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Increased Circulating Adrenomedullin, a Novel Vasodilatory Peptide, in Sepsis*

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ABSTRACT

Human adrenomedullin (hAM), a potent vasodilatory peptide originally identified in pheochromocytoma, has been shown to be present in various human tissues and circulate in human plasma. We measured plasma concentrations of immunoreactive hAM in patients with sepsis who had been admitted to intensive care unit (ICU). Plasma hAM concentrations in 12 septic patients upon entering the ICU were extremely elevated (107 ± 139 fmol/ml: mean ± SD) compared to those of 16 age-matched normal subjects (7.9 ± 3 fmol/mL). Among 10 patients with normal renal function, plasma hAM levels either decreased or increased during the hospital course; the former group survived and the latter group succumbed. Two patients with acute renal failure had markedly elevated plasma hAM levels during the early course, which declined rapidly during the recovery course. High performance liquid chromatography of plasma extracts from one patient with acute renal failure revealed a single major component of immunoreactive hAM coeluting with authentic hAM (1–52) during acute and recovery phase. Plasma hAM concentration showed positive correlations with heart rate, right atrial pressure, and serum creatinine concentration, but not with other hemodynamic variables. These data suggest that a marked increase in circulating hAM in sepsis may be caused by its decreased clearance and/or its enhanced synthesis by multiple organ dysfunction, and that increased endogenous hAM may be involved in the mechanism of cardiovascular abnormalities associated with sepsis.*J Clin Endocrinol Metab* **81:** 1449–1453, 1996)

THE SYSTEMIC response to infection has been termed sepsis. Recently, it has been proposed that sepsis is defined as systemic inflammatory response syndrome caused by infection and noninfectious insult (1). Sepsis-induced hypotension, septic shock, and multiple organ dysfunction syndrome have been reported to be the main cause of death in the intensive care unit (ICU) (1). A cascade of inflammatory mediators identified in sepsis are responsible for abnormalities in vasoregulation and coagulation. These include cytokines such as tumor necrosis factor (TNF) and interleukins (IL), platelet activating factor, leukotriens, prostaglandins, nitric oxide (NO), and so forth (2). Bacterial toxins, *i.e.* lipopolysaccharide (LPS), stimulate the release of these endogenous mediators from plasma, inflammatory cells (neutrophiles, macrophages), and vascular endothelial cells (EC), which in turn lead to refractory hypotension and progressive failure of multiple organ systems (2). Among them, it has been clinically emphasized that excessive production and release of NO from vascular smooth muscle cells (SMC) and EC stimulated by LPS and cytokines largely contribute to the development of septic shock.

Human adrenomedullin (hAM) is a novel vasodilatory peptide with 52-amino-acid residue, originally identified in pheochromocytoma (3). hAM, which has a slight homology with calcitonin gene-related peptide (CGRP), caused a potent and sustained hypotensive effect in rats. Recent studies have shown that AM transcripts and protein are expressed not only in adrenal medulla, but in a variety of tissues, including heart, lung, aorta, and kidney (4, 5). Subsequently, it has been demonstrated that cultured vascular EC and SMC of several species express abundant AM messenger (m) RNA and release immunoreactive (ir) AM into media, which are augmented by TNF-α (6, 7). Furthermore, it has been shown that ir-hAM circulates in normal human plasma (8), and plasma ir-hAM levels are elevated in patients with essential hypertension and chronic renal failure (9). These data suggest that circulating AM may be involved in the regulation of cardiovascular function.

These observations led us to speculate that endogenous hAM may play some pathophysiological role in sepsis. Therefore, the present study was undertaken to measure plasma ir-hAM concentrations in patients with sepsis who entered into ICU to investigate whether circulating hAM has any relation to hemodynamic changes and renal function associated with sepsis, and to characterize circulating ir-hAM during acute and recovery phase of acute renal failure by reverse-phase high performance liquid chromatography (HPLC).

Methods

Patients

Twelve septic patients (11 men, 1 woman, 50–78 yr old, mean age 64 ± 8.9) who entered ICU of Tokyo Medical and Dental University Hospital were studied. Sepsis was diagnosed by the criteria recently defined (1). The systemic response was manifested by two or more of the following conditions as a result of infection: temperature over 38C or under 36C; heart rate more than 90 beats/min; respiratory rate more than 20

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breaths/min or $PaCO_2$ less than 32 torr; WBC more than 12,000/mm^3, less than 4,000/mm^3, or more than 10% immature (band) forms. The study was approved by the Institutional Review Board, and informed consent was obtained from the patients' surrogates. Their underlying diseases and complications are listed in Table 1. Except for two patients (cases 11 and 12), ten patients who had sepsis-induced hypotension and septic shock (systolic blood pressure < 90 mmHg, or reduction > 40 mmHg from the baseline) received inotropic or vasopressor agents (dopamine, dobutamine, and/or norepinephrine). Because of hypoxemia, all patients were treated with mechanical ventilation to maintain PaO_2 of at least 75 mmHg. Two patients (cases 3 and 5) had acute renal failure upon entry to ICU; one patient (case 3) was treated once with hemodialysis on the second admission day. Otherwise, the remaining ten patients had serum creatinine concentrations less than 1.2 mg/dL during the entire hospital days.

Measurements of hemodynamic parameters

A Swan-Ganz catheter was inserted after admission to the ICU in all patients except two (cases 7 and 12). Heart rate was monitored with ECG. Mean arterial pressure was monitored via radial arterial catheter. Right atrial pressure, mean pulmonary arterial pressure, and pulmonary capillary wedge pressure were measured via Swan-Ganz catheter. Cardiac output was measured by the thermodilution method; the mean value of at least three measurements was calculated. Systemic vascular resistance index and pulmonary vascular resistance index were calculated. These measurements were started on admission to ICU and repeated every 24 h during 4–19 admission days.

Measurement of plasma immunoreactive (ir)-hAM

Blood samples were obtained from an indwelling radial arterial catheter immediately after admission to ICU and every 1–3 days thereafter. Blood samples were also obtained from 16 age-matched normal subjects (10 men, 6 women, age 60–69 yr, mean age 65 ± 5) via antecubital vein. Blood samples were collected into a chilled polypropyrene tube containing EDTA-2K, centrifuged at 4C, and plasma samples were stored at − 40C until assayed.

Two-mL aliquot of plasma samples was acidified with an equal volume of 0.1% trifluoroacetic acid (TFA) and centrifuged. The supernatant was loaded on a Sep-pak C18 cartridge (Waters Inc., Milford, MA). After washing with 0.1% TFA twice and 10% acetonitrile/0.1% TFA, the adsorbed materials were eluted with 70% acetonitrile/0.1% TFA. The eluates were lyophilyzed and dissolved in assay buffer. The recovery of synthetic hAM added during the extraction procedure was 87%.

Radioimmunoassay (RIA) for hAM was performed essentially in the same method as recently reported (8). RIA buffer consisted of 0.05M sodium phosphate buffer (pH7.4), containing 0.08M NaCl, 0.01M EDTA, 0.1% bovine serum albumin, 0.1% Triton X-100, and 0.01% NaN_3. Briefly, samples or standard synthetic hAM (Peptide Institute Inc., Osaka, Japan) and anti-hAM serum (final dilution, 1:32000; Peptide Institute Inc.) were incubated at 4C for 24 h, followed by the late addition of ^{125}I-hAM and further incubation at 4C for 24 h. Separation of bound from free ligands was performed by the double-antibody method. The antibody had 100% cross-reactivities with intact hAM(1–52), 10% with hAM(13–52) and hAM(1–10), less than 0.1% with hAM(33–52), CGRP and amylin, and none with various vasoactive polypeptides. The minimum detectable dose in RIA was 0.5 fmol/tube (95% confidence), and the 50% binding intercept was 4.5 fmol/tube. The intraassay and interassay coefficients of variation (n = 5) were 4.8% and 8.3%, respectively.

Reverse-phase HPLC

Reverse-phase HPLC was performed using an octadecylsilica column (0.46 × 25 cm, Tosoh, Tokyo, Japan) eluted with a linear gradient (10–60%) of acetonitrile in 0.1% TFA for 1 h with a flow rate of 1 mL/min; 1 mL fractions were collected. The recovery of hAM during the chromatographic procedure was 95%.

Statistical analysis

Linear regression analysis was used to determine correlations between plasma ir-hAM levels and hemodynamic parameters and serum creatinine levels. Statistical analysis was performed by unpaired Student's *t* test. A *P* value less than 0.05 was considered statistically significant. All data are shown as mean ± SD.

TABLE 1. Clinical characteristics and hemodynamic parameters of 12 septic patients upon entry to intensive care unit

Case	Age/Sex	Complication (Underlying disease)	Heart rate (beat/m)	Mean arterial pressure (mmHg)	Cardiac index (1/min/m^2)	Mean pulmonary arterial pressure (mmHg)	Right atrial pressure (mmHg)	Pulmonary capillary wedge pressure (mmHg)
1	60/F	peritonitis (perforation of colon) postoperative pulmonary failure	125	99	3.6	17	5	8
2	57/M	postoperative pneumonia (esophageal ca)	100	80	5.8	25	9	11
3	65/M	postoperative pneumonia, renal failure (rupture of dissecting aortic aneurysma)	139	72	3.7	28	11	18
4	59/M	postoperative pulmonary failure (hepatocellular ca)	128	76	7.3	36	16	17
5	77/M	peritonitis (suppurative cholangitis) renal failure	140	72	4.7	26	13	17
6	50/M	postoperative septic shock (oral cavity tumor)	107	67	5.5	20	4	8
7	78/M	postoperative septic shock (perforation of appendicitis)	128	73	–	–	6	–
8	75/M	postoperative shock (rupture of dissecting aortic aneurysma)	95	83	3.2	23	11	10
9	61/M	postoperative septic shock (esophageal ca)	122	82	3.3	23	11	12
10	66/M	postoperative pulmonary failure (esophageal ca)	98	73	4.2	27	8	19
11	55/M	postoperative pyrothorax (esophageal ca)	133	71	5.4	24	12	14
12	65/M	postoperative pneumonia (esophageal ca)	84	83	–	–	–	–
Mean ± SD			116 ± 18.8	78 ± 8.5	4.7 ± 1.3	25 ± 5	9.6 ± 3.6	13.4 ± 4.1

Ca; carcinoma.

Results

Plasma ir-hAM concentrations in all 12 patients with sepsis upon entry to the ICU were markedly elevated (107 ± 139 fmol/mL, mean ± SD), which were significantly ($P < 0.01$) greater than those in 16 age-matched normal subjects (7.9 ± 8 fmol/mL). Changes of plasma ir-hAM levels in 10 septic patients with normal renal function during the entire ICU admission days are shown in Fig. 1. Plasma ir-hAM levels in 5 patients who recovered from sepsis (cases 2,6,7,9,12) gradually decreased (Fig. 1, *upper panel*), whereas those in 5 patients who died (cases 1,4,8,10,11), although fluctuated, rose from the initial values (Fig. 1, *lower panel*). There was no significant difference of the initial plasma ir-hAM levels between survivors (52 ± 11.6 fmol/mL) and nonsurvivors (137 ± 168 fmol/mL). Changes of plasma ir-hAM and serum creatinine concentrations in 2 patients with acute renal failure (cases 3 and 5) are shown in Fig. 2. Plasma ir-hAM levels drastically increased in these two patients during 1–2 days after admission to the ICU and rapidly decreased during recovery course, while serum creatinine levels were gradually decreased. Both patients were completely recovered from sepsis and discharged from the hospital; their plasma ir-hAM levels became normalized (< 10 fmol/mL).

Elution profiles by HPLC of plasma extracts from a patient with acute renal failure (case 5) during acute phase (serum creatinine: 2.9 mg/dL) and recovery phase (serum creatinine:

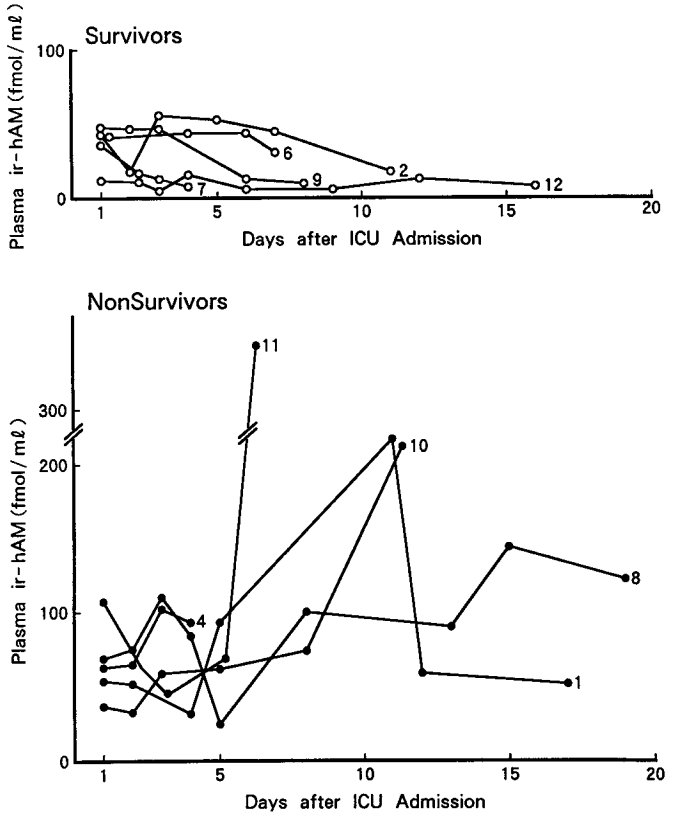

FIG.1. Sequential changes of plasma levels of ir-hAM in 10 septic patients with normal renal function during ICU admission. *Upper panel* shows plasma ir-hAM levels in 5 septic patients (cases 2,6,7,9,12) who survived, and *lower panel* shows those of 5 septic patients (cases 1,4,8,10,11) who died. Each number represents case.

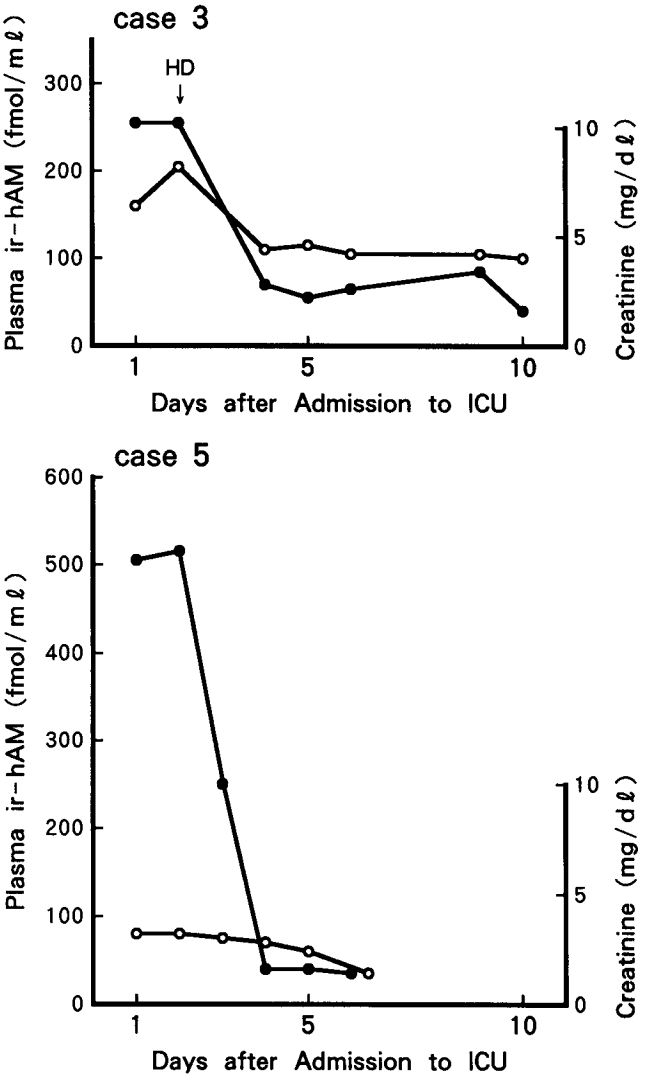

FIG.2. Sequential changes of plasma levels of ir-hAM and serum creatinine levels in 2 septic patients with acute renal failure (cases 3 and 5) during ICU admission. (●) ir-hAM; (○) creatinine; (↓) hemodialysis.

1.5 mg/dL) are shown in Fig. 3. A single major ir-hAM component was eluted in the position corresponding to that of authentic hAM(1–52), whose elution pattern was essentially similar during acute and recovery course.

Plasma ir-hAM concentrations in 12 septic patients serially obtained during the entire ICU course showed significant ($P < 0.05$) correlations with heart rate (r = 0.35) and right atrial pressure (r = 0.31), but not with mean arterial pressure, mean pulmonary arterial pressure, pulmonary capillary wedge pressure, cardiac index, systemic vascular resistance, or pulmonary vascular resistance ($P > 0.05$). There was a significant correlation (r = 0.48) between plasma ir-hAM and serum creatinine concentrations ($P < 0.01$).

Discussion

The present study clearly demonstrated that circulating ir-hAM, a novel vasodilatory peptide, was markedly elevated in 12 septic patients admitted to the ICU, showing positive cor-

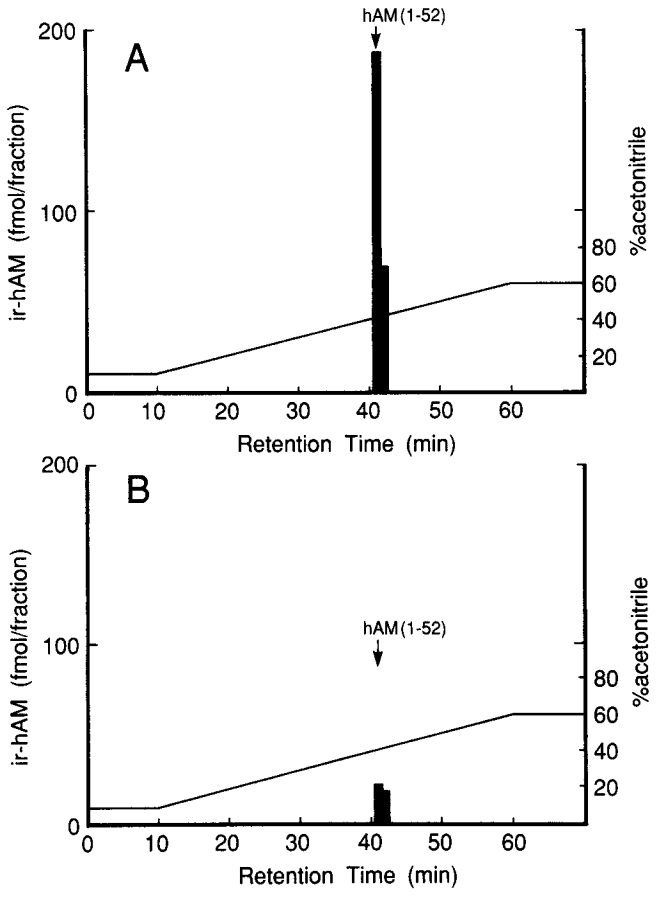

FIG.3. Reverse-phase HPLC of plasma extracts from one septic patient with acute renal failure. Plasma extracts of one patient (case 5) obtained from (A) acute phase (first admission day) and (B) recovery phase (6th admission day) were subjected to reverse-phase HPLC eluted with a linear gradient (10–60%) of acetonitrile (*solid line*). *Closed columns* show ir-hAM concentrations in each eluate. *Arrow* indicates the elution position of standard hAM (1–52).

relations with hemodynamic parameters (heart rate, right atrial pressure) and serum creatinine concentrations.

In sepsis syndrome, a variety of vasoconstrictors such as cathecolamine, angiotensin, thromboxane A_2, and endothelin-1 are secreted to increase systemic vascular resistance as a compensatory mechanism to maintain systemic blood pressure (2). These vasoconstrictors, on the other hand, induce pulmonary vasoconstriction, thereby leading to the development of pulmonary hypertension associated sepsis. In fact, our septic patients had pulmonary hypertension with increased mean pulmonary arterial pressure (25 ± 5 mmHg). Increase in right atrial pressure (9.6 ± 3.6 mmHg) is likely caused by the increased right ventricular afterload, although fluid administration may also have increased preload. A positive correlation of plasma ir-hAM levels with right atrial pressure in this study may suggest its compensatory role in reducing pulmonary vasoconstriction associated with sepsis. Since hAM has significant pulmonary vasodilatory activity in the rat (10), it would be of interest to study specific diseases, such as pulmonary hypertension and acute respiratory distress syndrome.

Our septic patients had increased heart rate (116 ± 18.8 beats/min) and cardiac output (4.7 ± 1.3 1/min/m^2). The direct action of AM on the heart remains unknown at present. However, CGRP with a slight structural similarity to AM has been shown to share the common CGRP/AM receptors (11, 12). Furthermore, CGRP has a direct positive chronotropic and inotropic action on myocardium (13). Therefore, it is reasonable to speculate that circulating hAM may play some role in increased heart rate associated with sepsis. However, increased heart rate may be caused by baroreflex sympathetic activation resulting from sepsis-induced hypotension. Furthermore, it remains unknown what influence, if any, the inotropic and vasopressor agents affect circulating ir-hAM levels. In this respect, it would be interesting to study an additional group of patients with shock not caused by sepsis, such as cardiogenic and hypovolemic shock, in determining the effect of hypotension *vs.* other sepsis-associated effects.

Characterization of plasma ir-hAM in one septic patient by reverse-phase HPLC revealed that circulating hAM consists of a single major component coeluting with authentic hAM (1–51). This patient had acute renal failure at admission, but the renal function normalized after treatment. The molecular form of circulating ir-hAM during acute and recovery phase of renal failure appeared essentially similar. These data suggest that the major circulating hAM in sepsis, irrespective of renal dysfunction, is an intact molecule. It should be noted that N-terminal and C-terminal fragments of hAM molecule, and a linear peptide after cleavage of intramolecular cyclic structure are devoid of biological activity (14). Thus, circulating hAM should retain its vasodilatory property.

The precise mechanism(s) by which circulating hAM is increased in sepsis remains unknown. Since plasma ir-hAM concentration showed a positive correlation with serum creatinine concentration, decreased clearance of circulating hAM by the kidney may be responsible for the raised plasma ir-hAM levels in patients with sepsis. In fact, two septic patients with acute renal failure had markedly elevated plasma ir-hAM levels, which fell rapidly during recovery course and normalized before discharge, while serum creatinine concentrations gradually decreased. Our data are consistent with those of a recent report showing that a close relationship exists between concentrations of plasma ir-hAM and serum creatinine in patients with chronic renal failure (9). However, patients with end-stage renal failure had only about 5-fold greater plasma ir-hAM levels than those of normal subjects, which did not change after hemodialysis (15). Thus, the kidney is not the sole organ to remove circulating hAM. An alternative site of its clearance may be the lung. It has been reported that plasma ir-hAM concentrations from aorta is slightly lower than those from pulmonary artery during selective catheter sampling (16). Furthermore, it has recently been shown that rat lung has the most abundant binding sites for ^{125}I-AM among the various rat tissues examined (17). Therefore, impaired removal of circulating hAM during pulmonary circulation resulting from sepsis-associated lung injury may partly contribute to the elevation of plasma ir-hAM concentrations.

The possible mechanism of increased circulating hAM other than its decreased clearance in sepsis is its excessive synthesis and secretion. It has been demonstrated that AM mRNA expression and ir-AM synthesis are not confined only

to adrenal medulla, but also to a wide variety of tissues, including heart, aorta, lung, and kidney (4, 5), and that AM mRNA and protein are abundantly expressed by cultured EC and SMC of many species (6, 7), which are augmented by bacterial LPS and several cytokines (18). Therefore, elevated circulating AM in sepsis may be derived from enhanced secretion from the LPS- and cytokines-activated vascular EC and SMC. We (11) and other investigators (12) have demonstrated that AM exerts its direct vasodilatory action via receptor-mediated activation of adenylate cyclase in vascular SMC. Local production of AM in vascular EC and SMC coupled with the presence of AM receptor in SMC suggests that AM regulates local vascular tonus via an autocrine/paracrine manner.

In summary, the present study demonstrates that septic patients had markedly increased circulating hAM, possibly caused by its decreased clearance and/or its increased synthesis by the affected multiple organs, suggesting its possible involvement in the development of sepsis-related cardiovascular abnormalities.

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