obtained. Thirty-five patients, undergoing cardiovascular surgery using CPB were studied. They were ASA physical status III–IV, aged 51–70 years and weighed 45–60 kg. General anesthesia was induced with 5 mg of midazolam, 6–10 μg/kg fentanyl and vecuronium 10 mg and maintained with fentanyl, midazolam, propofol and inhalational anesthetics. The inhalational agents were not administered during study but, if necessary, were given after the study. The lungs were mechanically ventilated with a tidal volume of 10 ml/kg and the respiratory rate was adjusted to maintain $PaCO_2$ at 35–45 mmHg. A radial arterial catheter, a pulmonary catheter, and a central venous pressure (CVP) catheter were placed using a sterile technique. Systemic and pulmonary arterial pressures, CVP, heart rate, peripheral oxygen saturation, and end-tidal carbon dioxide concentration were monitored continuously throughout the operation. Cardiac output was measured using the thermodilution technique. Acetated or lactated Ringer's solution was infused at a rate of 10–15 ml/kg/h to maintain a CVP of 5–7 mmHg and pulmonary capillary wedge pressure (PCWP) of 8–12 mmHg until CPB was introduced. The protocol of CPB in our institute included a nonpulsatile pump flow of 2.0–3.0 l/min m^{-2} and mild hypothermia (32–34°C nasopharyngeal temperature). $PaCO_2$ was not corrected for temperature (α-stat regulation). The hematocrit was maintained at 20–25% during CPB. Mechanical ventilation was interrupted and the lungs remained collapsed during cross-clamping of the aorta and were resumed after declamping of the aorta and the return of spontaneous cardiac rhythm. A continuous infusion of catecholamines, if required, was given during weaning from CPB. Both radial and pulmonary artery blood were sampled to determine arterial pH, PaO_2, $PaCO_2$, HCO_3, base excess, arterial oxygen saturation, hemoglobin concentration and hematocrit, using a blood gas analyzer (ABL-625; Raiometer, Copenhagen, Denmark).

To investigate the role of the pulmonary circulation in the clearance of adrenomedullin, we obtained simultaneous blood samples from the left atrium by direct puncture using a 22-gauge needle and the pulmonary artery through the pulmonary artery catheter. The blood sampling and data collection were performed at the following intervals: just before systemic heparinization (period A); during pulmonary reperfusion and before clamping of the bypass lines (drainage from bicaval cannulation was reduced and CVP was maintained about 4 mmHg) (period B); and after CPB and just before the sternum was closed (period C). Data collection included hemodynamic variables (systemic blood pressure, CVP, pulmonary artery pressure, heart rate), cardiac output, body temperature and hematocrit. Pulmonary plasma flow was calculated using the hematocrit value. For measurement of adrenomedullin, 3 ml of blood was collected into chilled glass tubes containing disodium ethylenediaminetetraacetic acid (1 mg/ml) and aprotinin (500 U/ml) and were then centrifuged at 4°C for 15 min. The plasma samples were stored at −40°C until assay of adrenomedullin. Plasma adrenomedullin concentrations were determined by two immunoradiometric assay kits specific for human total and mature adrenomedullin (13). We calculated intermediate adrenomedullin as the difference between total adrenomedullin and mature adrenomedullin. The pulmonary gradient of adrenomedullin was calculated as $(AM_{PA} - AM_{LA})/AM_{PA}$, where AM_{PA} and AM_{LA} are adrenomedullin concentrations in the pulmonary artery and left atrium. This parameter shows relative change of adrenomedullin during pulmonary circulation. On the other hand, in order to evaluate absolute change of adrenomedullin during pulmonary circulation, the pulmonary clearance quantity of adrenomedullin was $(AM_{PA} - AM_{LA}) \times$ pulmonary plasma flow.

The data are expressed as the mean ± SEM. The results of the multiple intervals (period A, B and C) were analyzed by one-way analysis of variance and comparisons between intervals were assessed using the Student–Newman–Keuls test. Differences in plasma adrenomedullin concentrations between the pulmonary artery and left atrium at each period and pulmonary clearance quantity of adrenomedullin between period A and C were compared using a paired or unpaired *t*-test, as appropriate. Regression analysis was performed to examine the relationship between left atrial adrenomedullin concentrations and pulmonary or systemic hemodynamic variables, or pulmonary clearance quantity of adrenomedullin and the duration of aortic cross-clamping. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics are shown in Table 1. Plasma mature adrenomedullin concentrations in the pulmonary artery were significantly higher than those in the left atrium before CPB and before sternal closing but not during pulmonary reperfusion (Fig. 1). Plasma intermediate adrenomedullin concentrations in the pulmonary artery were significantly higher than those in the left atrium before CPB (period A), but there was no significant difference in the plasma

Table 1

Patient demographics.

	Period A	Period B	Period C
n	9	13	13
Age (year)	63 ± 5	63 ± 2	63 ± 3
Height (cm)	162 ± 2	163 ± 2	155 ± 8
Weight (kg)	64 ± 3	58 ± 2	68 ± 8
Gender (M:F)	7:2	10:3	10:3
Temperature (°C)	36.3 ± 0.3	35.4 ± 0.1	35.7 ± 0.3
Primary diagnosis			
Coronary artery disease	6	7	5
Valve disease	3	6	8
ASA class			
II	4	6	5
III	4	6	7
IV	1	1	1
Smoking	1	3	2
Pre-operative medication			
Ca blocker	4	6	6
β Blocker	1	3	3
Nitrates	5	7	7
Intra-operative catecholamine			
Dopamine	0	11	11
None	9	2	2
Intra-operative vasodilators			
Nitroglycerine	7	11	9
Diltiazem	4	1	1
None	2	2	4

n = the number of observations.

intermediate adrenomedullin concentrations between the two sampling sites during pulmonary reperfusion (period B) and before sternal closing (period C) (Fig. 2). The pulmonary gradient of mature, but not intermediate, adrenomedullin after cardiopulmonary bypass was restored to the level comparable to that before cardiopulmonary bypass (Table 2). The pulmonary clearance quantity of mature adrenomedullin after CPB significantly increased compared with that before CPB, although the pulmonary clearance quantity of intermediate adrenomedullin after CPB did not change significantly (Table 2). There was no significant correlation between the pulmonary clearance quantity of any type of adrenomedullin and the duration of aortic cross-clamping after CPB (data were not shown) and there was no significant

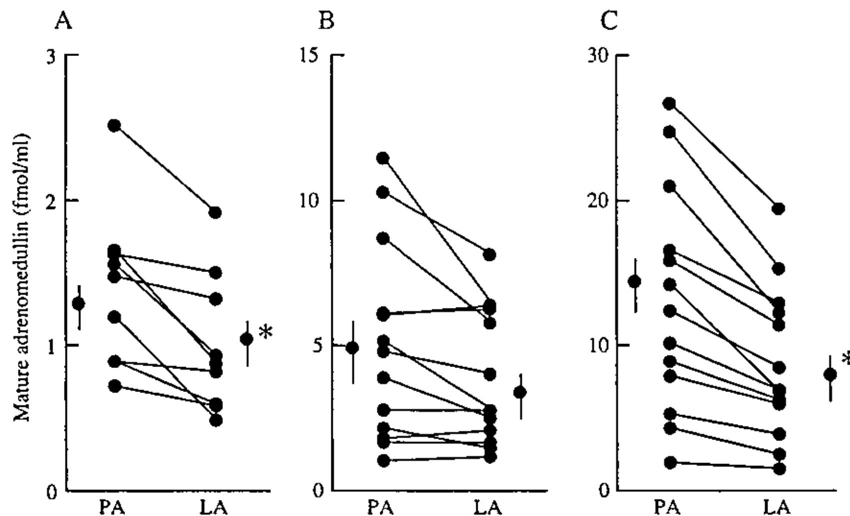

*Fig. 1. Plasma mature adrenomedullin concentration in the pulmonary artery and the left atrium at the three study periods. A. just before systemic heparinization, B. during pulmonary reperfusion and before weaning from cardiopulmonary bypass, C. just before the sternum was closed. Values are mean ± SEM. Orders of magnitude on the y-axis are different in each period. *P < 0.05 compared with the value of the pulmonary artery.*

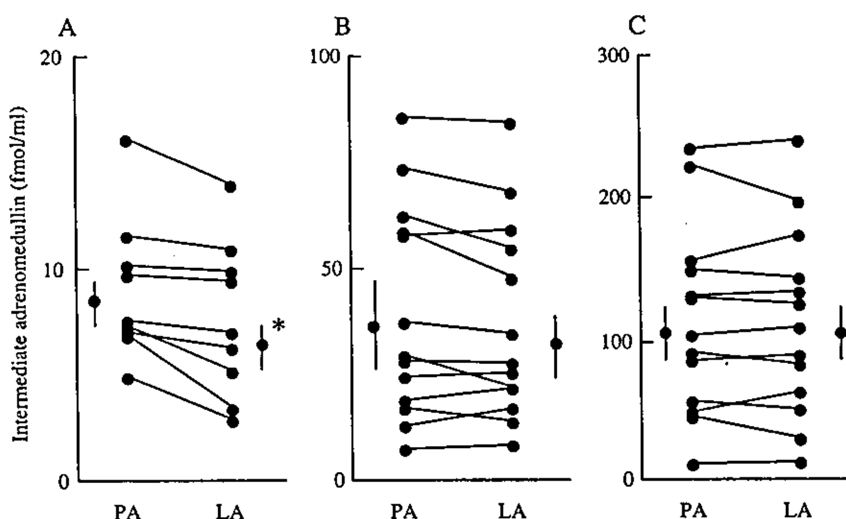

*Fig. 2. Plasma intermediate adrenomedullin concentration in the pulmonary artery and the left atrium at the three study periods. A. just before systemic heparinization, B. during pulmonary reperfusion and before weaning from cardiopulmonary bypass, C. just before the sternum was closed. Values are mean ± SEM. Orders of magnitude on the y-axis are different in each period. *$P < 0.05$ compared with the value of the pulmonary artery.*

correlation between pulmonary artery or left atrial adrenomedullin concentrations and pulmonary or systemic hemodynamic variables, respectively (data not shown). Furthermore, there was no significant correlation between the pulmonary clearance quantity of any type of adrenomedullin and the dosages of catecholamine (dopamine) or vasodilators after CPB (period C) (data were not shown).

Discussion

Adrenomedullin is a novel hypotensive peptide and is known to have various physiological effects, including diuresis and natriuresis, inhibition of aldosterone secretion and increase of cardiac output (14–16). Furthermore, the adrenomedullin concentration has been reported to be increased in proportion to the severity of cardiovascular diseases, including myocardial infarction, congestive heart failure and systemic hypertension (2–5). These data suggest that adrenomedullin may contribute to the regulation of hemodynamic homeostasis as a circulating hormone as well as a paracrine and autocrine factor.

Our results show that the lungs are a clearance site of both mature and intermediate adrenomedullin before CPB; this clearance was lost during pulmonary reperfusion, and the pulmonary clearance of mature adrenomedullin, but not intermediate adrenomedullin, was recovered after CPB. In addition, the absolute pulmonary clearance of mature adrenomedullin after CPB significantly increased after CPB.

Table 2

Pulmonary gradient (upper panel) and pulmonary clearance quantity (lower panel) of adrenomedullin (AM) just before systemic heparinization (period A), during pulmonary reperfusion and before weaning from cardiopulmonary bypass (period B), and after cardiopulmonary bypass and just before the sternum was closed (period C) (Mean ± SEM; the number of observations are shown in parentheses).

	Period A (9)	Period B (13)	Period C (13)
Pulmonary gradient			
Mature adrenomedullin*	0.27 ± 0.06	0.13 ± 0.06	0.32 ± 0.03
Intermediate adrenomedullin*	0.18 ± 0.06	0.03 ± 0.05†	0.01 ± 0.04†
Pulmonary clearance quantity (nmol/min)			
Mature adrenomedullin	1.26 ± 0.32	-	14.4 ± 2.82†
Intermediate adrenomedullin	4.19 ± 1.06	-	−4.04 ± 12.24

Pulmonary clearance fraction of AM = $(AM_{PA} - AM_{LA})/AM_{PA}$, where AM_{PA} and AM_{LA} are AM concentrations in the pulmonary artery and left atrium.
Pulmonary clearance quantity of AM = $(AM_{PA} - AM_{LA}) \times$ pulmonary plasma flow.
*$P < 0.05$ significant correlation with period multiples.
†$P < 0.05$ compared with the value at period A.

It is well known that the lungs have a significant metabolic function for many substances, including catecholamines and peptides, and this function is altered by various kinds of lung injuries (17, 18). In clinical situations, cardiopulmonary bypass is one of the most common procedures to promote lung injuries (19, 20) and several mechanisms including complement activation, endotoxin release and cytokine activation are known to be involved in the injury (21, 22). In addition, it may be possible that pulmonary ischemia during CPB and reperfusion after CPB affect the pulmonary metabolism. Nishikimi et al. (9) systemically examined the sites of secretion and clearance of adrenomedullin in humans and demonstrated that the lung is a site of clearance of adrenomedullin. Recently, Hirayama et al. (10) reported pulmonary clearance of mature adrenomedullin in humans. The present study also showed that both mature and intermediate adrenomedullin were removed during pulmonary circulation before CPB (period A) (Figs 1, 2). By contrast, the pulmonary clearance of adrenomedullin was not observed during pulmonary reperfusion (period B) (Figs 1, 2), suggesting that lung injury due to CPB impaired the pulmonary clearance of adrenomedullin. Furthermore, complete recovery of pulmonary clearance of mature, but not of intermediate adrenomedullin was observed after CPB (Figs 1, 2, Table 2). Considering that mature adrenomedullin is a biologically active type of adrenomedullin (11), the metabolic function of lungs for physiologically active substances may recover before that for inactive substances after CPB.

Although the pulmonary gradient of mature adrenomedullin after CPB was comparable with that before CPB, pulmonary clearance quantity of mature adrenomedullin after CPB was significantly enhanced after CPB because of increased mature adrenomedullin concentration (Fig. 1, Table 2). On the other hand, pulmonary clearance quantity of intermediate adrenomedullin after CPB was not significantly different from that before CPB (Table 2). These data also indicate that absolute clearance during pulmonary circulation is exclusively enhanced for the biologically active type of adrenomedullin.

The detailed mechanism involved in decreased pulmonary clearance during reperfusion and increased clearance after CPB of mature adrenomedullin is obscure. Oman et al. (23) reported that saturation of pulmonary adrenomedullin receptors by increased plasma adrenomedullin is one possible mechanism involved in reducing pulmonary clearance of adrenomedullin during sepsis. In addition, they demonstrated up-regulation of pulmonary adrenomedullin receptors as a compensatory mechanism to facilitate clearance of the increased plasma adrenomedullin. These phenomena may explain our data during pulmonary reperfusion and after CPB, that is, increased plasma adrenomedullin during reperfusion may facilitate saturation of adrenomedullin receptors and decreased pulmonary clearance. On the other hand, up-regulation of adrenomedullin receptors after CPB may induce the increased clearance of mature adrenomedullin.

In conclusion, cardiopulmonary bypass affects the transpulmonary gradient of adrenomedullin in humans. Both mature and intermediate adrenomedullin are removed during pulmonary circulation before cardiopulmonary bypass; these metabolic functions are lost during pulmonary reperfusion, and the pulmonary clearance of mature, but not intermediate adrenomedullin is recovered after CPB. In addition, the absolute pulmonary clearance of mature, but not immature, adrenomedullin is enhanced after cardiopulmonary bypass.

References

1. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 1993; **192**: 553–60.
2. Ishimitsu T, Nishikimi T, Saito Y, Kitamura K, Eto T, Kangawa K et al. Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. *J Clin Invest* 1994; **94**: 2158–61.
3. Jougasaki M, Grantham JA, Redfield MM, Burnett JC Jr. Regulation of cardiac adrenomedullin in heart failure. *Peptides* 2001; **22**: 1841–50.
4. Kobayashi K, Kitamura K, Hirayama N, Date H, Kashiwagi T, Ikushima I et al. Increased plasma adrenomedullin levels in chronic congestive heart failure. *Am Heart J* 1996; **131**: 994–8.
5. Nishikimi T, Saito Y, Kitamura K, Ishimitsu T, Eto T, Kangawa K et al. Increased plasma levels of adrenomedullin in patients with heart failure. *J Am Coll Cardiol* 1995; **26**: 1424–31.
6. Yoshibayashi M, Kamiya T, Kitamura K, Saito Y, Kangawa K, Nishikimi T et al. Plasma levels of adrenomedullin in primary and secondary pulmonary hypertension in patients < 20 years of age. *Am J Cardiol* 1997; 79: 1556–8.
7. Nishikimi T, Hayashi Y, Iribu G, Takishita S, Kosakai Y, Minamino N et al. Increased plasma adrenomedullin concentrations during cardiac surgery. *Clin Sci (Lond)* 1998; **94**: 585–90.
8. Inoue S, Hayashi Y, Ohnishi Y, Kikumoto K, Minamino N, Kangawa K et al. Cerebral production of adrenomedullin after hypothermic cardiopulmonary bypass in adult cardiac surgical patients. *Anesth Analg* 1999; **88**: 1030–5.
9. Nishikimi T, Kitamura K, Saito Y, Shimada K, Ishimitsu T, Takamiya M et al. Clinical studies on the sites of production and clearance of circulating adrenomedullin in human subjects. *Hypertension* 1994; **24**: 600–4.

10. Hirayama N, Kitamura K, Imamura T, Kato J, Koiwaya Y, Eto T. Secretion and clearance of the mature form of adrenomedullin in humans. *Life Sci* 1999; **64**: 2505–9.
11. Kitamura K, Kato J, Kawamoto M, Tanaka M, Chino N, Kangawa K et al. The intermediate form of glycine-extended adrenomedullin is the major circulating molecular form in human plasma. *Biochem Biophys Res Commun* 1998; **244**: 551-5.
12. Hirayama N, Kitamura K, Imamura T, Kato J, Koiwaya Y, Tsuji T et al. Molecular forms of circulating adrenomedullin in patients with congestive heart failure. *J Endocrinol* 1999; **160**: 297–303.
13. Ohta H, Tsuji T, Asai S, Tanizaki S, Sasakura K, Teraoka H et al. A simple immunoradiometric assay for measuring the entire molecules of adrenomedullin in human plasma. *Clinica Chimica Acta* 1999; **287**: 131–43.
14. Ebara T, Miura K, Okumura M, Natsuura T, Kim S, Yukimura T et al. Effect of adrenomedullin on renal hemodynamics and functions in dogs. *Eur J Pharmacol* 1994; **263**: 69–73.
15. Yamaguchi T, Baba K, Doi Y, Yano K. Effect of adrenomedullin on aldosterone secretion by dispersed rat adrenal zona glomerulosa cells. *Life Sci* 1995; **56**: 379–87.
16. Fukuhara M, Tsuchihashi T, Abe I, Fujishima M. Cardiovascular and neurohormonal effects of intravenous adrenomedullin in conscious rabbits. *Am J Physiol* 1995; **269**: R1289–93.
17. Gills CN. Pharmacological aspects of metabolic processes in the pulmonary microcirculation. *Ann Rev Pharmacol Toxicol* 1986; **26**: 183–200.
18. Mellins RB. Metabolic function of the lungs and their clinical relevance. *Am J Roentogenol* 1982; **138**: 999–1009.
19. Moat NE, Shore DF, Evans TW. Organ dysfunction and cardiopulmonary bypass: the role of complement and complement regulatory proteins. *Eur J Cardiothorac Surg* 1993; **7**: 563–73.
20. Ratliff NB, Young WG, Hachel DB, Mikat E, Wilson JW. Pulmonary injury secondary to extracorporeal circulation; an ultrastructural study. *J Thorac Cardiovasc Surg* 1973; **65**: 425–32.
21. Boyle EM, Pohlman TH, Johnson MC, Verrier ED. Endothelial cell injury in cardiovascular surgery: the systemic inflammatory response. *Ann Thorac Surg* 1997; **63**: 269–76.
22. Hall RI, Stafford M, Rocker G. The systemic inflammatory response to cardiopulmonary bypass: Pathophysiological, therapeutic, and pharmacological considerations. *Anesth Analg* 1997; **85**: 766–82.
23. Ornan DA, Chaudry IH, Wang P. Saturation of adrenomedullin receptors plays an important role reducing pulmonary clearance of adrenomedullin during the late stage of sepsis. *Biochim Biophys Acta* 2002; **1586**: 199–306.

Address:
Yukio Hayashi
Department of anesthesiology
Osaka University Faculty of Medicine (D-7)
2-2, Yamada-oka
Suita Osaka 565-0871
Japan
e-mail: yhayashi@anes.med.osaka-u.ac.jp

Different Secretion Patterns of Two Molecular Forms of Cardiac Adrenomedullin in Pressure- and Volume-Overloaded Human Heart Failure

TOSHIO NISHIKIMI, MD, PhD,[1] HIROSHI ASAKAWA, MD, PhD,[1] HIROSHI IIDA, MD, PhD,[2] YASUSHI MATSUSHITA, MD, PhD,[2] IKUKO SHIBASAKI, MD, PhD,[2] KAZUYOSHI TADOKORO, MD,[1] YOSUKE MORI, MD, PhD,[1] HIDEAKI MORI, MD, PhD,[2] YOSHIHIKO MOCHIZUKI, MD, PhD,[2] YOSHITAKA OKAMURA, MD, PhD,[2] SHINICHIRO MIYOSHI, MD, PhD,[2] KENJI KANGAWA, PhD,[3] AND HIROAKI MATSUOKA, MD, PhD[1]

Tochigi, Japan; Osaka, Japan

ABSTRACT

Background: In the final step of production of adrenomedullin (AM), an inactive intermediate form of glycine-extended AM (AM-glycine) is converted to the active mature form of adrenomedullin (AM-mature) by enzymatic amidation. Recent studies have revealed that AM-mature and AM-glycine circulate in human plasma. In this study, we investigated the differences of the concentrations of cardiac AM between pressure-overloaded (PO) heart failure (HF) and volume-overloaded (VO)-HF in humans.
Methods and Results: We measured AM-mature and AM-glycine by immunoradiometric assays in pericardial fluid and plasma in 38 patients who underwent valve replacement surgery (PO-HF: aortic stenosis, n = 14; VO-HF: aortic or mitral regurgitation, n = 24). Stable coronary artery disease with normal left ventricular function served as the control (n = 24). Plasma AM-mature (VO-HF: +59%, PO-HF: +65%, $P < .05$) and AM-glycine (VO-HF: +43%, PO-HF: +50%, $P < 0.05$) were similarly higher in the 2 HF groups than in the control group. Interestingly, pericardial fluid AM-mature was markedly higher than that in plasma (control: +789%, VO-HF: +1050%, PO-HF: +1745%, all $P < .001$). Pericardial fluid AM-mature was higher in VO-HF (+106%, $P < .01$) than in controls and they were further increased in PO-HF (+243%, $P < .05$). Pericardial fluid molecular forms of AM correlated with left ventricular systolic pressure, but not with left ventricular end-diastolic volume index in PO-HF. In contrast, they correlated with left ventricular end-diastolic volume index, but not with left ventricular systolic pressure in VO-HF.
Conclusion: These results suggest that cardiac AM is differently regulated from plasma AM and that cardiac AM production is upregulated in both types of HF in response to each different stimulus.
Key Words: Adrenomedullin, Heart failure, Plasma, Pericardial fluid, Valvular, Pressure overload, Volume overload.

From the [1]Department of Hypertension and Cardiorenal Medicine and [2]Department of Thoracic Surgery, Dokkyo University School of Medicine, Mibu, Tochigi, Japan; [3]Research Institute, National Cardiovascular Center, Fujishirodai, Suita, Osaka, Japan.

Manuscript received May 30, 2003; revised manuscript received October 31, 2003; revised manuscript accepted December 1, 2003.

Reprint requests: Toshio Nishikimi, MD, PhD, Department of Hypertension and Cardiorenal Medicine, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan.

Supported in part by Scientific Research Grant-in-Aid 14570692 from the Ministry of Education, Culture, Sports, Science and Technology, by the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan, and by the Seki Minato Prize.

1071-9164/$ - see front matter

© *2004 Elsevier Inc. All rights reserved.*

doi:10.1016/j.cardfail.2003.12.002

A novel vasodilating peptide, adrenomedullin (AM) and its receptor are highly expressed in the heart.[1,2] Recent studies demonstrated that cardiac myocytes and fibroblasts produce and secrete AM[3,4] and that AM inhibits collagen and DNA synthesis in cardiac nonmyocytes and inhibits myocyte hypertrophy.[5,6] Furthermore, it is reported that AM has positive inotropic effects in vitro and in vivo.[7,8] These results suggest that AM locally released in the heart plays a role in the maintenance of cardiac function or in cardiac structural remodeling as an autocrine or paracrine factor. Indeed, we and other groups recently reported that cardiac AM and its gene expression are increased in pressure-overloaded (PO) and volume-overloaded (VO) heart failure (HF) in rats.[9-11]

On the other hand, recent studies revealed that 2 molecular forms of AM, an active form of mature AM (AM-mature) and an intermediate inactive form of glycine-extended AM (AM-glycine), circulate in human plasma and that the major circulating form is AM-glycine.[12,13] We reported very recently that the AM-mature/AM-total (AM-total = AM-mature + AM-glycine) ratio is much higher in cardiac tissue than in plasma in rats and that the cardiac tissue AM-mature/AM-total ratio is increased in cardiac hypertrophy or failing heart compared with that in the normal heart.[14,15]

However, little information is available about the active and inactive forms of AM of cardiac origin in human HF. It is generally considered that pericardial fluid is not only an ultrafiltrate of plasma, but also a transudate from the cardiac interstitium.[16,17] Indeed, we recently reported that AM levels in pericardial fluid are higher in plasma in patients with acute coronary syndrome.[18] Thus the purpose of the present study was: (1) to characterize the 2 molecular forms of AM in plasma and pericardial fluid in PO-HF and VO-HF and (2) to investigate their relations to hemodynamic parameters to obtain clinical evidence that AM acts as autocrine and paracrine factor in the failing myocardium in human PO-HF and VO-HF.

Methods

Patients

We studied 38 consecutive Japanese patients (26 men, 12 women) ages 37 to 80 years (mean age ± SD, 66 ± 10 years) who had valvular heart disease and underwent single valve replacement. The diagnoses of aortic stenosis, aortic regurgitation, and mitral regurgitation were determined by cardiac catheterization and echocardiography. In this study, aortic stenosis served as PO-HF and aortic regurgitation or mitral regurgitation served as VO-HF. Stable patients with ischemic heart disease with normal left ventricular function (LVEF >55) served as controls (66 ± 8 years; 15 men, 9 women). The diagnosis of ischemic heart disease was determined by history, electrocardiography, and coronary angiography.

All patients were evaluated routinely before the operation by left- and right-sided cardiac catheterization including coronary angiography. Left ventriculography was also performed in all cases except for the emergent cases or infective endocarditis (aortic stenosis: n = 3, aortic regurgitation: n = 2, mitral regurgitation: n = 1). Right atrial pressure, pulmonary arterial systolic pressure, pulmonary capillary wedge pressure, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure, aortic pressure, and cardiac index were measured. Pressure gradient across the aortic valve and aortic valve area was calculated. LVEF, left ventricular end-diastolic volume index (LVEDVI), and left ventricular end-systolic volume index (LVESVI) were calculated using the area-length method. We excluded patients with decompensated congestive HF, renal dysfunction (serum creatinine level >283 μmol/L), and primary lung disease.

The investigation conformed with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from each patient and the protocol was approved by the ethics committee of our institute.

Sampling of Plasma and Pericardial Fluid

In the operating room, immediately after the incision of the pericardium, undiluted samples of pericardial fluid were obtained without heparinization.[16–18] Blood was simultaneously withdrawn from the cannulated brachial artery. Samples were added to sterile chilled tubes containing EDTA (1 mg/mL) and aprotinin (500 KIU/mL) and immediately centrifuged at 3000 rpm (4°C) for 10 minutes and stored in sample tubes at −80°C.

Assays for AM-Mature, AM-total, Atrial Natriuretic Peptide, Brain Natriuretic Peptide, and Others

Both AM-mature and AM-total were measured by immunoradiometric assays using specific kits (AM mature RIA SHIONOGI, AM RIA SHIONOGI, Shionogi Co, Ltd, Osaka, Japan).[19,20] These assays measure human AM-mature or AM-total by sandwiching it between 2 monoclonal antibodies without plasma extraction. The assay's minimal detectable quantity of human AM-mature or AM-total is .5 pmol/L for both kits. AM-glycine was calculated with the following formula: AM-glycine = AM-total − AM-mature.[12,13] The pericardial fluid and plasma concentrations of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were measured using specific immunoradiometric assay kits (Shionogi Co., Ltd., Osaka, Japan) as previously reported.[21] Total protein and albumin levels in pericardial fluid were measured by standard technique.

Statistical Analysis

All values are expressed as mean ± SD. When we compared 3 groups (control, VO-HF, and PO-HF), we used 1-way analysis of variance. If the F value was found to be significant, the data were compared using Bonferroni's multiple comparison test. Correlation coefficients were calculated using linear regression analysis. Log transformation was used to normalize the distribution of pericardial fluid and plasma ANP and BNP levels because ANP and BNP are not normally distributed. When we compared plasma AM and pericardial AM in each group, we used paired Student's t-test. A value of $P < .05$ was considered significant. Statistical analysis was performed using STATVIEW version 5 (Abacus Concepts, Berkeley, CA).

Results

Clinical Characteristics in PO-HF and VO-HF

The clinical characteristics of the control, VO-HF, and PO-HF groups are presented in Table 1. there were no significant differences in age or sex among the 3 groups. Both HF groups had lower LVEF, and higher LVESVI, pulmonary arterial systolic pressure, and pulmonary capillary wedge pressure compared with the control group. Patients with VO-HF had regurgitation >3/4 (Sellers' grade). The VO-HF group was characterized by higher LVEDVI and LVESVI than the other 2 groups, whereas the PO-HF group was characterized by higher LVSP, left ventricular end-diastolic pressure, and pressure gradient across the aortic valve than the other 2 groups. Thus, each HF group had characteristic hemodynamic features.

Molecular Forms of AM in Pericardial Fluid and Plasma in Patients with PO-HF and VO-HF

Mean total protein level, albumin level, and albumin/total protein ratios in pericardial fluid from all the patients were

Table 1. Patients Characteristics and Hemodynamic Parameters

Parameters	Control (n = 24)	Volume-Overloaded HF (n = 24)	Pressure-Overloaded HF (n = 14)
Age (y)	66 ± 8	64 ± 10	68 ± 10
Sex (M/F)	(15/9)	(17/7)	(8/6)
LVEF (%)	67 ± 10	61 ± 9*	59 ± 12*
LVEDVI (mL/m^2)	67 ± 22	113 ± 34**	76 ± 23††
LVESVI (mL/m^2)	24 ± 12	44 ± 19**	32 ± 17*†
RA (mm Hg)	4.7 ± 2.0	5.6 ± 3.1	5.4 ± 4.0
PA SysP (mm Hg)	27 ± 6	36 ± 14*	37 ± 18**
PCWP (mm Hg)	9.0 ± 3.5	14.5 ± 8.9*	15.1 ± 7.9**
LVSP (mm Hg)	136 ± 19	137 ± 26	217 ± 28**††
LVEDP (mm Hg)	14.2 ± 6.4	13.8 ± 8.5	20.9 ± 11.0**††
AO (mm Hg)	135 ± 20	137 ± 26	131 ± 32
CI (L.min.m^2)	2.7 ± 0.7	2.8 ± 0.8	2.5 ± 0.5
Pressure gradient across the aortic valve (mm Hg)	2 ± 1	4 ± 2	86 ± 42**††
Aortic valve area (cm^2)	ND	ND	0.54 ± 0.22

Values are means ± SD.

LVEF, left ventricular ejection fraction; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; RA, right atrial pressure; PA SysP, pulmonary arterial systolic pressure; PCWP, pulmonary capillary wedge pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; AO, aortic pressure; CI, cardiac index; ND, not determined.

*$P < .05$ vs. control.

**$P < .01$ vs. control.

$^{\dagger}P < .05$ vs. volume-overloaded.

$^{\dagger\dagger}P < .01$ vs. volume-overloaded.

3.5 ± 0.5 g/dL, 2.4 ± 0.3 g/dL, and 0.70 ± 0.05%, respectively. There were no differences in total protein level, albumin level, or albumin/total protein ratio among the 3 groups (data not shown).

Figure 1 shows the AM-total, AM-mature, AM-glycine, and AM-mature/AM-total ratio levels in the pericardial fluid and plasma in the 3 groups. Plasma AM-mature, AM-glycine, and AM-total were similarly higher in the 2 HF groups than in the control group; however, there was no difference in the AM-mature/AM-total ratio among the 3 groups. In pericardial fluid, AM-total, AM-mature, and AM-glycine were higher in the VO-HF group than in the control group, and AM-total and AM-mature were further increased in the PO-HF group, whereas there was no difference in AM-glycine between the 2 HF groups. The AM-mature/AM-total ratio was only elevated in the PO-HF group compared with the control group.

Table 2 shows comparisons of mean AM-total, AM-mature, AM-glycine levels, and AM-mature/AM-total ratios between plasma and pericardial fluid in each group. AM-total, AM-mature, and the AM-mature/AM-total ratio were higher in pericardial fluid than in plasma in all 3 groups. In particular, AM-mature levels were markedly higher in pericardial fluid than that in plasma (control: +789%, VO-HF: +1050%, PO-HF: +1745%, all $P < .001$). In contrast, AM-glycine was higher in pericardial fluid than in plasma only in the PO-HF group (Table 2).

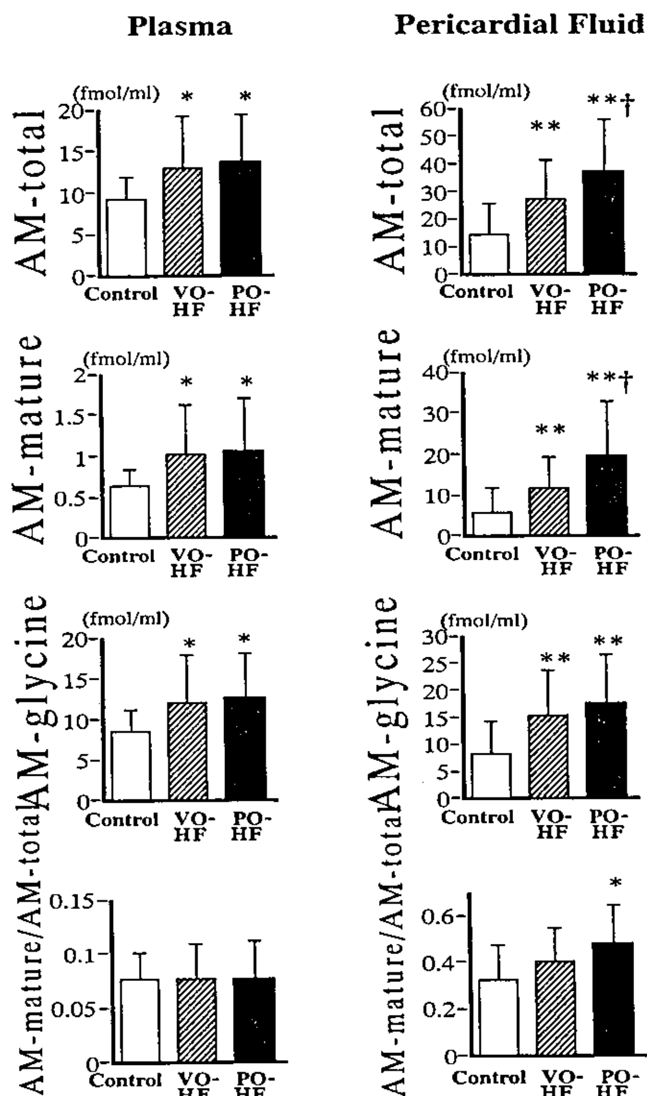

Fig. 1. Adrenomedullin (AM)-total, AM-mature, AM-glycine, and AM-mature/AM-total ratio in plasma, pericardial fluid, and the pericardial fluid/plasma ratios of these values in control (open columns), patients with volume-overloaded heart failure (VO-HF) (hatched columns), and patients with pressure-overloaded (PO)-HF (closed columns). Values are mean ± SD. *$P < .05$ versus control, **$P < .01$ versus control, †$P < .05$ versus VO-HF.

Figure 2 shows the plasma and pericardial fluid ANP and BNP levels in the 3 groups. Plasma and pericardial ANP and BNP were higher in VO-HF than in controls, and they were further increased in PO-HF.

Relationships Between the Levels of the Molecular Forms of AM in Pericardial Fluid and Plasma and Hemodynamic Indices in PO-HF and VO-HF

Table 3 shows the correlation coefficients between molecular forms of AM in plasma and pericardial fluids and hemodynamic indices in patients with PO-HF. Plasma AM-total and AM-glycine correlated significantly with LVSP and

Table 2. Comparisons of Mean Plasma and Pericardial Fluid AM-Total, AM-Mature, AM-Glycine Values, and AM-Mature/AM-Total in Control, Volume-Overloaded HF, and Pressure-Overloaded Heart Failure Groups

	Control			Volume-Overloaded HF			Pressure-Overloaded HF		
	Plasma	Pericardial Fluid	*P* value	Plasma	Pericardial Fluid	*P* value		Pericardial Fluid	*P* value
AM-total (fmol/ml)	9.1	14.0	.039	13.0	27.0	.0016	13.7	37.1	.0002
AM-mature (fmol/ml)	0.64	5.7	.0004	1.02	11.7	<.0001	1.06	19.6	<.0001
AM-glycine (fmol/ml)	8.5	8.3	.903	12.1	15.3	.123	12.7	17.6	.034
AM-mature/AM-total	0.08	0.32	<.0001	0.08	0.40	<.0001	0.08	0.48	<.0001
ANP (pg/mL)	31	17	.0004	75	81	.395	127	149	.114
BNP (pg/mL)	42	161	<.0001	120	1059	<.0001	688	2378	<.0001

AM, adrenomedullin; HF, heart failure; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.

LVEF in PO-HF, whereas pericardial fluid AM-total, AM-mature, and AM-glycine correlated significantly with these indices with better correlation coefficients than those in plasma. Interestingly, only AM-mature/AM-total ratio in pericardial fluid, not in plasma, correlated significantly with LVSP in PO-HF. Both plasma and pericardial fluid AM-total and AM-glycine correlated significantly with plasma ANP and BNP levels (Table 3). Molecular forms of AM neither in plasma nor in pericardial fluid correlated with LVEDVI in PO-HF.

Table 4 shows the correlation coefficients between molecular forms of AM in plasma and pericardial fluid and hemodynamic indices in patients with VO-HF. Plasma AM-total correlated significantly only with EDVI, whereas plasma AM-mature, AM-glycine, or the AM-mature/AM-total ratio did not correlate with any hemodynamic indices in VO-HF. In contrast, pericardial fluid AM-total and AM-glycine correlated significantly with LVEF and EDVI in VO-HF. AM-mature or AM-mature/AM-total ratio in pericardial fluid did not correlated with any hemodynamic indices. Both plasma and pericardial fluid AM-total and AM-glycine correlated significantly with plasma ANP and BNP levels in VO-HF (Table 4). Molecular forms of AM neither in plasma nor in pericardial fluid correlated with LVSP in VO-HF.

ANP and BNP in pericardial fluid as well as those in plasma similarly correlated with characteristic hemodynamic indices in each type of HF (Table 3 and 4).

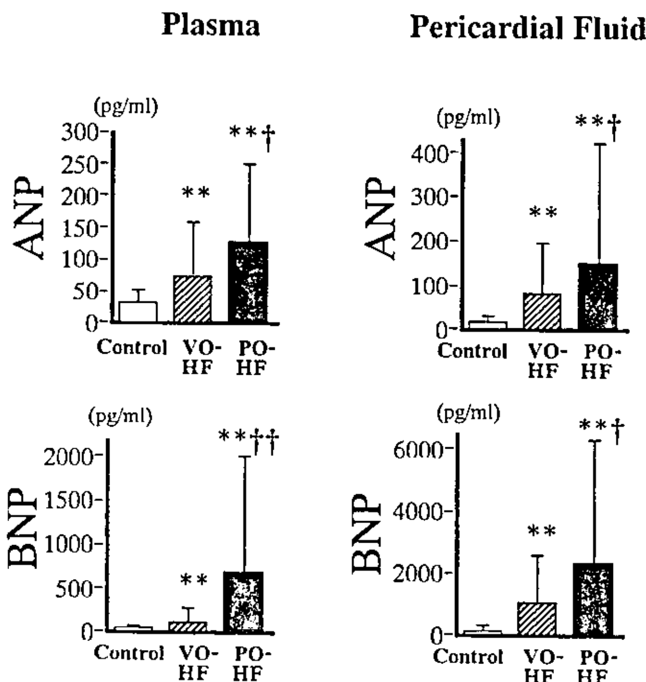

Fig. 2. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in plasma, pericardial fluid, and the pericardial fluid/plasma ratio of these values in control (open columns), patients volume-overloaded heart failure (VO-HF) (hatched columns), and pressure-overloaded (PO)-HF (closed columns). Values are mean ± SD. $^{**}P < .01$ versus control, $^{\dagger}P < .05$ versus VO-HF, $^{\dagger\dagger}P < .01$ versus VO-HF.

Discussion

Regulatory peptides are generated from larger precursors via a variety of posttranslational modifications,[22] 1 of which is the amidation of the peptide carboxy-terminal amino acid. In addition to resistance to carboxypeptidase degradation, amidation frequently confers biologic activity on the peptide.[23,24] AM-mature is produced from AM precursor of 185 amino acids by a 2-step enzymatic reaction.[25] First, AM precursor is converted to AM-glycine, a 53–amino acid peptide that is an intermediate, inactive form. Subsequently, AM-glycine is converted to the active form of AM-mature, a 52–amino acid peptide with a C-terminal amide structure, by enzymatic amidation.[12] We and other groups recently reported that both AM-mature and AM-glycine circulate in human plasma and that the major circulating form is AM-glycine in various cardiovascular diseases.[12,25,26] Plasma AM levels are increased in HF,[27] and a recent study showed that both AM-mature and AM-glycine are increased progressively in proportion to the severity of HF.[28] The findings of higher levels of AM-mature and AM-glycine in both types of HF than in controls and 7% to 8% of an AM-mature/AM-total ratio in plasma are consistent with the results of previous studies.[12,13,26,28,29] The reasons for the lower AM-mature/AM-total ratio in the plasma are considered to be as follows: (1) AM-mature produced in the tissues binds and acts in situ, and little AM-mature is released into the circulation; (2) AM-glycine has low biological activity and cannot

Table 3. Correlation Coefficients Between Molecular Forms of AM in Plasma and Pericardial Fluids and Hemodynamic Indices in Patients With Pressure-Overloaded Heart Failure

	Parameters	Plasma ANP	Plasma BNP	LVEF	LVSP	LVEDVI
Plasma	AM-total	0.70 ($P < .01$)	0.66 ($P < .01$)	−0.34 ($P < .05$)	0.50 ($P < .01$)	0.20 (NS)
	AM-mature	0.29 (NS)	0.27 (NS)	−0.28 (NS)	0.42 ($P < .05$)	0.11 (NS)
	AM-glycine	0.72 ($P < .01$)	0.67 ($P < .01$)	−0.33 ($P < .05$)	0.48 ($P < .01$)	0.20 (NS)
	AM-mature/AM-total	−0.20 (NS)	−0.16 (NS)	−0.13 (NS)	−0.03 (NS)	0.14 (NS)
	ANP	—	0.90 ($P < .01$)	−0.46 ($P < .01$)	0.70 ($P < .01$)	0.26 (NS)
	BNP	—	—	−0.58 ($P < .01$)	0.73 ($P < .01$)	0.32 (NS)
Pericardial fluid	AM-total	0.59 ($P < .01$)	0.43 ($P < .01$)	−0.44 ($P < .01$)	0.63 ($P < .01$)	0.06 (NS)
	AM-mature	0.47 ($P < .01$)	0.35 ($P < .05$)	−0.39 ($P < .05$)	0.60 ($P < .01$)	−0.06 (NS)
	AM-glycine	0.66 ($P < .01$)	0.48 ($P < .01$)	−0.43 ($P < .01$)	0.56 ($P < .01$)	0.22 (NS)
	AM-mature/AM-total	0.25 (NS)	0.16 (NS)	−0.25 (NS)	0.40 ($P < .01$)	−0.12 (NS)
	ANP	0.74 ($P < .01$)	0.67 ($P < .01$)	−0.45 ($P < .01$)	0.36 ($P < .05$)	0.09 (NS)
	BNP	0.73 ($P < .01$)	0.58 ($P < .01$)	−0.56 ($P < .01$)	0.40 ($P < .05$)	0.27 (NS)

LVEF, left ventricular ejection fraction; LVSP, left ventricular systolic pressure; LVEDVI, left ventricular end-diastolic volume index. All other abbreviation, see Table 2.

bind to the AM receptor on cells, and therefore AM-glycine produced in cells is almost completely released into the circulation; and (3) the half-life of AM-mature is shorter than that of AM-glycine in plasma, because AM-mature, but not AM-glycine, is extracted in the pulmonary circulation.[30] As the origin of plasma AM is now thought to be the vascular wall,[13,31,32] the decreased AM-mature/AM-total ratio in plasma may be explained by autocrine and paracrine actions of AM in the vascular wall or the longer half-life of AM-glycine in the circulation as noted above.

We previously reported that the mRNA and peptide levels of AM are increased in the failing rat myocardium.[9] In addition, Yoshihara et al recently reported that ventricular AM levels in the PO and VO failing myocardium are well-correlated with the degree of fetal cardiac gene expression,[33] suggesting that cardiac AM level is a marker of the failing heart. However, little information is available about the cardiac AM synthesis in human HF, and there have been no studies investigating differences of the production of cardiac AM between human PO-HF and VO-HF. We measured the levels of different molecular forms of AM and natriuretic peptides in pericardial fluid to investigate their cardiac production, because it is generally considered that pericardial fluid is not only an ultrafiltrate of plasma, but also a transudate from the cardiac interstitium.[16,17] To support this hypothesis, we measured total protein and albumin level in pericardial fluid and found that pericardial fluid is a very stable transudate, which reflects interstitial myocardial concentrations. Indeed, the levels of vasoactive peptide of cardiac origin are reported to be higher in pericardial fluid than in plasma.[16–18] In this study, we found that AM-mature and the AM-mature/AM-total ratio were markedly higher in pericardial fluid than in plasma. Furthermore, the AM-total, AM-glycine, and AM-mature levels in pericardial fluid correlated with hemodynamic indices in both types of HF with relatively good correlation efficients compared with those in plasma. These results raise the possibility that pericardial fluid AM reflects the cardiac production of AM and that pericardial fluid AM increases in proportion to the severity of each type of HF. We very recently reported that cardiac tissue AM-mature and AM-mature/AM-total ratio in rats are markedly higher than those in plasma.[14,15] and that cardiac

Table 4. Correlation Coefficients Between Molecular Forms of AM in Plasma and Pericardial Fluids and Hemodynamic Indices in Patients With Volume-Overloaded Heart Failure

	Parameters	Plasma ANP	Plasma BNP	LVEF	LVEDVI	LVSP
Plasma	AM-total	0.41 ($P < .01$)	0.53 ($P < .01$)	−0.25 (NS)	0.33 ($P < .05$)	−0.09 (NS)
	AM-mature	0.23 (NS)	0.59 ($P < .01$)	−0.22 (NS)	0.20 (NS)	−0.11 (NS)
	AM-glycine	0.31 ($P < .05$)	0.37 ($P < .05$)	−0.05 (NS)	0.28 (NS)	−0.02 (NS)
	AM-mature/AM-total	0.02 (NS)	0.06 (NS)	−0.24 (NS)	−0.02 (NS)	0.001 (NS)
	ANP	—	0.76 ($P < .01$)	−0.17 (NS)	0.42 ($P < .01$)	0.13 (NS)
	BNP	—	—	−0.37 ($P < .05$)	0.43 ($P < .01$)	0.08 (NS)
Pericardial fluid	AM-total	0.34 ($P < .05$)	0.37 ($P < .05$)	−0.32 ($P < .05$)	0.40 ($P < .01$)	−0.02 (NS)
	AM-mature	0.24 (NS)	0.35 ($P < .05$)	−0.28 (NS)	0.29 (NS)	−0.01 (NS)
	AM-glycine	0.42 ($P < .01$)	0.36 ($P < .05$)	−0.33 ($P < .05$)	0.48 ($P < .01$)	−0.02 (NS)
	AM-mature/AM-total	0.08 (NS)	0.20 (NS)	−0.13 (NS)	0.10 (NS)	−0.04 (NS)
	ANP	0.49 ($P < .01$)	0.53 ($P < .01$)	−0.31 ($P < .05$)	0.40 ($P < .01$)	0.25 (NS)
	BNP	0.58 ($P < .01$)	0.65 ($P < .01$)	−0.44 ($P < .05$)	0.48 ($P < .01$)	0.08 (NS)

For abbreviations, see previous tables.

tissue AM-mature and AM-mature/AM-total ratio are further increased in the failing heart and they correlated with the severity of HF.[14] Our present findings are very consistent with these experimental results. In addition, we showed that AM-mature, AM-glycine, and AM-total levels in pericardial fluid were higher in both types of HF than in controls and that AM-mature and AM-total were more markedly increased in PO-HF than in VO-HF. Furthermore, AM-mature/AM-total ratio correlated with LVSP in PO-HF. A very recent study showed that cardiac tissue AM-mature/AM-total ratio increased in PO cardiac hypertrophy induced by malignant hypertension and that antihypertensive therapy decreased it,[34] suggesting that cardiac amidating activity increased in PO cardiac hypertrophy. These results suggest that not only cardiac production of AM is increased in HF, but also cardiac amidating activity is increased in PO-HF. Thus, PO may be a stronger stimulator of amidating enzyme activity in the heart than VO.

It remains unclear how endogenous cardiac AM functions in the failing heart in VO-HF and PO-HF. AM has recently been reported to increase myocardial contractility in vivo[7] and to exert a direct inotropic effect in vitro.[8] Thus 1 possibility is that the increased cardiac AM observed in pericardial fluid may function as an endogenous positive inotropic substance that opposes deterioration of cardiac performance. Another possibility involves the antiremodeling effect of AM. Previous studies have demonstrated that AM inhibits cardiac hypertrophy and fibrosis in vivo and in vitro.[4–6] These results suggest that upregulation of the cardiac AM levels in HF may be a compensatory mechanism against cardiac hypertrophy and remodeling. Recent studies in vitro and in vivo demonstrated that ANP and BNP are autocrine or paracrine antihypertrophic factors in the heart.[35,36] Thus AM and natriuretic peptides may form a sort of antihypertrophic mediator group. Further studies are necessary to elucidate the exact role of increased AM in PO-HF and VO-HF.

In conclusion, molecular forms of AM in pericardial fluid are differently regulated in those in plasma and that cardiac AM production is increased in both types of HF in response to each different stimulus.

Acknowledgments

We wish to thank Ms. Kyoko Tabei, Ms. Masako Minato, and Ms. Machiko Sakata for technical assistance.

References

1. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Matsuo H, Eto T. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993;192:553–60.
2. Owji AA, Smith DM, Coppock HA, Morgan DG, Bhogal R, Ghatei MA, et al. An abundant and specific binding site for the novel vasodilator adrenomedullin in the rat. Endocrinology 1995;136:2127–34.
3. Nishikimi T, Horio T, Yoshihara F, Nagaya N, Matsuo H, Kangawa K. Effect of adrenomedullin on cAMP and cGMP levels in rat cardiac myocytes and nonmyocytes. Eur J Pharmacol 1998;353:337–44.
4. Tsuruda T, Kato J, Kitamura K, Kuwasako K, Imamura T, Koiwaya Y, et al. Adrenomedullin: a possible autocrine or paracrine inhibitor of hypertrophy of cardiomyocytes. Hypertension 1998;31:505–10.
5. Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K. Effects of adrenomedullin on cultured rat cardiac myocytes and fibroblasts. Eur J Pharmacol 1999;382:1–9.
6. Tsuruda T, Kato J, Kitamura K, Kawamoto M, Kuwasako K, Imamura T, et al. An autocrine or a paracrine role of adrenomedullin in modulating cardiac fibroblast growth. Cardiovasc Res 1999;43:958–67.
7. Szokodi I, Kinnunen P, Tavi P, Weckstrom M, Toth M, Ruskoaho H. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. Circulation 1998;97:1062–70.
8. Charles CJ, Rademaker MT, Richards AM, Cooper GJ, Coy DH, Jing NY, et al. Hemodynamic, hormonal, and renal effects of adrenomedullin in conscious sheep. Am J Physiol Regul Integr Comp Physiol 1997;272:R2040–7.
9. Nishikimi T, Horio T, Sasaki T, Yoshihara F, Takishita S, Miyata A, et al. Cardiac production and secretion of adrenomedullin are increased in heart failure. Hypertension 1997;30:1369–75.
10. Morimoto A, Nishikimi T, Yoshihara F, Horio T, Nagaya N, Matsuo H, et al. Ventricular adrenomedullin levels correlate with the extent of cardiac hypertrophy in rats. Hypertension 1999;33:1146–52.
11. Romppanen H, Marttila M, Magga J, Vuolteenaho O, Kinnunen P, Szokodi I, et al. Adrenomedullin gene expression in the rat heart is stimulated by acute pressure overload: blunted effect in experimental hypertension. Endocrinology 1997;138:2636–9.
12. Kitamura K, Kato J, Kawamoto M, Tanaka M, Chino N, Kangawa K, et al. The intermediate form of glycine-extended adrenomedullin is the major circulating molecular form in human plasma. Biochem Biophys Res Commun 1998;244:551–5.
13. Nishikimi T, Matsuoka H, Shimada K, Matsuo H, Kangawa K. Production and clearance sites of two molecular forms of adrenomedullin in human plasma. Am J Hypertens 2000;13:1032–4.
14. Nishikimi T, Tadokoro K, Mori Y, Wang X, Akimoto K, Yoshihara F, et al. Ventricular adrenomedullin system in the transition from LVH to heart failure in rats. Hypertension 2003;41:512–8.
15. Tadokoro K, Nishikimi T, Mori Y, Wang X, Akimoto K, Matsuoka H. Altered gene expression of adrenomedullin and its receptor system and molecular forms of tissue adrenomedullin in left ventricular hypertrophy induced by malignant hypertension. Regul Pept 2003;112:71–8.
16. Iwakura A, Fujita M, Hasegawa K, Sawamura T, Nohara R, Sasayama S, et al. Pericardial fluid from patients with unstable angina induces vascular endothelial cell apoptosis. J Am Coll Cardiol 2000;35:1785–90.
17. Tanaka T, Hasegawa K, Fujita M, Tamaki SI, Yamazato A, Kihara Y, et al. Marked elevation of brain natriuretic peptide levels in pericardial fluid is closely associated with left ventricular dysfunction. J Am Coll Cardiol 1998;31:399–403.
18. Nishikimi T, Shibasaki I, Iida H, Asakawa H, Matsushita Y, Mori H, et al. Molecular forms of adrenomedullin in pericardial fluid and plasma in patients with ischaemic heart disease. Clin Sci 2002;102:669–77.
19. Ohta H, Tsuji T, Asai S, Sasakura K, Teraoka H, Kitamura K, et al. One-step direct assay for mature-type adrenomedullin with monoclonal antibodies. Clin Chem 1999;45:244–51.
20. Ohta H, Tsuji T, Asai S, Tanizaki S, Sasakura K, Teraoka H, et al. A simple immunoradiometric assay for measuring the entire molecules of adrenomedullin in human plasma. Clin Chim Acta 1999;87:131–43.
21. Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994;90:195–203.
22. Sossin WS, Fisher JM, Scheller RH. Cellular and molecular biology of neuropeptide processing and packaging. Neuron 1989;2:1407–17.

23. Cuttitta F. Peptide amidation: signature of bioactivity. Anat Rec 1993; 236:87–95.
24. Merkler DJ. C-terminal aminated peptides: production by the in vitro enzymatic amidation of glycine-extended peptides and the importance of the amide to bioactivity. Enzyme Microb Technol 1994;16:450–6.
25. Kitamura K, Sakata J, Kangawa K, Kojima M, Matsuo H, Eto T. Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. Biochem Biophys Res Commun 1993;194:720–7.
26. Asakawa H, Nishikimi T, Suzuki T, Hara S, Tsubokou Y, Yagi H, et al. Elevation of two molecular forms of adrenomedullin in plasma and urine in patients with acute myocardial infarction treated with early coronary angioplasty. Clin Sci 2001;100:117–26.
27. Nishikimi T, Saito Y, Kitamura K, Ishimitsu T, Eto T, Kangawa K, et al. Increased plasma levels of adrenomedullin in patients with heart failure. J Am Coll Cardiol 1995;26:1424–31.
28. Hirayama N, Kitamura K, Imamura T, Kato J, Koiwaya Y, Tsuji T, et al. Molecular forms of circulating adrenomedullin in patients with congestive heart failure. J Endocrinol 1999;160:297–303.
29. Nishikimi T, Horio T, Kohmoto Y, Yoshihara F, Nagaya N, Inenaga T, et al. Molecular forms of plasma and urinary adrenomedullin in normal, essential hypertension and chronic renal failure. J Hypertens 2001;19:765–73.
30. Nishikimi T, Nagata S, Sasaki T, Yoshihara F, Nagaya N, Horio T, et al. The active molecular form of plasma adrenomedullin is extracted in the pulmonary circulation in patients with mitral stenosis: possible role of adrenomedullin in pulmonary hypertension. Clin Sci 2001;100:61–6.
31. Nishikimi T, Kitamura K, Saito Y, Shimada K, Ishimitsu T, Takamiya M, et al. Clinical studies on the sites of production and clearance of circulating adrenomedullin in human subjects. Hypertension 1994;24: 600–4.
32. Charles CJ, Lainchbury JG, Lewis LK, Rademaker MT, Richards AM, Yandle TG, et al. The role of adrenomedullin. Am J Hypertens 1999;12:166–73.
33. Yoshihara F, Nishikimi T, Horio T, Yutani C, Nagaya N, Matsuo H, Ohe T, et al. Ventricular adrenomedullin concentration is a sensitive biochemical marker for volume and pressure overload in rats. Am J Physiol Heart Circ Physiol 2000;278:H633–42.
34. Wang X, Nishikimi T, Akimoto K, Tadokoro K, Mori Y, Minamino N. Upregulation of ligand, receptor system, and amidating activity of adrenomedullin in left ventricular hypertrophy of severely hypertensive rats: effects of angiotensin-converting enzyme inhibitors and diuretic. J Hypertens 2003;21:1171–81.
35. Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K. Inhibitory regulation of hypertrophy by endogenous atrial natriuretic peptide in cultured cardiac myocytes. Hypertension 2000;35:19–24.
36. Knowles JW, Esposito G, Mao L, Hagaman JR, Fox JE, Smithies O, et al. Pressure-independent enhancement of cardiac hypertrophy in natriuretic peptide receptor A-deficient mice. J Clin Invest 2001; 107:975–84.

ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT

Peptides 25 (2004) 609–614

PEPTIDES

www.elsevier.com/locate/peptides

Disassociated increases of adrenomedullin in the rat cerebrospinal fluid and plasma after salt loading and systemic administration of lipopolysaccharide

Lei Chen[a], Seiichi Hashida[b], Kazuo Kitamura[c], Tanenao Eto[c], Kenji Kangawa[d], Ryota Serino[a], Bela Kis[a,e], Hiroshi Yamashita[a], Yoichi Ueta[a,*]

[a] *Department of Physiology, University of Occupational and Environmental Health, Iseigaoka 1-1, Yahatanishi-ku, Kitakyushu 807-8555, Japan*

[b] *Department of Biochemistry, Miyazaki Medical College, Miyazaki, Japan*

[c] *Department of Internal Medicine, Miyazaki Medical College, Miyazaki, Japan*

[d] *National Cardiovascular Center Research Institute, Osaka, Japan*

[e] *Department of Physiology and Pharmacology, Wake Forest University Winston-Salem, NC, USA*

Received 12 August 2003; received in revised form 28 January 2004; accepted 2 February 2004

Available online 10 March 2004

Abstract

To determine the role of adrenomedullin (AM) in the fluid electrolyte homeostasis and endotoxin shock, cerebral spinal fluid (CSF) and plasma were sampled from rats after respective challenges. The AM levels were measured by a highly sensitive immunoassay. The AM levels in the CSF of the rats anesthetized with ether (10.7 ± 0.60 fmol/ml) were significantly higher than those with isoflurane (5.17 ± 0.70 fmol/ml, $P < 0.01$), while the plasma level did not differ significantly. The CSF levels of the rats received 2% saline drinking increased to 3 and 4 folds at day 5 and day 7, respectively, while the plasma levels did not differ from controls at both time points. The AM levels in CSF or plasma increased to 1.5 and 3 folds at 1.5 h after intraperitoneal (i.p.) administration of lipopolysaccharide (LPS, 5 mg/kg), reached 6.5 and 30 folds at 6 h, respectively, while no change was observed in the controls. The present findings suggest that AM in the CSF is regulated independently from that in the plasma, the centrally synthesized AM plays and important role in the regulation of the fluid electrolyte homeostasis. Furthermore, the circulatory AM plays an important role in the endotoxin shock.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Adrenomedullin; CSF; Plasma; Salt loading; Endotoxin shock; Stress

1. Introduction

Adrenomedullin (AM) has been found diffusely in a variety of organs [45] with multiple functions [6], such as control of circulation [3], body fluid volume and electrolyte homeostasis [6,29,30,39,40]. Although the amino-acid sequence of AM has been identified 50 in the rat, whereas 52 in human, the functional cyclic region formed by an internal disulfide bridge are consistent between the species. AM levels in the human plasma increase during pregnancy [24], aging [13], and exercises [38]. More significant rises were found under pathological conditions such as heart failure [38], renal failure [10,38], hypertension [3,38], head trauma [28], and subarachnoid hemorrhage (SAH) [4,15]. Some of these findings have been supported by animal experiments [7,14,27]. In rats, AM levels in the plasma increased significantly in response to intravenous (i.v.) administration of endotoxin [21,36,46].

Previous studies suggested that AM levels in the cerebral spinal fluid (CSF) were increased in pregnant [24], head trauma [28], and SAH [4] subjects. The concentration of AM in the CSF is regulated independently from that in the plasma [24]. Thus, AM levels in the CSF can be an index of the central nervous system (CNS) activities. However, the relationship of the AM levels in the CSF and plasma is still unclear. In the present study, rat CSF and plasma were sampled to determine the AM levels, and the relevant changes are supposed to reflect the AM functions in the circulation and the CNS.

* Corresponding author. Tel.: +81-93-691-7420; fax: +81-93-692-1711. *E-mail address:* yoichi@med.uoeh-u.ac.jp (Y. Ueta).

0196-9781/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2004.02.001

2. Materials and methods

2.1. Animals

Adult male Wistar rats (200–350 g) were purchased from Seac Yoshitomi (Japan), used for CSF and plasma collections, with the permission of the Ethics Committee of the University of Occupational and Environmental Health, Japan. The animals were housed three per plastic cage at 24–25 °C in a light controlled room (lights on 7:00 and off 19:00 h) with free access to food and tap water at least 3 days prior to the experiment.

2.2. Experimental procedure

In the preliminary study, the AM levels in the CSF and plasma were measured in rats anesthetized with isoflurane ($n = 9$) or ethyl ether ($n = 11$).

2.2.1. Chronic salt loading

To examine the effects of chronic salt loading, the rats ($n = 11$) were given 2% saline to drink and were allowed free access to dry food that contained 0.3% sodium. For control experiments, the rats ($n = 9$) were allowed free access to tap water and dry food. The CSF and plasma sampling were performed at day 5 and day 7 during salt loading.

2.2.2. Systemic administration of lipopolysaccharide (LPS)

To examine the effects of systemic administration of LPS, the animals ($n = 6$–8) were intraperitoneally (i.p.) administered with LPS (5 mg/kg) from *Escherichia coli* (serotype 0111:B4) (Sigma, St Louis, USA). LPS was dissolved in pyrogen-free 0.9% saline with 1 mg/ml. Control rats ($n = 6$–8) were administered with 1 ml of pyrogen-free 0.9% saline. CSF and plasma were sampled at 0.5, 1, 1.5, and 6 h after i.p. administrations of LPS or saline.

2.3. Sampling of CSF and plasma

All the rats were anesthetized with isoflurane with the 400 Anesthesia Unit (Univentor Ltd., Zeftun, Malta) except the preliminary experiment. The anesthetized rats showed neither response to the squeeze of the tail nor change of respiratory pattern in the sampling process. The procedure of CSF tap was described in detail previously [5]. The anesthetized rats were set in a prone position with a small pillow under the cervix to stretch the posterior neck region. The neck was shaved and disinfected with ethanol. A 26G needle with a 1 ml syringe (Terumo, Tokyo, Japan) was punctured percutaneously into the cisternal magna after the determination of the foramen magnum by touch. CSF (50–200 μl/rat) were sampled from individual rats within 2 min. Blood (0.5–1 ml/rat) was collected from rat tail artery under anesthesia, supplemented with EDTA (Nacalai Tesque Inc., Kyoto, Japan) and Aprotinin (Bayer, I'laç Fabrikalar, Germany) to 1.5 mg/ml and 500 KIU/ml, respectively, then the plasma were collected after centrifuge at 3000 rpm for 10 min. The CSF and plasma samples were put into siliconized microtubes and at −80 °C until the assay. To test the effects of LPS, the rat rectum temperature was measured with a BAT-12 Physitemp (Physitemp Instruments Inc., Clinton, USA) during the sampling process under endotoxin shock. The AM levels in the CSF and plasma were determined using a highly sensitive enzyme immunoassay as described previously [14,45].

2.4. Statistical analysis

All the values are expressed as mean ± S.E.M. Statistical analysis was carried out by a one-way analysis of variance (ANOVA) and the correlation of AM levels in the CSF and plasma were determined by Pearson's test. A level of $P < 0.05$ was considered statistically significant.

3. Results

For the whole study, a total 125 CSF and 84 plasma samples were collected from 88 rats. No inflammatory signs were found at the sites of puncture and no rats died during the experiment. The levels of AM in the CSF in the rats anesthetized with ether (10.7 ± 0.60 fmol/ml, $n = 11$) showed 2 fold higher than those with isoflurane (5.17 ± 1.5 fmol/ml, $n = 9$, $P < 0.01$, Fig. 1A). No significant variance was seen

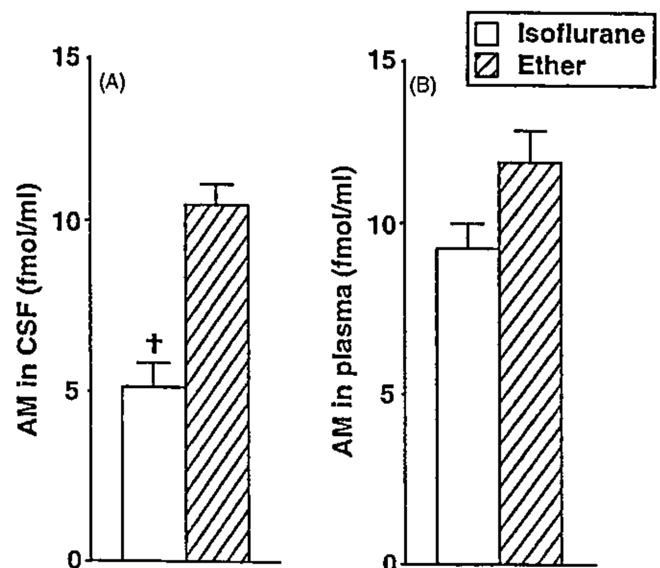

Fig. 1. Effects of isoflurane and ethyl ether anesthesia on the adrenomedullin levels in the rat cerebrospinal fluid (A) and plasma (B). Rats were anesthetized with isoflurane ($n = 9$) or ethyl ether ($n = 11$) respectively. Data are expressed as mean ± S.E.M.; † $P < 0.01$.

in the plasma (12.0±0.90 fmol/ml versus 9.3±1.3 fmol/ml, Fig. 1B). The correlated increases of AM in the CSF and plasma were seen in the group anesthetized with ether ($P < 0.05$) (Fig. 1).

3.1. Effects of chronic salt loading

Chronically salt loaded rats showed polydipsia and polyuria. On day 5, the AM levels in the CSF of the salt loaded rats ($n = 11$) or control ($n = 9$) were 15.4 ± 2.2 fmol/ml and 5.17 ± 0.70 fmol/ml ($P < 0.05$) respectively, the plasma levels were 6.00 ± 0.70 fmol/ml and 5.72 ± 1.5 fmol/ml, respectively. On day 7, the CSF levels were 23.4 ± 3.9 fmol/ml and 5.38 ± 1.0 fmol/ml ($P < 0.01$) respectively, the plasma levels were 6.38 ± 0.6 fmol/ml and 10.2 ± 1.1 fmol/ml, respectively (Fig. 2).

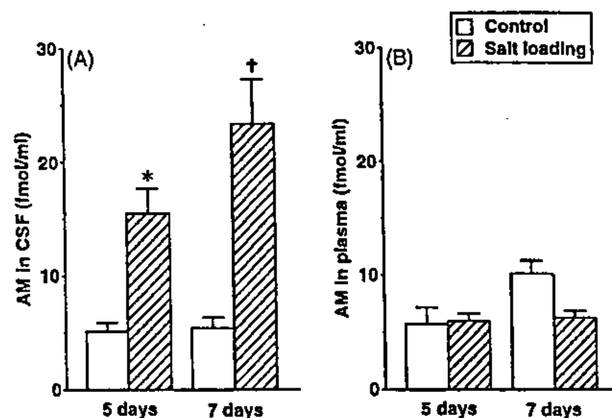

Fig. 2. Effects of chronic salt loading on the adrenomedullin levels in the rat cerebrospinal fluid (A) and plasma (B). The rats fed with dry food that contains 0.3% sodium were given tap water ($n = 9$) or 2% saline ($n = 11$) respectively. CSF and plasma samples of rats were collected at day 5 and day 7. Data are expressed as mean ± S.E.M.; * $P < 0.05$, † $P < 0.01$.

3.2. Effects of systemic administration of LPS

LPS administered rats showed sickness and reddish of ears, and the rectum temperature increased to 37.7±0.30 °C at 6 h, significantly higher than that of the control group (36.4 ± 0.20 °C, $P < 0.01$). The AM levels in the CSF of the LPS administered rats ($n = 6$–8) or the controls ($n = 6$–8) at 0.5, 1, 1.5, and 6 h were 7.50 ± 1.6 fmol/ml versus 4.4 ± 0.6 fmol/ml, 4.42 ± 1.8 fmol/ml versus 5.63 ± 1.0 fmol/ml, 8.3 ± 1.6 fmol/ml versus 6.67 ± 0.5 fmol/ml, 66.15 ± 15.1 fmol/ml versus 10.2 ± 1.1 fmol/ml ($P < 0.01$) respectively. The plasma levels at each time points were 4.20 ± 1.2 fmol/ml versus 7.94 ± 1.8 fmol/ml, 5.87 ± 1.7 fmol/ml versus 7.57±2.2 fmol/ml, 50.41±15.6 fmol/ml versus 15.5 ± 6.8 fmol/ml ($P < 0.05$), 278 ± 25 fmol/ml versus 9.27 ± 0.86 fmol/ml ($P < 0.01$) respectively. Another correlated increases in the plasma ($n = 6$) and CSF ($n = 6$) were seen in the LPS administered group at 1.5 h ($P < 0.01$) (Fig. 3).

4. Discussion

The present study demonstrated that the centrally synthesized AM are regulated independently from that in the circulation, and the AM may play different role in these two compartments. Peripherally, AM has high expression in adrenal gland, lung and cardiac atrium, and the blood vessels that have been suggested to be the main source of plasma AM [9]. Calcitonin-like receptor with receptor activity modifying protein (RAMP)2 as AM receptors has been found in different cell types [22].

In rats, 50–70% of CSF is secreted from the choroid plexus, and the reminder is formed around vessels and along the ventricular walls. In vitro studies have identified that cultured choroid plexus carcinoma cells [37], rat cerebral endothelial cells (CECs) [16,17], and astrocytes [33] could

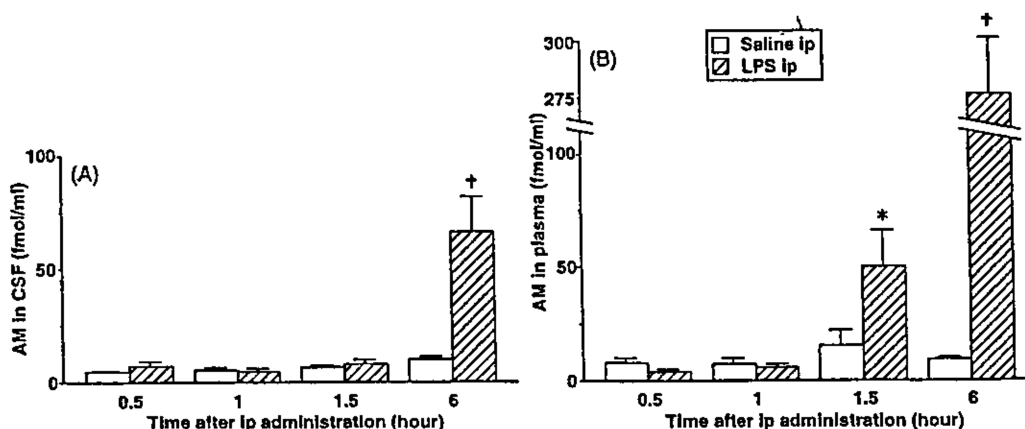

Fig. 3. Effects of intraperitoneal administration of lipopolysacchaide on the adrenomedullin levels in the rat cerebrospinal fluid (A) and plasma (B). Samplings were performed at 0.5, 1, 1.5, and 6 h after i.p. administration of saline ($n = 6$–8) or LPS (5 mg/kg) ($n = 6$–8) respectively. Data are expressed as mean ± S.E.M.; * $P < 0.05$, † $P < 0.01$.

produce and secret AM. The AM secreted from choroid plexus has been considered as one of the major source of AM in the CSF [19]. A previous study has revealed an extensive distribution of AM-immunoreactivity in neurons and processes in all regions of rat CNS [33], with stronger expression along the hypothalamo-neurohypophysial tract [26,32,42–44]. AM receptors have been identified in the rat cerebral microvessels [18] and diffusely in the rat brain, with the enrichment seen in the choroid plexus and lining of the third, fourth and lateral ventricles, basolateral amygdaloid nuclei, posterior pituitary, the trigeminal nerves and in the granular cell layer of the cerebellum [11].

In the preliminary experiment, samples from the rats anesthetized with ether showed AM levels over 2 fold higher than those from rats anesthetized with isoflurane, while the plasma levels did not differ significantly. Ether has been known for its stressful effects on animals [20,25]. In the parvocellular neurons of the paraventricular nucleus (PVN), acute ether stress can induce a rapid induction of the croticotropin-releasing hormone (CRH) [20], suggesting short-term exposure of ether can activate the parvocellular PVN (pPVN). Therefore, the increase in the CSF should be owned to the activation of the PVN and subsequent AM synthesis induced by ether anesthesia.

AM had been considered to play an important role in fluid and electrolyte homeostasis [6,39] for its strong natriuresis and uronesis effects [6]. In our study, the rats showed polydipsia and polyuria, suggesting both high volume and salt loading, which are the proper stimulators for AM secretion. Accordingly, our findings showed that AM levels in the CSF increased significantly after salt loading, while the plasma levels did not change until day 7, suggesting the central source of the increases in the CSF. This is consistent with other study in that, the AM levels of plasma of the rat received high salt diet (8% NaCl) did not increase up to 14 days. Thereafter they increased to 2.5 folds after 28 days salt loading, at that time the AM mRNA expression in the adrenal gland and kidney increased to 3 and 1.5 folds [2]. Other study showed that water deprivation for 24 h decreased preproadrenomedullin (ppAM) gene expression in the magnocellular PVN (mPVN) and the supraoptic nucleus (SON) [35]. As AM has direct actions in the hypothalamus to decrease vasopressin secretion and in the pituitary gland to inhibit ACTH release [26], the decreased AM expression in this regulatory mechanism suggests the selectivity to guarantee the circulatory volume as a priority by reducing the urine secretion in the acute response to water restriction [35]. In rats, central administration of AM could inhibit saline drinking and the water intake resulted from water deprivation [23,39] while peripheral administration of the same dose of AM did not significantly alter drinking in response to this mixed hypovolemic/hyperosmostic challenge [39]. In addition, in the rat adrenal zona glomerulosa, AM showed a reverse adjustive role on aldosterone secretion [1]. These studies have clearly demonstrated that AM plays an important role in the regulation of fluid and electrolyte homeostasis by resetting the hypothalamus–pituitary–adrenal axis, and the action sites are CNS and adrenal. More specifically, centrally synthesized AM is engaged in the quick response and short-term regulation of fluid and electrolyte.

Furthermore, AM also plays an important role in the pathophysiology of sepsis and vascular tone adjustment in the endotoxin shock. In our study, AM levels in the plasma reached 3 and 30 folds at 1.5 and 6 h after i.p. administration of LPS, while the CSF levels were 1.5 and 6.5 folds only than control.

The blood vessels have been suggested to be the main source of circulatory AM [9], and in vitro studies have shown LPS is a potent stimulant to AM secretion by vascular smooth muscle cells, fibroblasts, endothelial cells and microphages [41]. Our study has identified rat CECs as a major source of AM, the AM is secreted primarily at the luminal side into the blood, and the production of AM could not be elevated by cytokines, bacterial LPS or thrombin [16,17]. Thus, the AM production by CECs is unlikely to contribute to the increased plasma AM levels in sepsis. Other study has shown that AM can cross from blood to brain with a influx constant which much faster than that of albumin, and no blood-to-brain saturation is seen [12]. However, ^{125}I labeled AM showed high reversible association with the brain vessels and excess AM showed self-inhibition of the transportation [12], suggesting circulatory AM can induce certain but limited effects to the levels at the brain side of the blood–brain barrier (BBB).

Another possible origin of AM in the CSF is choroid plexus. As it is sited in the brain side of the BBB, the LPS could only show the effects through prostaglandins (PG) and some cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF) by activating microglia and tissue macrophages in the meninges and along penetrating blood vessels [31]. In a previous study, IL-1, TNF-α, combined with IL-γ could only induce AM mRNA expression to 339% after 24 h challenge [37]. Other study has shown that ^{125}I labeled AM exited the brain with the bulk reabsorption of CSF at an efflux rate comparable to that of albumin [12]. Therefore, the synthesis of AM from choroid plexus could only have limited contribution to the dramatic increases in the CSF seen at 6 h after LPS administration.

In addition, the integrity of the PVN is essential for LPS induced fever [8,31], suggesting the PVN is engaged in the febrile response in an autonomic and endocrine manner and the increased temperature can be an index of the PVN activity in the central response to LPS [8]. In our study, the temperature of LPS challenged rats reached significantly higher than those of the control ($P < 0.01$) at 6 h, suggesting the activation of PVN. However, this response is induced by cytokines results from LPS administration, but not LPS itself, and the ventromedial preoptic area, the key regulatory site of temperature, which extends some excesses to the PVN, is upstream of this reaction [31]. Moreover, the circulatory AM has been identified as an activator of PVN, especially in the dorsolateral medial parvocellular division,

and the inhibition of ppAM gene expression by LPS in the pPVN, the mPVN and the SON where have been shown rich of AM containing neurons, has been identified [35].

Taken together, the increases in the CSF should be majorly owned to the entry of circulatory AM from some BBB absent areas, such as the area postema, of which the ablation will attenuate the response of PVN neurons to circulatory AM [34]. The associated increases of AM levels in the CSF and plasma at 1.5 h also support this viewpoint.

In conclusion, AM is regulated independently in the CSF and plasma, the centrally synthesized AM may play an important role in the fluid and electrolyte homeostasis. Furthermore, the circulatory AM also plays an important role in the endotoxin shock.

Acknowledgments

We thank Dr. Yoshida Y. (Stanford University, USA), Dr. Fujiki N. (Gifu University, School of Medicine, Japan) for their technical advice and Dr. Govindan Dayanithi (INSERM, Montpellier, France) for his critical review of this paper. This study was supported by Grant-in-Aid for Scientific Research on Priority Areas (B), no. 10218210 for Y.U., Scientific Research (B) no. 10218210, from the Ministry of Education, Exploratory Research no. 14657020, Culture, Sports, Science and Technology, a research grant from the Ministry of Health and Welfare and from Ajinomoto Co. Ltd. (Japan).

References

[1] Albertin G, Malendowicz LK, Tortorella C, Mazzocchi G, Nussdorfer GG. Evidence for a paracrine role of adrenomedullin in the physiological resetting of aldosterone secretion by rat adrenal zona glomerulosa. Peptides 2000;21:413–7.

[2] Cao YN, Kitamura K, Kato J, Kuwasako K, Ito K, Onitsuka H, et al. Chronic salt loading upregulates expression of adrenomedullin and its receptors in adrenal glands and kidneys of the rat. Hypertension 2003;42(3):369–72.

[3] Eto T, Kitamura K, Kato J. Biological and clinical roles of adrenomedullin in circulation control and cardiovascular diseases. Clin Exp Pharmacol Physiol 1999;26:371–80.

[4] Fujioka M, Nishio K, Sakaki T, Minamino N, Kitamura K. Adrenomedullin in patients with cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Stroke 2000;31:3079–83.

[5] Fujiki N, Yoshida Y, Ripley B, Honda K, Mignot E, Nishino S. Changes in CSF hypocretin-1 (orexin A) levels in rats across 24 h and in response to food deprivation. Neuroreport 2001;12:993–7.

[6] Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. Endocr Rev 2000;21:138–67.

[7] Hirano S, Imamura T, Matsuo T, Ishiyama Y, Kato J, Kitamura K, et al. Differential responses of circulating and tissue adrenomedullin and gene expression to volume overload. J Card Fail 2000;6:120–9.

[8] Horn T, Wilkinson MF, Landgraf R, Pittman QJ. Reduced febrile responses to pyrogens after lesions of the hypothalamic paraventricular nucleus. Am J Physiol 1994;267:R323–8.

[9] Hwang IS, Tang F. Peripheral distribution and gene expression of adrenomedullin in the rat: possible source of blood adrenomedullin. Neuropeptides 2000;34:32–7.

[10] Ishihara T, Yokota N, Hisanaga S, Fujimoto S, Hirayama N, Kato J, et al. Increased plasma levels of mature form of adrenomedullin in patients with chronic renal failure. Clin Nephrol 1999;52:119–23.

[11] Juaneda C, Dumont Y, Chabot JG, Quirion R. Autoradiographic distribution of adrenomedullin receptors in the rat brain. Eur J Pharmacol 2001;421:R1–2.

[12] Kastin AJ, Akerstrom V, Hackler L, Pan W. Adrenomedullin and the blood–brain barrier. Horm Metab Res 2001;33:19–25.

[13] Kato J, Kitamura K, Uemura T, Kuwasako K, Kita T, Kangawa K, et al. Plasma levels of adrenomedullin and atrial and brain natriuretic peptides in the general population: their relations to age and pulse pressure. Hypertens Res 2002;25:887–92.

[14] Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993;192:553–60.

[15] Kikumoto K, Kubo A, Hayashi Y, Minamino N, Inoue S, Dohi K, et al. Increased plasma concentration of adrenomedullin in patients with subarachnoid hemorrhage. Anesth Analg 1998;87:859–63.

[16] Kis B, Abraham CS, Deli MA, Kobayashi H, Niwa M, Yamashita H, et al. Adrenomedullin, an autocrine mediator of blood–brain barrier function. Hypertens Res 2003;26(Suppl):S61–70.

[17] Kis B, Kaiya H, Nishi R, Deli MA, Abraham CS, Yanagita T, et al. Cerebral endothelial cells are a major source of adrenomedullin. J Neuroendocrinol 2002;14:283–93.

[18] Kobayashi H, Minami S, Yamamoto R, Masumoto K, Yanagita T, Uezono Y, et al. Adrenomedullin receptors in rat cerebral microvessels. Mol Brain Res 2000;81:1–6.

[19] Kobayashi H, Shiraishi S, Minami S, Yokoo H, Yanagita T, Saitoh T, et al. Adrenomedullin receptors in rat choroid plexus. Neurosci Lett 2001;297:167–70.

[20] Kovacs KJ, Sawchenko PE. Regulation of stress-induced transcriptional changes in the hypothalamic neurosecretory neurons. J Mol Neurosci 1996;7:125–33.

[21] Matsui E, Kitamura K, Yoshida M, Kato J, Asada Y, Sumiyoshi A, et al. Biosynthesis and secretion of adrenomedullin and proadrenomedullin N-terminal 20 peptide in a rat model of endotoxin shock. Hypertens Res 2001;24:543–9.

[22] Muff R, Born W, Fischer JA. Adrenomedullin selectivity of calcitonin-like receptor/receptor activity modifying proteins. Hypertens Res 2003;26:S3–8.

[23] Murphy TC, Samson WK. The novel vasoactive hormone, adrenomedullin, inhibits water drinking in the rat. Endocrinology 1995;136:2459–63.

[24] Nagata N, Kato J, Kitamura K, Kawamoto M, Tanaka N, Eto T, et al. Dissociation of adrenomedullin concentrations in plasma and cerebrospinal fluid in pregnant and non-pregnant women. Eur J Endocrinol 1998;139:611–4.

[25] Nevo I, Becker C, Hamon M, Benoliel JJ. Stress- and yohimbine-induced release of cholecystokinin in the frontal cortex of the freely moving rat: prevention by diazepam but not ondansetron. J Neurochem 1996;66:2041–9.

[26] Nussdorfer GG. Proadrenomedullin-derived peptides in the paracrine control of the hypothalamo–pituitary–adrenal axis. Int Rev Cytol 2001;206:249–84.

[27] Rademaker MT, Charles CJ, Lewis LK, Yandle TG, Cooper GJ, Coy DH, et al. Beneficial hemodynamic and renal effects of adrenomedullin in an ovine model of heart failure. Circulation 1997;96:1983–90.

[28] Robertson CL, Minamino N, Ruppel RA, Kangawa K, Wisniewski SR, Tsuji T, et al. Increased adrenomedullin in cerebrospinal fluid after traumatic brain injury in infants and children. J Neurotrauma 2001;18:861–8.

[29] Samson WK. Adrenomedullin and the control of fluid and electrolyte homeostasis. Annu Rev Physiol 1999;61:363–89.

[30] Samson WK, Murphy TC. Adrenomedullin inhibits salt appetite. Endocrinology 1997;138:613–6.

[31] Saper CB. Neurobiological basis of fever. Ann N Y Acad Sci 1998;856:90–4.

[32] Serino R, Ueta Y, Hara Y, Nomura M, Yamamoto Y, Shibuya I, et al. Centrally administered adrenomedullin increases plasma oxytocin level with induction of c-fos messenger ribonucleic acid in the paraventricular and supraoptic nuclei of the rat. Endocrinology 1999;140:2334–42.

[33] Serrano J, Uttenthal LO, Martinez A, Fernandez AP, Martinez de Velasco J, Alonso D, et al. Distribution of adrenomedullin-like immunoreactivity in the rat central nervous system by light and electron microscopy. Brain Res 2000;853:245–68.

[34] Shan J, Krukoff TL. Area postrema ablation attenuates activation of neurones in the paraventricular nucleus in response to systemic adrenomedullin. J Neuroendocrinol 2000;12:802–10.

[35] Shan J, Krukoff TL. Distribution of preproadrenomedullin mRNA in the rat central nervous system and its modulation by physiological stressors. J Comp Neurol 2001;432:88–100.

[36] Shoji H, Minamino N, Kangawa K, Matsuo H. Endotoxin markedly elevates plasma concentration and gene transcription of adrenomedullin in rat. Biochem Biophys Res Commun 1995;215:531–7.

[37] Takahashi K, Satoh F, Hara E, Murakami O, Kumabe T, Tominaga T, et al. Production and secretion of adrenomedullin by cultured choroid plexus carcinoma cells. J Neurochem 1997;68:726–31.

[38] Tanaka M, Kitamura K, Ishizaka Y, Ishiyama Y, Kato J, Kangawa K, et al. Plasma adrenomedullin in various diseases and exercise-induced change in adrenomedullin in healthy subjects. Intern Med 1995;34:728–33.

[39] Taylor MM, Samson WK. Adrenomedullin and the integrative physiology of fluid and electrolyte balance. Microsc Res Tech 2002; 57:105–9.

[40] Taylor MM, Samson WK. Ribozyme compromise of adrenomedullin mRNA reveals a physiological role in the regulation of water intake. Am J Physiol 2002;282:R1739–45.

[41] Tomoda Y, Isumi Y, Katafuchi T, Minamino N. Regulation of adrenomedullin secretion from cultured cells. Peptides 2001;22: 1783–94.

[42] Ueta Y, Hara Y, Setiadji VS, Isse T, Shibuya I, Kitamura K, et al. Adrenomedullin-like immunoreactivity in the rat hypothalamo-neurohypophysial tract. Peptides 1999;20:199–204.

[43] Ueta Y, Kitamura K, Isse T, Shibuya I, Kabashima N, Yamamoto S, et al. Adrenomedullin-immunoreactive neurons in the paraventricular and supraoptic nuclei of the rat. Neurosci Lett 1995;202:37–40.

[44] Ueta Y, Serino R, Shibuya I, Kitamura K, Kangawa K, Russell JA, et al. A physiological role for adrenomedullin in rats a potent hypotensive peptide in the hypothalamo-neurohypophysial system. Exp Physiol 2000;85:163S–9S.

[45] Yamaga J, Hashida S, Kitamura K, Tokashiki M, Aoki T, Inatsu H, et al. Direct measurement of glycine-extended adrenomedullin in plasma and tissue using an ultrasensitive immune complex transfer enzyme immunoassay in rats. Hypertens Res 2003;26:S45–53.

[46] Yang S, Zhou M, Chaudry IH, Wang P. The role of lipopolysaccharide in stimulating adrenomedullin production during polymicrobial sepsis. Biochim Biophys Acta 2001;1537:167–74.

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT

PEPTIDES

Peptides 25 (2004) 1321–1326

www.elsevier.com/locate/peptides

Adrenomedullin as a sensitive marker for coronary and peripheral arterial complications in patients with atherosclerotic risks

Yoshihiko Suzuki [a,b], Takeshi Horio [a,*], Hiroshi Nonogi [a], Tokio Hayashi [a], Kazuo Kitamura [b], Tanenao Eto [b], Kenji Kangawa [c], Yuhei Kawano [a]

[a] *Department of Medicine, National Cardiovascular Center, 5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan*
[b] *First Department of Internal Medicine, Miyazaki Medical College, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan*
[c] *National Cardiovascular Center Research Institute, 5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan*

Received 25 February 2004; received in revised form 23 April 2004; accepted 23 April 2004

Available online 1 June 2004

Abstract

Plasma adrenomedullin (AM) levels are elevated in various pathological states including cardiovascular and inflammatory diseases. The present study investigated whether an increased AM level is a marker of vascular complications in patients with atherosclerotic risks. In 114 patients with cardiovascular risks and/or diseases including ischemic heart disease (IHD) and peripheral arterial disease (PAD), plasma AM concentration and other inflammatory markers such as high sensitive C-reactive protein (CRP) and interleukin (IL)-6 were examined. The plasma AM level was not altered by the absence or presence of each of four major risk factors, i.e., hypertension, diabetes mellitus, hyperlipidemia, and smoking and its level was not significantly correlated with blood pressure, plasma glucose, or serum lipid levels. The patients with IHD had a significantly higher concentration of plasma AM than those without IHD. The AM level in subjects with PAD was also increased significantly compared with those without PAD. The plasma AM was strongly correlated with inflammatory parameters such as CRP and IL-6. Among AM, CRP, and IL-6, however, only AM was an independent predictor for both IHD and PAD by multiple logistic regression analysis. Our findings suggest the possibility that plasma AM is a novel sensitive marker for the presence of vascular lesions in patients with atherosclerotic risks.
© 2004 Elsevier Inc. All rights reserved.

Keywords: Hormone; Peptide; Atherosclerosis; Vascular disease; Inflammation

1. Introduction

Adrenomedullin (AM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma [13]. AM is widely distributed in various organs and tissues, including the cardiovascular system [8,31,32]. The plasma levels of AM have been shown to be elevated in various pathological states, such as acute myocardial infarction [14], congestive heart failure [11,23], chronic renal failure [10,22], essential hypertension [10,16,22], and diabetes mellitus [4].

It is currently recognized that vascular inflammation plays an important role in the development of atherosclerosis [17]. Many studies suggest that inflammatory markers such as C-reactive protein (CRP) and interleukin (IL)-6 are independent predictors of future cardiovascular events [15,19,26–29]. Inflammation is also a powerful inductive factor of AM production, because many kinds of inflammation-related cytokines such as IL, tumor necrosis factor, and interferon stimulate AM production and secretion from vascular and cardiac cells [6,8,33]. In fact, a marked increase in plasma AM levels was observed in patients with sepsis, a severe systemic inflammatory disorder [5,38]. However, little is known about the production and pathophysiological role of AM in patients with vascular atherosclerosis, probably accompanied with a low-grade inflammatory signature.

Thus, we conducted this study to examine the plasma concentration of AM in patients with atherosclerotic risk factors and diseases such as ischemic heart disease (IHD) and peripheral arterial disease (PAD), and investigate the association of plasma AM with atherosclerotic risks, vascular lesions, and inflammatory parameters. We further evaluated the potential usefulness of plasma AM as a marker for vascular complications in these patients.

* Corresponding author. Tel.: +81 6 6833 5012; fax: +81 6 6872 8091.
E-mail address: thorio@ri.ncvc.go.jp (T. Horio).

0196-9781/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2004.04.007

2. Methods

2.1. Study subjects

A total of 114 patients with cardiovascular risks and/or diseases were enrolled in the present study. All subjects were inpatients who were admitted to our hospital for the examination and treatment of hypertension, diabetes mellitus, and cardiovascular diseases including IHD and PAD. Patients with congestive heart failure or chronic renal failure were excluded from the study. Hypertension was defined as a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure of ≥90 mmHg by repeated measurements or when subjects had already been treated with antihypertensive drugs. Diabetes mellitus was diagnosed according to the American Diabetes Association criteria (a fasting plasma glucose of ≥126 mg/dL and/or a plasma glucose level at 2 h after 75 g oral glucose load of ≥200 mg/dL), or when medication was taken for treatment of hyperglycemia. Diagnosis of hyperlipidemia required a serum total cholesterol level of ≥220 mg/dL and/or a serum triglyceride level of ≥150 mg/dL or the use of lipid-lowering drugs. IHD, including angina pectoris and old myocardial infarction, was diagnosed by electrocardiographic, radioisotope cardiographic, and coronary angiographic criteria. Diagnosis of PAD was performed based on clinical symptoms, low ankle-brachial index, and findings of magnetic resonance angiography. All subjects gave their informed consent to participate in the present study.

2.2. Sample collection

Blood samples were taken from a peripheral vein at rest in the supine position. Blood was immediately transferred into ice-chilled glass tubes containing disodium EDTA (1 mg/mL) and aprotinin (500 U/mL) and centrifuged for 10 min at 4 °C. Plasma samples were frozen and stored at −80 °C until assayed.

2.3. Measurement of AM

Plasma concentration of human AM was measured by immunoradiometric assay using a specific kit (AM RIA SHIONOGI, Shionogi Pharmaceutical Co., Ltd., Osaka, Japan), as described previously [25].

2.4. Inflammatory and other laboratory parameters

Plasma level of IL-6 was measured by enzyme-linked immunosorbent assay using a commercially available assay kit (Quantikine HS, R&D Systems, Minneapolis, MN). High sensitive CRP was measured by nephelometry (SRL Inc., Tokyo, Japan). Fasting plasma glucose, hemoglobin A1c, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and serum creatinine were determined by standard laboratory measurements.

2.5. Statistical analysis

Values were expressed as mean±S.D. Unpaired Student's *t*-test was used for comparison between the two groups. The significance of differences among the three groups was evaluated by an unpaired ANOVA with subsequent Fisher's multiple comparison test. Relations between variables were assessed using a univariate linear regression analysis and Pearson's correlation coefficient. A multiple logistic regression analysis was applied to identify independent predictors for the presence of IHD and PAD. The difference in incidences of IHD and PAD between the subject groups was tested by χ^2 analysis. A value of $P < 0.05$ was accepted as statistically significant.

3. Results

Clinical characteristics of the study patients were shown in Table 1. The present study subjects had a high percentage of atherosclerotic risk factors such as hypertension, diabetes mellitus, hyperlipidemia, and smoking habit, although their blood pressure, plasma glucose, and serum lipid levels were well controlled by adequate treatments. Fifty-one (45%) or 43 (38%) patients had a complication of IHD or PAD, respectively.

We investigated the relation of cardiovascular risks to plasma AM concentration in the present subjects. The plasma level of AM was not altered by the absence or presence of each of four major risk factors (Fig. 1). We divided all subjects into four groups by the number of cardiovascular risk factors that they had, and compared the plasma AM concentration among the groups. However, there was

Table 1
Clinical characteristics of study patients

N	114
Age (years)	67 ± 10
Sex (male/female)	79/35
Hypertension (%)	81
Diabetes mellitus (%)	43
Hyperlipidemia (%)	55
Smokers (current or past, %)	69
IHD (%)	45
PAD (%)	38
Systolic blood pressure (mmHg)	136 ± 22
Diastolic blood pressure (mmHg)	73 ± 13
Fasting plasma glucose (mg/dL)	104 ± 27
Hemoglobin A1c (%)	6.2 ± 1.7
Total cholesterol (mg/dL)	191 ± 31
Triglycerides (mg/dL)	113 ± 53
HDL cholesterol (mg/dL)	45 ± 13
Serum creatinine (mg/dL)	0.8 ± 0.3

Values are mean ± S.D. or percentage.