Production and Clearance Sites of Two Molecular Forms of Adrenomedullin in Human Plasma

Toshio Nishikimi, Hiroaki Matsuoka, Ken-ei Shimada, Hisayuki Matsuo, and Kenji Kangawa

Human adrenomedullin (AM) precursor is converted to glycine-extended AM (AM-Gly), an inactive intermediate form of AM. Subsequently, AM-Gly is converted to active mature AM (AM-m) by enzymatic amidation. A recent study showed that two molecular forms of adrenomedullin (AM) are present in human plasma. In this study we investigated the production and clearance sites of two molecular forms of adrenomedullin in humans. We measured plasma levels of AM-m and AM-Total (T) (AM-m + AM-Gly) by immunoradiometric assay and calculated plasma levels of AM-Gly in blood samples taken from various sites during cardiac catheterization in patients with ischemic heart disease. Plasma AM-m levels were significantly lower in left-sided sites after passing through pulmonary circulation than in right-sided sites, whereas there were no significant differences in AM-Gly levels between left-sided sites and right-sided sites. These results suggest that AM-m produced in many organs is released into veins and that the main clearance sites of AM-m are the lungs. Considering that AM preferentially dilates pulmonary vessels rather than systemic vessels, a possible role of this peptide is suggested in the regulation of pulmonary vascular tonus. Am J Hypertens 2000; 13:1032–1034 © 2000 American Journal of Hypertension, Ltd.

KEY WORDS: Adrenomedullin, molecular form, clearance, production.
(AM-m), a 52-amino acid peptide with C-terminal amide structure, by enzymatic amidation. Recently Kitamura et al. reported that two molecular forms of AM, AM-m and AM-Gly, circulate in human blood.

In the present study we investigated the sites of production and clearance of two molecular forms of AM in human plasma.

**MATERIALS AND METHODS**

Informed consent was obtained from each patient and the protocol was approved by the ethical committee of our institute.

Fifteen patients with ischemic heart disease (12 men and three women, aged 67 ± 10 years) were studied. Right and left cardiac catheterization was performed in the morning after overnight fasting. After the pressure recordings, a blood sample was taken from the pulmonary capillary wedge, pulmonary artery, right ventricle, and right atrium from the Swan-Ganz catheter. Then the catheter was also positioned in the infrarenal and suprarenal inferior vena cava under fluoroscopic control and blood samples were obtained. Another catheter was inserted and positioned in the proximal portion of the coronary sinus and right renal vein under fluoroscopic control for blood sampling. Blood was immediately transferred into chilled glass tubes containing disodium EDTA (1 mg/mL) and aprotinin (500 U/mL) and centrifuged immediately at 4°C and the plasma was frozen and stored at −80°C until assayed.

Both AM-m and AM-T were measured by immunoradiometric assay (RIA) using specific kits (AM mature RIA Shionogi, AM RIA Shionogi) developed by the Diagnostic Science Department, Shionogi Pharmaceutical Co., Ltd., Osaka, Japan. These assay systems use two monoclonal antibodies against human AM, one recognizing a ring structure of human AM in both kits and the other recognizing the carboxy-terminal sequence in the AM-m kit or AM (25-36) in the AM-T kit. The assay measures human AM-m or AM-T by sandwiching it between the two antibodies without the extraction of plasma. The assay’s minimal detectable quantity of human AM-m or AM-T is 0.5 pmol/L in both kits. The coefficients of variation of the intraassay and interassay values in several blood samples were 4.4% to 8.2% and 5.5% to 8.3% in the AM-m kit and 3.4% to 7.3% and 5.3% to 9.0% in the AM-T kit, respectively. The recovery rate of 5 to 100 pmol/L of human AM added to several plasma samples was 91% to 118% in the AM-m kit and 93% to 118% in the AM-T kit, respectively. A reverse-phase high-performance liquid chromatography analysis revealed that the major peak of immunoreactive AM in the plasma detected by each immunoradiometric assay kit for AM-m and AM-T was identical to synthetic human AM (1-52). AM-Gly was calculated with the following formula: (AM-Gly) = (AM-T) − (AM-m).

All data were expressed as the means ± SD. Comparisons of plasma adrenomedullin concentrations between aorta and veins were done by one-way repeated measures of ANOVA. P < .05 was considered significant.

**RESULTS**

Fig. 1 shows the plasma AM-T, AM-m, and AM-Gly levels and AM-m/AM-T ratio at various sites in human plasma. Plasma AM-m concentrations were significantly lower in left-sided sites after passing through the pulmonary circulation than in right-sided sites, whereas there were no differences in plasma AM-Gly levels between left-sided and right-sided sites, suggesting that only AM-m is extracted in the
pulmonary circulation. Plasma AM-T concentrations and the AM-m/AM-T ratio, as a result, were lower in left-sided than in right-sided sites.

**DISCUSSION**

In this study we demonstrated for the first time that plasma AM-m and AM-T concentrations taken from the various veins were similar, and that they were significantly higher than those of the aorta, whereas there were no differences in plasma AM-Gly between left-sided and right-sided sites. These findings suggest that two molecular forms of AM are present in plasma, and that the major molecular form of plasma AM is AM-Gly. AM-m is produced in various organs and the main clearance sites of plasma AM-m may be the lungs.

In this study, venous plasma AM-m, AM-Gly, and AM-T levels were similar at various sites including coronary sinus and renal vein, whereas plasma AM-T levels were slightly lower in left-sided sites than in right-sided sites. There were marked step-downs in plasma AM-m levels between pulmonary artery and pulmonary capillary, although there were no differences in plasma AM-Gly levels between pulmonary artery and pulmonary capillary. A previous autoradiographic study using venous injection of $^{125}$I-AM showed that $^{125}$I-AM was strongly taken up by the lungs, suggesting that the lung has abundant binding sites for AM. In addition, a previous study reported that AM binding sites were highly concentrated in the lung. We also reported that plasma AM was partially metabolized in the pulmonary circulation of patients with primary and secondary pulmonary hypertension. Moreover, a previous study demonstrated that AM preferentially dilates pulmonary vessels over systemic vessels. Taken together, these findings suggest that AM-m is produced in the various organs, including heart and kidneys, that it is released into the veins, and that the main target organ of AM-m may be the lungs. Thus, extracted AM in the pulmonary circulation may be involved in the regulation of pulmonary vascular tonus. Further studies are necessary to elucidate the exact role of plasma AM in the pulmonary circulation.

**REFERENCES**


