sites to investigate the clearance and production of circulating adrenomedullin in humans. There were no obvious sites of adrenomedullin secretion in the subjects studied, in contrast to the established sites of hormone secretion in humans. This suggests that adrenomedullin may be produced and metabolized as a local hormone. There is a significant reduction in plasma adrenomedullin concentrations in the left side of the heart relative to the right side. Pulmonary circulation may be one site of endogenous adrenomedullin clearance, and adrenomedullin receptors may be widely distributed to the lungs. Pharmacokinetics of adrenomedullin including degradation and receptor distribution and analysis will be necessary.

Acknowledgments

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References

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