E. 結論

ヒトにおいて、AMによる非常に強力かつ直接的なアルドステロン分泌抑制効果が確認された。一方、AMの炎症反応調節因子としての意義は、今後更なる検討が必要と思われる。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表　別紙４

2. 学会発表　別紙４

H. 知的財産権の出願・登録状況

1. 特許取得　別紙４

2. 実用新案登録　なし

3. その他　なし

分担研究報告書

急性心筋梗塞症におけるｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝの心筋保護治療の臨床評価

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研究要旨　急性心筋梗塞症に対して急性期にｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝを投与し、その心筋保護効果を検討した。少数例ではあるが慢性期の左室造影での駆出率には差はなかったが、核磁気共鳴装置(MRI)による解析では梗塞サイズの縮小が認められ、ｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝによる adjunctive therapy の可能性が示唆された。

A. 研究目的　ｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝは、抗炎症作用・抗ｱﾎﾟﾄｰｼｽ作用、血管新生作用などその多機能性から薬物として臨床への応用が期待されている生理活性物質である。本研究では急性心筋梗塞症例を対象として、合成ﾋﾄｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝを投与した際の安全性ならびに心筋保護作用による心機能改善効果について検討する。

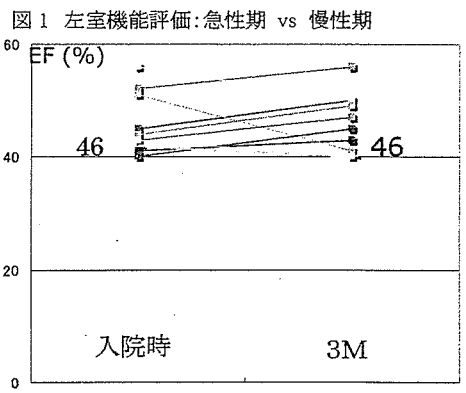
B. 研究方法　対象を30歳以上75歳以下の初回急性心筋梗塞で発症12時間以内に来院した症例とし、冠動脈再疎通療法(PCI)施行前からｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝを経静脈的に投与する。まず少数例で投与量と使用方法を確認する予備試験として0.025μg/kg 体重/min(6例)にて行い、安全性を評価する。その後 0.025μg/kg 体重/min にて3時間の投与、引き続き0.0125μg/kg 体重/min にて9時間の投与(合計 12 時間)する方法で、心臓カテーテル検査(CAG(再狭窄の有無), LVG(biplane撮影))、心臓超音波検査、核医学(RI)検査、核磁気共鳴(MRI)検査、等を急性期と慢性期に行い安全性、再灌流障害抑制効果、梗塞サイズ縮小効果、慢性期心機能改善効果を評価する。

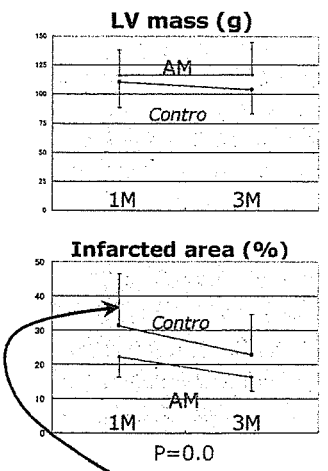
(倫理面への配慮)対象となる患者の人権は最大限に尊守される。具体的には検査・治療前にはその内容、被験者の得る利益、不利益についての十分な説明を行い、文書にて同意を得る。またいったん同意した後でも患者本人の申し出があればいつでも撤回することができる。患者のﾌﾟﾗｲﾊﾞｼｰ保護についても十分配慮し、個人を特定できる資料についてはその一切を公表しない。

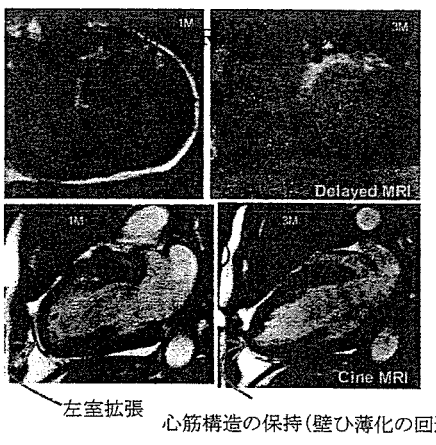
C. 研究結果　低用量0.025μg/kg 体重/min を6例に対して使用したが、収縮血圧 100mmHg 未満になる症例が 4 例にみられ、右室梗塞を合併した下壁心筋梗塞の一例では56mmHgまで低下した。このため安全性の面から右室梗塞合併例を除外し、ｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝ

の投与方法を0.025μg/kg 体重/min にて3時間、引き続き0.0125μg/kg 体重/min にて9時間の投与(合計 12 時間)とした。この容量設定にて血圧は穏徐に低下し、ｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝを安全に投与することができた。慢性期ﾃﾞｰﾀ収集が終了した10症例の検討では急性期と慢性期(3 ヶ月後)の左室造影での左室機能評価では駆出率に差はなかった(図 1)が、核磁気共鳴装置(MRI)による解析では梗塞ｻｲｽﾞの縮小が認められた。

図1 左室機能評価:急性期 vs 慢性期







初回AMI (n=8);年齢, EF, peak CPKをマッチさせた症例群

D. 考察　急性心筋梗塞症に対するPCIは、心機能および予後の改善に多大な貢献をしてきた。一方で再灌流に伴う傷害や梗塞後ﾘﾓﾃﾞﾘﾝｸﾞにより十分な改善が得られない症例もあり、adjunctive therapy が求められている。ｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝは内因性生理活性物質で多機能性を特徴とし、過去の動物実験においても強い心筋保護効果が報告されている。臨床において有用性が証明されればその価値は高く心筋梗塞の長期予後の改善、死亡率の低下が期待できる。本研究において急性心筋梗塞症に対しての経静脈的ｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝ投与による心筋保護目的の adjunctive therapy の可能性が示唆された。

E. 結論　急性心筋梗塞症に対するｱﾄﾞﾚﾉﾒﾃﾞｭﾘﾝ経静脈的投与を2段階の用量を設定し行った。投与方法は許容できるものであり、今後局所壁運動の改善・心筋ﾘﾓﾃﾞﾘﾝｸﾞ抑制など期待される効果についての検討対照群を設定した比較試験にて明らかにする必要がある。

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研究成果の刊行に関する一覧表（18年度）

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CHAPTER

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Adrenomedullin and Its Related Peptides

KAZUO KITAMURA AND JOHJI KATO

ABSTRACT

Adrenomedullin (AM) is a potent vasodilator peptide that exerts major effects on cardiovascular function. AM, initially isolated from human pheochromocytoma tissue, is biosynthesized in a wide variety of organs and cells. In addition to AM, proadrenomedullin N-terminal 20 peptide (PAMP) is found to be processed from the AM precursor. Both AM and PAMP show hypotensive effects in anesthetized rats but exhibit different hypotensive mechanisms. Further, AM possesses multiple biological effects closely related to cardiovascular homeostasis. Plasma AM concentration is increased in patients with several cardiovascular diseases such as hypertension, congestive heart failure, renal failure, and septic shock. It has been recognized that AM is one of the important vasoactive peptides involved in the physiology and pathophysiology of circulation and body fluid control.

DISCOVERY AND STRUCTURE OF AM

We have been searching for peptides that may be relevant to circulation control, using an assay system that monitors the elevating activity of rat platelet cAMP. By isolating and sequencing all of the bioactive peaks in high-performance liquid chromatography (HPLC) analysis of a human pheochromocytoma tissue extract, we were able to discover the novel biologically active peptide. Because this peptide is also abundant in normal adrenal medulla, it was designated adrenomedullin (AM) [1, 7].

Human AM consists of 52 amino acids and has one intramolecular disulfide bond [1, 7]. In addition, the C-terminal Tyr is amidated, which has been observed in a number of other biologically active peptides, including calcitonin gene–related peptide (CGRP) and amylin, with which AM shares some structural homology. As shown in Fig. 1, the sequence homology of AM with human CGRP and amylin is not high, although they share the C-terminal amide and a six-residue ring structure formed by the intramolecular disulfide linkage. Nevertheless, given the slight sequence homology and pharmacological activities that are similar to those of CGRP, it is likely that AM belongs to the CGRP superfamily. In addition to the human peptide, the amino acid sequences of AM from murine, canine, porcine, and bovine species have now been determined. Porcine AM is nearly identical to the human peptide, with a single substitution (Gly for Asn) at position 40. Rat AM has 50 amino acids, with two deletions and six substitutions, as compared with the human peptide. Notably, among all these species, the ring structure and C-terminal amide, both of which are essential for biological activity, are well conserved.

Very recently a new member of AM family, adrenomedullin 2 (AM2)/intermedin was identified by two groups [18, 23]. Although the sequence identity between AM2/intermedin and AM is relatively low (approximately 30%), as shown in Fig. 1, the pharmacological activities are similar. One of the discoverers, Takei, discusses this peptide in the Renal Peptides Section of this Handbook.

STRUCTURE OF THE PRECURSOR mRNA/GENE

The precursor for human AM (human preproAM) consists of 185 amino acid residues, including the AM sequence [9]. The predicted sequence of proAM contains a Gly-Lys-Arg segment immediately adjacent to the C-terminal tyrosine residue of AM. GIy-X-Y, where X and Y are basic residues, can serve as signals for C-terminal amidation, a process in which the glycine

```
Adrenomedullin   H2N-YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNV-APRSKISPQGY-CONH2

CGRP                          H2N-ACDTATCVTHRLAGLLSRSGGVVKNNF-VPTNVGSKA-F-CONH2
                                    *  **    **          * *   *    *
Amylin                        H2N-KCNTATCATQRLANFLVHSSNNFGALL-SSTNVGSNT-Y-CONH2
                                    *  **  * **                      *   *

Adrenomedullin-2/      H2N-TQAQLLRVGCVLGTCQVQNLSHRLWQLMGPAGRQDSAPVDPSSPHSY-CONH2
Intermedin                   *   **  *** ** * *   *          **    **  *
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FIGURE 1. Comparison of amino acid sequence of human adrenomedullin with human CGRP, amylin, and adrenomedullin 2/intermedin.

residue donates an amide moiety to the free carboxylic acid group in a reaction catalyzed by the enzyme peptidylglycine α-amidating monooxygenase (PAM; EC 1.14.17.3).

In addition to AM, proadrenomedullin (proAM) contains a unique 20-amino-acid sequence followed by Gly-Lys-Arg, a typical amidation signal, in the N-terminal region. It is possible that a novel 20-residue peptide, termed proadrenomedullin N-terminal 20 peptide (PAMP), whose carboxy terminus is Arg-$CONH_2$, is processed from the AM precursor. We have clarified that PAMP exists in vivo and elicits a potent hypotensive activity in anesthetized rats.

The genes for human and mouse AM were isolated and its structure was determined [3, 16]. The genomic DNA of human AM consists of four exons and three introns, as shown in Fig. 2. The mature AM peptide is coded in the fourth exon, whereas PAMP is interposed by the second intron. In addition, the AM gene is found to be situated in a single locus of chromosome 11. The 5′ flanking region of the gene contains TATA, CAAT, and GC boxes, and there are multiple binding sites for activator protein 2, a cAMP-regulated enhancer element, nuclear factor-kappa (κ) B, and hypoxia response elements. These indicate that the human AM gene contains components for its functional expression and that the expression may be subject to the activity of protein kinase C and feedback from cAMP levels.

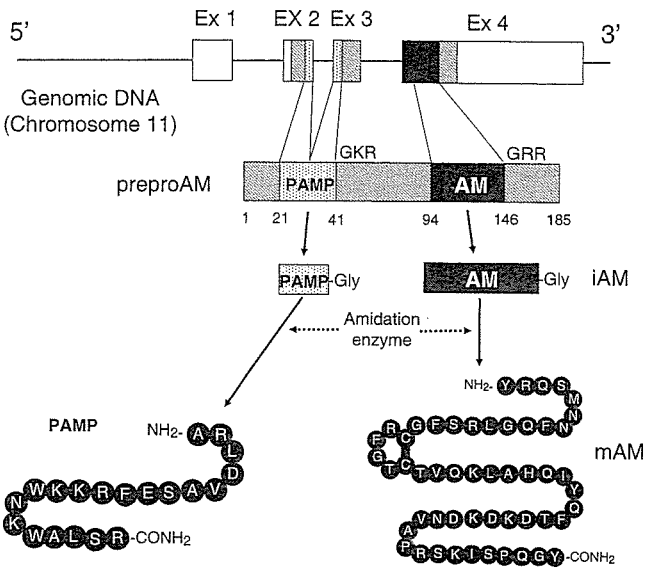

FIGURE 2. The schematic presentation of adrenomedullin (AM) gene and precursor, structures and biosynthesis of AM and proadrenomedullin N-terminal 20 peptide (PAMP).

DISTRIBUTION OF THE mRNA AND PEPTIDE IN THE CARDIOVASCULAR SYSTEM

Although AM was discovered from pheochromocytoma tissue arising from adrenal medulla, AM has been shown to be widely distributed in tissue, including cardiovascular organs. Figure 3 summarizes the distribution of AM mRNA and immunoreactivity in rat tissue. A high level of AM mRNA was found in cardiovascular tissues such as atrium, aorta, kidney, and lung as well as in adrenal gland. A high concentration of immunoreactive AM was observed in lung and atrium as well as adrenal gland. Immunoreactive AM was found ubiquitously in all tissue examined. The concentration of immunoreactive AM in aorta, ventricle, and kidney was less than 5% of that in adrenal gland, yet high levels of AM mRNA were found in these tissues [5]. This discrepancy may be explained by the possibility that AM biosynthesized in these tissues may be rapidly and constitutively secreted into the blood or function as an autocrine or paracrine regulator. In contrast, AM synthesized in adrenal medulla is thought to be stored in the granules and secreted in a regulatory pathway. Therefore, the biosynthetic and excretion systems of AM may be different from tissue to tissue.

Many different cultured cell lines produce AM. The reverse transcription polymerase chain reaction (RT-PCR) revealed the presence of AM in a variety of cells and tissues such as human pulmonary cells, pancreatic islet cells, cardiac myocytes, and vascular endothelial and smooth muscle cells [5]. Endothelial cells (ECs) actively synthesize and secrete AM [22]. In the cul-

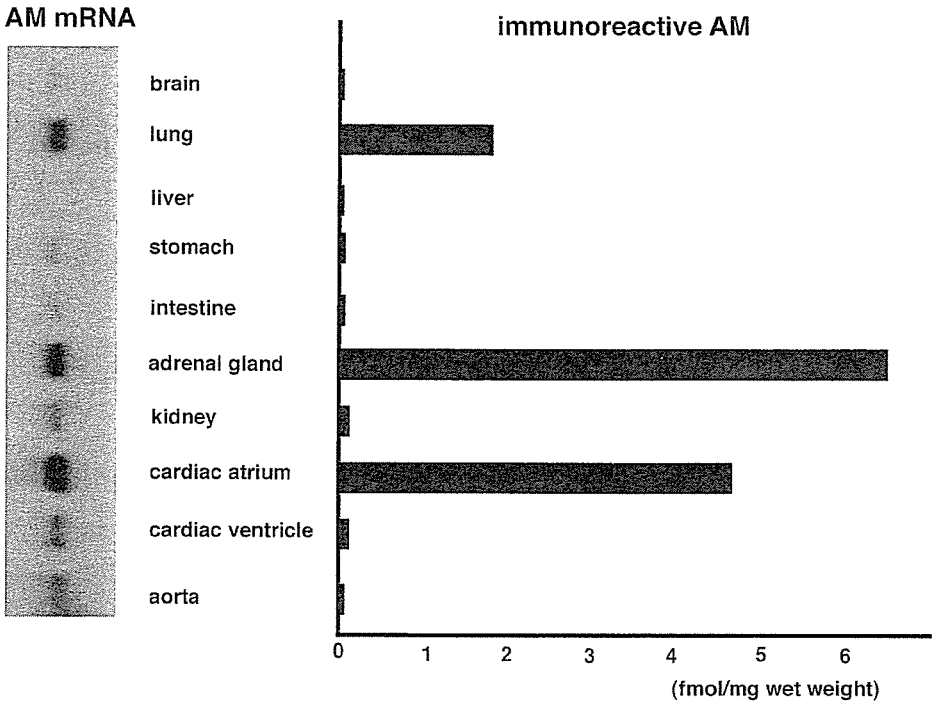

FIGURE 3. Distribution of AM mRNA and immunoreactive AM in rat tissue.

ture medium of rat ECs, the secretion rate of AM was almost comparable to that of endothelin-1. In addition to ECs, vascular smooth muscle cells (VSMCs) were found to produce AM. The presence of specific AM receptors on VSMCs and ECs is consistent with the notion that AM secreted from ECs and VSMCs functions as an autocrine or paracrine regulator in vascular cell communication.

RECEPTORS AND THEIR DISTRIBUTION IN THE CARDIOVASCULAR SYSTEM

AM has been shown to elevate intracellular cAMP levels in many but not all cells and tissues, including blood vessels, where it exerts biological actions, although identification of the AM receptor subtype has been controversial. McLatchie et al. identified three subtypes of receptor-activity-modifying protein (RAMP1–3), an accessory protein required for the transport of calcitonin-receptorlike receptor (CRLR) to the cell membrane [13]. CRLR was originally discovered as an orphan receptor that shows a 55% identity with calcitonin receptor. CRLR can function as either an AM receptor or a CGRP receptor, depending on the subtype of RAMP expressed [10]. RAMP2 enables CRLR to form an adrenomedullin (AM)-specific receptor that is sensitive to AM(22–52) (AM1 receptor). RAMP3 enables CRLR to form an AM receptor sensitive to both CGRP(8–37) and AM(22–52) (AM2 receptor), although rat and mouse AM2 receptors show a clear preference for CGRP(8–37) over AM(22–52). RAMP1 enables CRLR to form the CGRP(8–37)-sensitive CGRP1 receptor, which can also be activated by higher concentrations of AM.

CRLR mRNA is extremely abundant in the rat lung and is expressed in blood vessels by in situ hybridization studies. CRLR protein was also shown to be expressed in vascular endothelial cells by immunocytochemistry. As to the distributions of RAMPs mRNA expression in the human, rat, and mouse, RAMP1 is abundantly expressed in the brain, fat, thymus, and spleen and RAMP2 in the lung, spleen, fat, and aorta; RAMP3 is most abundant in the kidney and lung and is expressed ubiquitously [13]. Recently Kuwasako et al. clearly demonstrated that CRLR is endocytosed together with RAMPs via clathrin-coated vesicles, and both the internalized molecules are targeted to the degradative pathway [11].

BIOLOGICAL ACTIONS IN THE CARDIOVASCULAR SYSTEM

AM was discovered in human pheochromocytoma extract by monitoring activity that elevated rat platelet cAMP [7]. To date, however, it is known that AM is really multifunctional peptide. As summarized in Table 1, several biological effects of AM have been described

in vivo and in vitro. We describe here the biological actions of AM in cardiovascular systems.

The basic characteristic effect of AM is a potent, long-lasting hypotension that is dose-dependent in several species, including humans. AM dilates resistance vessels in the kidney, brain, lung, hind limbs, and mesentery in animals. In conscious sheep, Parkes has reported details on the cardiovascular and hemodynamic changes induced by human AM [17]. AM produced a dose-dependent decrease in blood pressure accompanied by an increase in heart rate and cardiac output. Decreased peripheral resistance and blood pressure (BP) induce reflex tachycardia; however, the heart rate increases less than after other vasodilators inducing comparable hypotension.

Several papers show that the vasodilating effect by AM is abrogated by blockade of nitric oxide (NO) synthase activity with L-NAME, suggesting that the decrease in total peripheral resistance subsequent to AM infusion is in part due to NO generation [19]. AM activates endothelial nitric oxide synthase (eNOS) by at least two mechanisms. First, AM elevates the intracellular calcium level, which increases eNOS activity. Second, ADM activates phosphatidylinositol 3-kinase (PI3K) and protein kinase B/Akt, which phosphorylate eNOS and increase its activity even at low calcium concentration. However, the diminution of vasodilation by L-NAME seems to vary greatly from study to study. Pulmonary vasodilator responses are significantly reduced by L-NAME in rats, but L-NAME had no significant effect in the pulmonary vascular bed of the cat [15]. Hence, it appears that nitric oxide may at least be an important mediator for AM despite its regional and interspecies variation.

In cultured cardiac myocytes and fibroblasts, AM may inhibit the protein synthesis and hypertrophy of myocytes, proliferation of fibroblasts, and production of extracellular matrix. Because AM is synthesized and secreted by cultured cardiac myocytes and fibroblasts, this peptide may regulate myocardial hypertrophy and remodeling in arterial hypertension or heart failure in a paracrine/autocrine manner.

AM may bidirectionally regulate VSMC proliferation. It stimulates the proliferation of quiescent VSMC in the absence of other stimulating factors, but inhibits proliferation induced by PDGF or fetal bovine serum. AM inhibits endothelial cell apoptosis induced by serum deprivation. This effect is mediated by nitric oxide, but is cGMP-independent. In addition, AM stimulates the proliferation of endothelial cells that may be involved in angiogenesis and reendothelialization of injured vessels. Considering the results on vascular tissue expression of AM mRNA, AM seems to regulate vascular proliferation and remodeling as well as vascular tone.

TABLE 1. Cardiovascular Actions of Adrenomedullin.

Vasculature	Hypotension, antiproliferation, survival factor inhibition of Ca increase by endothelin, decreased endothelin production
Heart	Positive chronotropism and inotropism, increased coronary blood flow, increased ANP gene transcription, antimitogenesis, increased hypertrophy
Lung	Vasodilation, bronchodilation, antiinflammatory
Adrenal gland	Inhibition of aldosterone secretion
Kidney	Increased renal flow, diuresis, natriuresis, inhibition of mesangial cell proliferation
Pituitary	Inhibition of ACTH secretion, inhibition of AVP secretion
Brain	Increased collateralization and cerebral blood flow, inhibition of thirst and salt appetite, stimulation of sympathetic outflow (hypertension)

PROADRENOMEDULLIN N-TERMINAL 20 PEPTIDE

PAMP consists of 1–20 amino acids of proadrenomedullin (pro-AM) whose C-terminus is Arg-CONH2. The distribution of PAMP in mammalian tissues is similar to AM, consistent with their origin from a common precursor. The PAMP/AM ratio in tissue extracts and cell culture homogenates varies depending on the cell studied, from 1–2% in the lung to ~50% in the heart atria. PAMP elicits a potent hypotensive effect in anesthetized rats [6]. An intravenous bolus injection of human PAMP causes a rapid and strong hypotensive effect in a dose-dependent manner, although this peptide is less potent than AM.

PAMP is found to inhibit carbachol-induced catecholamine secretion in cultured bovine adrenal medullary cells, but AM showed no effect on catecholamine secretion. Fujita et al. demonstrated that AM infused into pithed rat showed hypotensive action dose-dependently but PAMP did not [20]. After BP was increased to a level of 80–100 mmHg by electrical stimulation of the pithed rat, PAMP exhibited hypotensive effects. Furthermore, during augmentation of peripheral sympathetic nerve activity with periarterial electrical stimulation, norepinephrine released in the perfusate was measured as an indication of neural transmission. PAMP decreased norepinephrine overflow dose-dependently, whereas AM did not. These results suggest that the hypotensive effect of PAMP may be due to the inhibition of neural transmission at nerve endings

rather than via a direct vasodilating effect. Recently, it has been reported that PAMP may antagonize the stimulatory effect of AM on endothelial NO production, suggesting a more complex role of this peptide in the regulation of vascular tone [12]. Very recently, it has been reported PAMP exhibits an extremely potent angiogenic potential. Exposure of endothelial cells to PAMP increases gene expression of other angiogenic factors such as adrenomedullin, vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived growth factor C.

Consequently, PAMP as well as AM is biosynthesized from the AM precursor and may participate in circulation control in different mechanisms (Fig. 2).

INFORMATION ON MOLECULAR FORM OF AM

The mature form of AM (mAM, AM[1–52] NH2) is produced by C-terminal amidation of glycine-extended AM, AM-glycine (AM-Gly). AM-Gly, an intermediate form of AM (iAM), is processed from proAM. Biological activity is exerted only by mAM. We have found that most of the circulating AM in human plasma is not mAM but iAM [8]. The plasma concentration of mAM was much lower than that of AM-Gly or of total AM (tAM), which is mAM and iAM. All forms of AM increased significantly as the severity of congestive heart failure increased [2]. The ratio of mAM/tAM did not differ significantly in any of the study groups, suggesting that the amidation process of AM is unaffected in the patients with congestive heart failure. On the other hand, the major immunoreactive AM in tissue or cell is determined to be mAM.

CLINICAL IMPLICATION OF AM IN CARDIOVASCULAR DISEASES

AM circulates in human blood at a considerable concentration, and the plasma AM levels were increased in patients with a variety of diseases, including congestive heart failure, renal diseases, hypertensive diseases, diabetes mellitus, and septic shock [1].

The plasma AM concentration in patients with essential hypertension or primary aldosteronism was significantly higher than that in normotensive controls. The plasma AM levels increase with the severity of hypertension [4]. Furthermore, in the patients with malignant hypertension and renovascular hypertension, the increase in plasma AM is marked in comparison with the increases in primary aldosteronism and essential hypertension. AM, therefore, seems to act as a humoral regulator in blood pressure control and to counteract pressor factors that may cause and promote hypertension. In patients with hypertension complicated with chronic renal failure, the increment of AM is obvious and the plasma AM concentration was elevated in relation to the degree of renal failure.

In patients with congestive heart failure, the plasma AM was significantly correlated with pulmonary artery pressure, pulmonary capillary wedge pressure, left atrial dimension, plasma renin activity, and plasma concentrations of atrial and brain natriuretic peptides. Intravenous infusion of human AM into patients with congestive heart failure predominantly improved cardiac function [14]. The AM elicited dilatation of the resistance arteries and increases in cardiac stroke index and urinary sodium excretion. An improvement of the cardiac pre- and after-loads and cardiac contractility elicited by AM is the mechanism for recovery of function of the failing heart.

Intravenous injection of lipopolysaccharide (endotoxin) produces a marked increase in plasma immunoreactive AM in a dose-dependent fashion in the anesthetized rat, suggesting AM may be involved in sepsis. Actually, plasma AM concentration was markedly increased in patients with septic shock. As suggested by an experimental model of septic shock in transgenic mice [21], the large amount of plasma AM observed in patients with septic shock may play a role in protecting against peripheral circulatory failure, organ damage, and mortality characteristic of endotoxic shock.

In summary, regarding the clinical features of a variety of diseases, the plasma and tissue concentrations of AM are increased in response to pathological conditions such as hypervolemia, hypertension, tissue ischemia or hypoxia, and inflammatory damage by cytokines. The increased plasma and tissue concentrations of AM seems to function as a counterregulator for pathologically altered circulation, tissue ischemia, or tissue injury by toxic factors through its humoral or paracrine-autocrine actions.

CONCLUSION

AM is a potent vasodilatory peptide discovered in 1993 from the extract of human pheochromocytoma. AM consists of 52 amino acids and belongs to the calcitonin, CGRP, and amylin family. In addition to AM, the AM precursor generates another bioactive peptide, proadrenomedullin N-terminal 20 peptide (PAMP). Though AM and PAMP show hypotensive effect, their mechanisms are different. During the 12 years since its discovery, over 1600 papers concerning AM and PAMP have been published. Considering the multifunctional characteristics of AM and significant increase in plasma

immunoreactive AM levels in patients with cardiovascular diseases, AM should be recognized as an important factor regulating circulation and involved in cardiovascular diseases.

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Isolation and identification of proangiotensin-12, a possible component of the renin–angiotensin system

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Abstract

The renin–angiotensin (RA) system plays an important role in regulating blood pressure and fluid balance. In the search for bioactive peptides with an antibody binding to the N-terminal portion of angiotensin II (Ang II), we isolated a new angiotensinogen-derived peptide from the rat small intestine. Consisting of 12 amino acids, this peptide was termed proangiotensin-12 based on its possible role of an Ang II precursor. Proangiotensin-12 constricted aortic strips and, when infused intravenously, raised blood pressure in rats, while both the vasoconstrictor and pressor response to proangiotensin-12 were abolished by captopril and by CV-11974, an Ang II type I receptor blocker. Proangiotensin-12 is abundant in a wide range of organs and tissues including the small intestine, spleen, kidneys, and liver of rats. The identification of proangiotensin-12 suggests a processing cascade of the RA system, different from the cleavage of angiotensinogen to Ang I by renin.

Keywords: Proangiotensin-12; Renin–angiotensin system; Pressor peptide; Angiotensinogen; Processing

A number of mechanical or humoral mechanisms are involved in blood pressure and body fluid homeostasis in mammals. Among them, the renin–angiotensin (RA) system plays a pivotal role, regulating the vascular tone of resistant vessels and glomerular filtration or re-absorption of electrolytes in the kidneys. The protease enzyme renin secreted from kidneys cleaves specifically angiotensinogen circulating in the blood to produce angiotensin I (Ang I), a peptide consisting of 10 amino acid residues, which in the presence of angiotensin-converting enzyme (ACE), is in turn converted to Ang II, a potent pressor peptide mediating the major actions of the RA system as a circulating hormone [1,2]. On the other hand, recent research has revealed new aspects of the RA system. For example, the tissue RA system has been vigorously studied, and its activation is assumed to be regulated independently of the systemic RA system [3–5]. Another example is the identification of Ang II-derived peptides that are shorter than Ang II, such as Ang(1–7), Ang III, or Ang IV, having pharmacological properties different from those of Ang II [6–11]. Meanwhile, consisting of 10 amino acid residues, Ang I has been thought to be produced by renin directly from angiotensinogen, a protein of 452 amino acids for humans or 453 for rats [12,13], but there has been no report on the occurrence of angiotensinogen-derived peptides of amino acid sequences longer than Ang I. In the present study, on searching for peptides structurally related to Ang II, we purified an angiotensinogen-derived pressor peptide of 12 amino acids, which is thought to be one of the major components of the RA system.

Materials and methods

Reagents and animals. Ang I and Ang II were purchased from Peptide Institute Inc. (Osaka, Japan) and proangiotensin-12 was synthesized by Bex. Co., Ltd. (Tokyo, Japan). CV-11974 was kindly provided by Takeda

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Pharmaceutical Company Limited (Osaka, Japan). Wistar rats of 6–7 weeks of age were purchased from Charles River Laboratories (Kanagawa, Japan) and New Zealand white rabbits from Kyudo Co., Ltd. (Saga, Japan). The present study was performed in accordance with the Animal Welfare Act and with approval of the University of Miyazaki Institutional Animal Care and Use Committee (2004-100-2, 2004-101-2).

Detection of immunoreactive N-terminal Ang II. To prepare antiserum against the N-terminal of Ang II, synthesized Ang II-Cys was conjugated with *keyhole limpet* hemocyanin at room temperature over 2 h. After dialyses repeated four times in 50 mmol/L PBS, the conjugate solution was emulsified with an equal volume of Titer Max Gold (Sigma–Aldrich, Tokyo, Japan) and injected subcutaneously into New Zealand white rabbits every two weeks over three months. A specific radioimmunoassay (RIA) was prepared with the antiserum obtained from an immunized rabbit, as previously described [14,15]. This RIA cross-reacted with Ang I and Ang III at levels of 50% and 12.5%, respectively, without cross-reactions with Ang IV and Ang(1–7). To characterize the immunoreactive N-terminal Ang II in tissues, 1.0 g of sample from various rat tissues was immediately boiled for 10 min and acidified with acetic acid to a final concentration of 1.0 mol/L to inactivate proteases. Then the samples were homogenized and centrifuged for 20 min at 12,000 rpm, and the supernatant was applied to Sep-Pak C18 cartridges. After elution with 60% CH_3CN in 0.1% trifluoroacetic acid, the peptide extracts were subjected to reverse-phase high-performance liquid chromatography (RP-HPLC) with an ODS-120A column, and the immunoreactive N-terminal Ang II of each fraction was measured by the RIA.

Purification procedure. The peptide extract was obtained with the above-described method from 380 g of small intestine, a rat tissue showing the largest peak of immunoreactive N-terminal Ang II at a position clearly different from that of Ang I or Ang II in the RP-HPLC analysis. The sample was applied to a SP Sephadex C-25 column eluted with 2.0 mol/L pyridine, and subjected to gel filtration with Sephadex G-50 and an affinity column (Affi-Gel 10 Active Ester Agarose, Bio-Rad) which had been prepared with the anti-N-terminal Ang II antiserum. These purification steps were done while monitoring immunoreactive N-terminal Ang II with the RIA. The extract was further purified by three steps of HPLC with columns of ODS-120A, diphenyl, and Chemco sorb3-ODS-H. The amino acid sequence of the finally purified peptide was analyzed by a time-of-flight mass spectrometric method.

Mass spectrometry. To determine the amino acid sequence and molecular weight of the purified peptide, a tandem mass spectrometric analysis was performed with positive electrospray ionization using a QTof-2 quadrupole time of flight mass spectrometer (Micromass, UK). The sample was dissolved in a solution of water/methanol/acetic acid mixed at 49:49:2 by volume and nanosprayed from off-line MS emitters (Proxeon, Denmark) with a capillary voltage of 1.5 kV and a cone voltage of 30 V. The mass spectrum was acquired from m/z 200 to 2000. For tandem mass spectrometry, the triply charged ion with m/z 524.97 was subjected to collision-induced dissociation with argon gas in the 30 eV collision energy range. Data acquisition and processing were performed using MassLynx v4.0 (Micromass). The resultant tandem mass spectrum was subjected to a Mascot MS/MS ion search (Matrixscience, UK) and also interpreted by PepSeq (Micromass).

Radioimmunoassay for the C-terminal portion of proangiotensin-12. To specifically detect proangiotensin-12 in tissues and plasma, we developed a RIA, as previously described, with antiserum raised against the C-terminal portion of the peptide [14,15]. Synthetic proangiotensin-12 was conjugated with bovine thyroglobulin at room temperature for 15 min by the glutaraldehyde method. New Zealand white rabbits were immunized with the dialyzed conjugate, according to the procedure described above. After the immunization, specific antibody was purified from the antiserum using an affinity column (NHS-activated Sepharose 4 Fast Flow) with the C-terminal peptide of proangiotensin-12, Ile-His-Pro-Phe-His-Leu-Leu-Tyr. The proangiotensin-12 levels in tissues and plasma of male Wistar rats were determined by RIA with the purified antibody, following extraction with a Sep-Pak C18 cartridge, as described previously [14,15]. This RIA detected 1.6 and 3.1% of angiotensinogen(1–14) and angiotensinogen(1–17), respectively, but showed no cross-reactivity with Ang I, Ang II, Ang III, Ang IV, or Ang(1–7). The Ang I or Ang II levels in tissues and plasma were similarly determined by RIAs with anti-C-terminal of Ang I and Ang II antisera purchased from Miles and Cortex Biochem, Inc. (San Leandro, USA), respectively [16,17]. To characterize the immunoreactive C-terminal proangiotensin-12 in the extract of rat small intestines, a RP-HPLC analysis was done with an ODS-120A column as described above.

Pharmacological studies ex vivo and in vivo. We examined the effects of proangiotensin-12 on vascular tone with perfused aorta isolated from rats as reported previously [18]. The aortic rings were mounted under a passive tension of 1.0 g in organ baths containing Krebs–Henseleit solution oxidized with 95% O_2 and 5% CO_2 at 37 °C, and left for 60 min for equilibration. The viability of the aortic rings was confirmed by exposing the samples to 60 mmol/L KCl, 10^{-7} mol/L phenylephrine, and 10^{-6} mol/L acetylcholine. Contractions of the aortic samples were recorded before and after Ang I, Ang II, or proangiotensin-12 was added to the perfusion solution at the indicated concentration. The responses to these peptides were also tested in the presence of 10^{-7} mol/L captopril, an ACE inhibitor, or 10^{-8} mol/L CV-11974, an Ang II type 1 (AT1) receptor antagonist. Next, the effects of proangiotensin-12 on blood pressure were examined *in vivo* with rats fed a normal rat chow, as previously described [19]. Male Wistar rats weighing 220–260 g were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital sodium. Synthetic proangiotensin-12 dissolved in 100 μl saline was injected into the jugular vein at the bolus-dose indicated, and blood pressure and heart rate were monitored before and after the injection by a carotid artery catheter connected to a pressure transducer with the Power Lab system (MLT0699 Disposable BP Transducer, AD Instruments, Australia). The responses to proangiotensin-12 were similarly tested 2 min after the intravenous injection of 0.03 mg/kg captopril or 0.3 mg/kg CV-11974.

Statistical analysis. Comparisons of all data were made with an analysis of variance (ANOVA) followed by Scheffe's test. Values are presented as means ± SE and statistical significance was set at $P < 0.05$.

Results

Purification of proangiotensin-12

As a first step in the isolation of Ang II-related peptides, we analyzed immunoreactive N-terminal Ang II in peptide samples extracted from various tissues of rats with RP-HPLC and a RIA. In those analyses, we found three clear peaks of immunoreactive N-terminal Ang II in rat tissues including the small intestine (Fig. 1): the earliest peak and the next peak corresponded to Ang II and Ang I, respectively. Interestingly, the largest peak emerged later

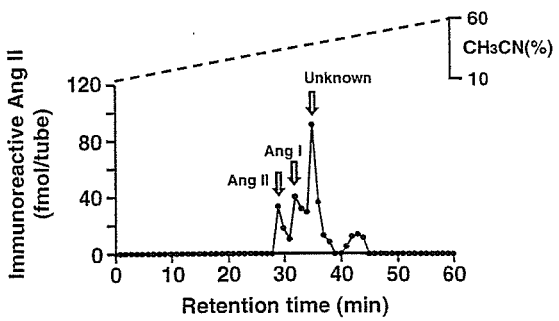

Fig. 1. Immunoreactive N-terminal Ang II in rat small intestine. An extract of the small intestine was subjected to RP-HPLC, where the immunoreactive N-terminal Ang II of each fraction was determined by specific radioimmunoassay, as described. An elution gradient of 10% to 60% CH_3CN was made in 0.1% trifluoroacetic acid over 60 min with a flow rate at 1 ml/min.

than Ang I in the small intestine, suggesting the presence of an unknown peptide similar in sequence to the N-terminal portion of Ang II. Therefore, we tried to purify this unknown peptide from 380 g of rat small intestine by several steps of ion-exchange, gel-filtration, and affinity chromatography, and RP-HPLC, as described in the Materials and methods section. Fig. 2A shows the final step of purification with RP-HPLC, where the unknown peptide was obtained as a single peak. The purified peptide sample was then subjected to the tandem mass spectrometry and found to consist of 12 amino acid residues (Fig. 2B). In comparison of the sequences of Ang I and the N-terminal portion of angiotensinogen, the unknown peptide was deduced to be a C-terminal extended form of Ang I (Fig. 3). We have termed this novel peptide proangiotensin-12, based upon the results of the tissue distribution and pharmacological experiments of the present study, suggesting a role for this peptide as a precursor of Ang II.

Measurement of tissue and plasma levels of proangiotensin-12

Next, we developed a radioimmunoassay specifically detecting the C-terminal portion of proangiotensin-12 to clarify the presence of this novel peptide in rat tissues including the small intestine. As shown in Table 1, proangiotensin-12 was abundantly detected in a variety of tissues, where its levels were higher than those of Ang I or Ang II, except for the lungs, adrenal glands, pancreas, and aorta. These tissue levels determined by the RIA specific to the C-terminal were found to be mostly identical to those estimated by an immunoreactive peak corresponding to proangiotensin-12 (Fig. 1) by RP-HPLC analyses with RIA detecting the N-terminal Ang II. In contrast to the tissue levels, the plasma concentration of proangiotensin-12 was lower than that of Ang I or

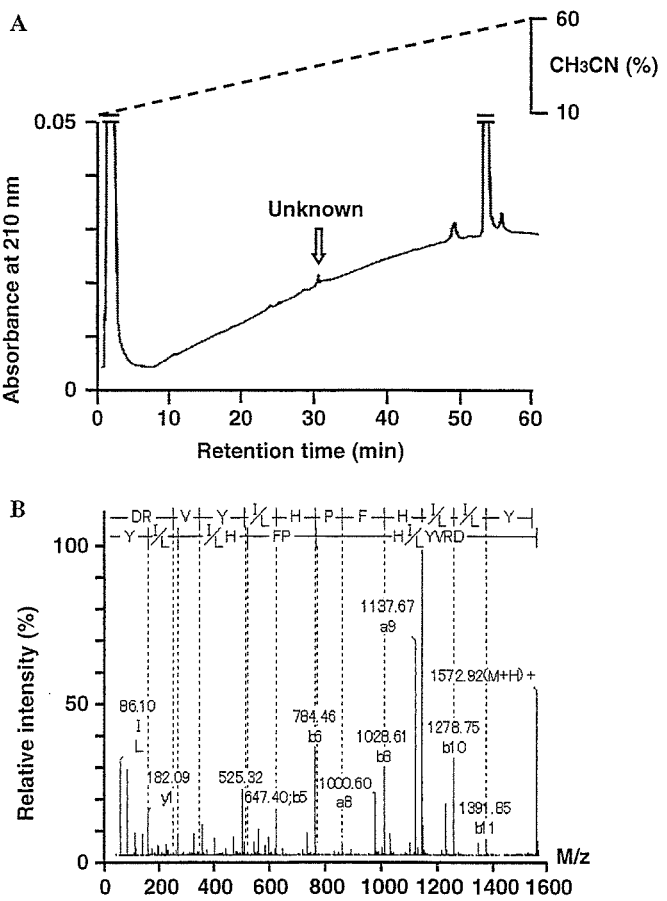

Fig. 2. Final purification by RP-HPLC (A) and tandem mass spectrometric analysis of the purified peptide (B).

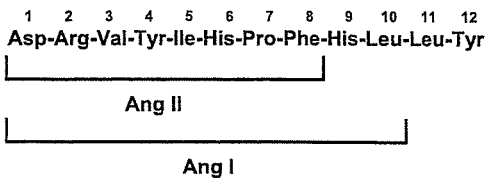

Fig. 3. Amino acid sequence of proangiotensin-12.

Table 1
Specific measurement of immunoreactive proangiotensin-12, Ang I, and Ang II levels in rat tissues and plasma

	Proangiotensin-12	Ang I	Ang II
Small intestine	663.1 ± 123.3	283.6 ± 43.0	254.9 ± 48.3
Spleen	419.1 ± 18.4	71.1 ± 4.0	302.0 ± 8.7
Kidneys	380.3 ± 27.0	365.2 ± 29.7	116.4 ± 6.9
Liver	252.0 ± 13.8	79.6 ± 2.6	87.9 ± 4.6
Stomach	241.7 ± 15.5	148.2 ± 23.0	81.5 ± 6.8
Lungs	227.8 ± 20.1	56.7 ± 5.7	236.2 ± 31.7
Adrenal glands	223.8 ± 8.2	121.4 ± 27.7	3049.2 ± 779.5
Heart	150.6 ± 11.3	84.9 ± 7.8	42.3 ± 7.3
Brain	147.4 ± 8.1	52.0 ± 2.7	29.6 ± 3.6
Pancreas	87.2 ± 13.8	37.8 ± 11.6	93.0 ± 17.6
Aorta	24.1 ± 7.5	39.6 ± 2.4	118.5 ± 6.6
Plasma	10.1 ± 2.7	382.5 ± 79.9	28.9 ± 8.8

The tissue or plasma levels of proangiotensin-12 were determined by a radioimmunoassay specifically detecting the C-terminal portion of the peptide as described in the Materials and methods section. The tissues are listed in order of immunoreactive proangiotensin-12 levels. The results are shown as means ± SE for eight rats examined (fmol/g tissue or fmol/ml plasma).

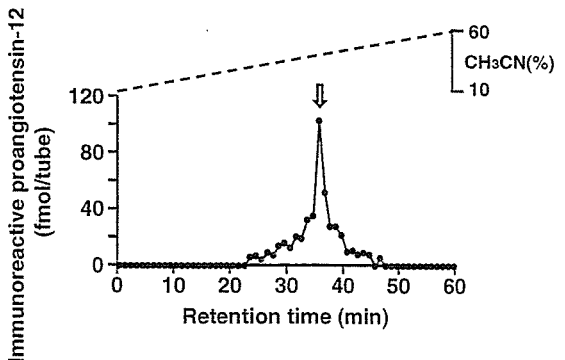

Fig. 4. Characterization of immunoreactive C-terminal proangiotensin-12 in rat small intestine by RP-HPLC and radioimmunoassay. RP-HPLC was conducted as described in the legend for Fig. 1. The arrow indicates the position of the full-length synthetic proangiotensin-12 peptide.

Ang II. The HPLC analysis revealed that immunoreactive proangiotensin-12 in the small intestine was eluted at the same position as the complete synthetic peptide (Fig. 4), further confirming the amino acid sequence and presence of proangiotensin-12.

Pharmacological studies ex vivo and in vivo

To study the biological actions of proangiotensin-12, we first looked at the effects on the vascular tonus of perfused rat aortic rings *ex vivo*. As shown in Fig. 5, proangiotensin-12 dose-dependently constricted the rat aorta as did Ang I or Ang II. Ang I and proangiotensin-12 had weaker vasoconstrictor effects than Ang II at concentrations of 3–30 nmol/L, but similar maximum contractions were obtained at 100 nmol/L of all three peptides. The constrictor action of proangiotensin-12 was mostly abolished in the presence of captopril, an ACE inhibitor, or CV-11974, an AT1 receptor blocker. Next, we examined the effects of proangiotensin-12 on blood pressure levels *in vivo* in anesthetized rats. A rise in arterial blood pressure was observed immediately after the intravenous injection of a bolus of proangiotensin-12 in rats, with a return to the basal level in 3–4 min (Fig. 6A). The pressor effects were dose-dependent and attenuated by pre-administration of captopril or CV-11974 (Figs. 6B and C), a result consistent with the *ex vivo* study.

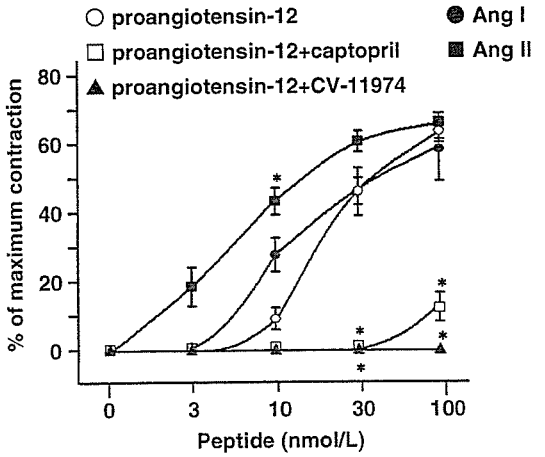

Fig. 5. Vasoconstrictor effects of Ang I, Ang II, and proangiotensin-12 on perfused rat aorta *ex vivo*. Captopril and CV-11974, an AT1 receptor antagonist, were used at 10^{-7} and 10^{-8} mol/L, respectively. The results are shown as means ± SE of % of maximum contraction induced by 60 mmol/L KCl for six to seven samples. $^{*}P < 0.05$ vs proangiotensin-12 alone.

Discussion

In this report, we describe the purification, sequence determination, tissue distribution, and vasoconstrictor properties of the novel angiotensinogen-derived peptide, Ang I-Leu-Tyr, which consists of 12 amino acid residues. We have termed this novel peptide proangiotensin-12, based upon the present results that suggest its role as a precursor of Ang II.

A concern may be raised over non-specific cleavage between Tyr-12 and Ser-13 of rat angiotensinogen, resulting in the occurrence of proangiotensin-12 as a non-specifically fragmented product during the extraction procedure; however, this possibility is unlikely based on the following reasons or findings. First, samples of rat small intestine were immediately boiled and acidified after resection in

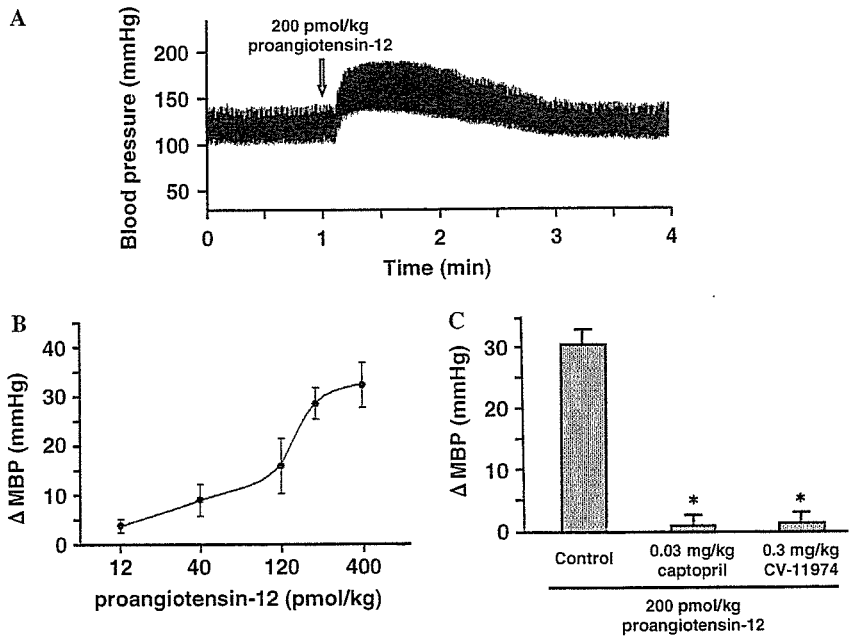

Fig. 6. (A) Representative blood pressure recording following the intravenous injection of a 200 pmol/kg bolus of proangiotensin-12 in anesthetized rats, (B) dose-dependent pressor effects, and (C) attenuation of the effects of proangiotensin-12 by captopril or CV-11974, an AT1 receptor blocker. ΔMBP indicates the maximum rise of mean blood pressure from the baseline. Either captopril or CV-11974 was intravenously injected at the indicated dose 2 min before the injection of proangiotensin-12. The results are shown as means ± SE for five rats examined. $^{*}P < 0.05$ vs proangiotensin-12 alone.

the extraction procedure. Non-specific cleavage of peptides can be avoided with this step which inactivates enzymes with proteolytic activity [20,21]. Second, the HPLC analysis of immunoreactive N-terminal Ang II (Fig. 1) showed three clear peaks, which correspond to Ang II, Ang I, and proangiotensin-12, without fragmentation of peptides. The specific radioimmunoassay (Table 1) also revealed that proangiotensin-12 is ubiquitously present in various organs and tissues, as are Ang I and Ang II. Last, the immunoreactive proangiotensin-12 in the small intestine was eluted at the position of the full-length peptide in the RP-HPLC analysis and radioimmunoassay detecting the C-terminal portion of proangiotensin-12 (Fig. 4). These findings are clearly indicative of the endogenous occurrence of proangiotensin-12.

A number of mechanisms, either mechanical or humoral, have been known to be involved in regulating blood pressure and body fluid balance in mammals. Among the humoral factors, the RA system plays a pivotal role: renin secreted mainly from kidneys cleaves angiotensinogen circulating in the blood to produce Ang I, which is then converted to Ang II exerting various effects particularly important for blood pressure and body fluid homeostasis. In the meantime, recent progress in research has revealed new aspects of the RA system. One example is identification of the Ang II-derived bioactive peptides with amino acid sequences shorter than Ang II such as Ang(1–7), Ang III or Ang IV [6–11]. Given this fact, it should be noted that proangiotensin-12 is a peptide longer than Ang I and such an angiotensinogen-derived peptide had not been identified. The second example is an active role of the tissue RA system probably independent of the systemic RA system [3–5]. In the present study, radioimmunoassay specifically detecting the C-terminal portion of proangiotensin-12 revealed ubiquitous presence of this novel peptide in various tissues and organs at the concentrations comparable with those of Ang I and Ang II, while the plasma concentration was lower than that of Ang I or Ang II. The relatively higher tissue levels suggest a significant role of proangiotensin-12 as a molecule of the tissue RA system.

In the present study, proangiotensin-12 exerted vasoconstrictor activity *ex vivo* on rat aortic rings, potency of which was similar to Ang I, while somehow weaker than Ang II at concentration of 3–30 nmol/L, showing the maximum contraction similar to Ang II. When injected intravenously in rats, proangiotensin-12 immediately raised blood pressure levels, and both the vasoconstrictor and pressor effects mostly disappeared following administration of captopril or CV-11974, an AT1 receptor blocker. These findings suggest prompt conversions of proangiotensin-12 to Ang I, and then Ang I to Ang II by ACE. Although the enzymes involved in the conversion to Ang I remain to be identified, the prompt cleavages of proangiotensin-12 to produce Ang II support the hypothesis of a significant role of proangiotensin-12 as an important molecule of the RA system.

Angiotensinogen is recognized to be produced and supplied to the blood mainly by the liver, though this precursor protein has been shown to be expressed widely in other tissues [22–25]. Consistent with this, according to our quantitative PCR, angiotensinogen mRNA was detected in various rat organs and tissues including the small intestine with the highest level in liver (data not shown). Because renin is an exclusively specific enzyme which cleaves angiotensinogen directly to produce Ang I, it is unlikely that renin is involved in the production of proangiotensin-12 [1,2]. An important question related to this is where the cleavage of angiotensinogen to proangiotensin-12 occurs: in tissues or in plasma. Clarifying the processing cascade of angiotensinogen would therefore provide us with information not only on the production of proangiotensin-12 but also on the mechanism activating the RA system and the role of proangiotensin-12 in blood pressure and body fluid homeostasis. Indeed, we are currently working on characterization and purification of enzymes that produce or cleave proangiotensin-12. Additionally, it should be clarified whether or not Ang I precursor peptides similar to proangiotensin-12 are present in other species including humans. Thus, the identification of proangiotensin-12 warrants future research on the RA system, which should be aimed at addressing a number of these unanswered questions.

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Roles of Adrenomedullin in Hypertension and Hypertensive Organ Damage

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Abstract: Adrenomedullin (AM) is a bioactive peptide having a wide range of biological actions such as vasodilatation, natriuresis, diuresis and inhibition of aldosterone secretion. Various organs and tissues, including the myocardium, vascular wall and kidneys, produce AM, and AM is also present in the bloodstream. Plasma levels of AM were elevated in patients with essential or secondary hypertension as compared with normotensive controls. When infused in a relatively short period of time, AM reduced blood pressure in humans and experimental animals largely through vasodilatation. The blood pressure of transgenic mice overexpressing AM was lower than that of their wild-type littermates; while heterozygotes of AM knockout mice showed higher blood pressure, suggesting a role for endogenous AM in the regulation of blood pressure. Studies with cultured cardiac cells suggest a role for AM in inhibiting hypertrophy or fibrosis of the heart as an autocrine or paracrine factor. Animal experiment studies showed that either prolonged infusion or virally mediated overexpression of AM ameliorated the cardiovascular and renal damage associated with hypertension. Thus, a body of evidence accumulated in this field suggests that AM functions to counteract the elevation in blood pressure and progression of hypertensive organ damage.

Key Words: Adrenomedullin, hypotensive peptide, hypertension, end organ damage.

INTRODUCTION

In mammalians, blood pressure and the water-electrolyte balance are regulated by a number of neurohumoral factors, among which, peptide hormones including angiotensin II, endothelins and natriuretic peptides have a particular importance. These peptides have been shown to not only participate in the mechanism regulating blood pressure but also modulate progression of the cardiovascular and renal diseases associated with elevations of blood pressure. In 1993, a new peptide hormone, adrenomedullin (AM), was isolated from tissue extract of human pheochromocytoma by monitoring cAMP levels in rat platelets [1]. Substantial levels of the AM peptide and gene expression were detected in the heart, blood vessels and kidneys, and AM was found to exert a wide range of biological actions related to cardiovascular and renal functions such as vasodilatation, natriuresis and inhibition of aldosterone secretion [1-3]. Ever since the discovery of AM, efforts have been made to clarify the role of this bioactive peptide and a substantial amount of basic and clinical data has been accumulated. In this review, after summarizing the biochemical and pharmacological features of AM, we comprehensively discuss the pathophysiological role of AM in hypertension and hypertensive organ damage.

BIOCHEMISTRY OF AM

Human AM is a 52-amino-acid peptide with a ring structure formed by a disulfide bond and amidated tyrosine at the C-terminal (Fig. (**1**)), portions essential for the binding to receptors and biological actions [1-3]. Based on sequence homology, AM is thought to belong to the calcitonin gene-related peptide (CGRP) superfamily [1-3]. Cloning of the cDNA encoding AM revealed the sequence of the AM precursor peptide preproAM of 185 amino acids, in which the C-terminal of AM is found to be followed by a pair of basic amino acids, Arg-Arg, a typical processing signal (Fig. (**2**)) [4]. In addition to AM, preproAM was found to contain another bioactive peptide, proadrenomedullin N-terminal 20 peptide (PAMP), in the N-terminal portion [4]. PAMP lowered blood pressure when injected intravenously, but the hypotensive action is weaker than that of AM and there has currently been little information as to the role of PAMP in regulating blood pressure [3, 4]. Ishimitsu *et al.* reported the sequence of genomic DNA for human preproAM [5], where AM is encoded in the fourth exon and PAMP in the second and third exons (Fig. (**2**)). When processed from preproAM, AM-Gly, an intermediate form (iAM), is produced, and then, iAM is converted by amidation enzyme to the mature form of AM (mAM) having an amide structure at the C-terminal [6]. The mature form of PAMP is thought to be produced by a similar process (Fig. (**2**)).

BIOLOGICAL ACTIONS OF AM

Soon after the discovery of AM peptide, a number of pharmacological studies were performed to see the biological actions of AM in experimental animals or human subjects [2, 3]. As listed in Table **1**, AM has been shown to exert a wide range of biological effects in various organs and tissues. Some of them are thought to occur at physiological levels of AM in plasma or tissue, but it is unclear whether or not the others do. Nonetheless, one can realize that many of the effects of AM are closely related to the regulation of blood pressure and the body fluid-electrolyte balance. We summarize the biological effects of AM below, based mainly on the results of pharmacological studies. The cardiovascular pro-

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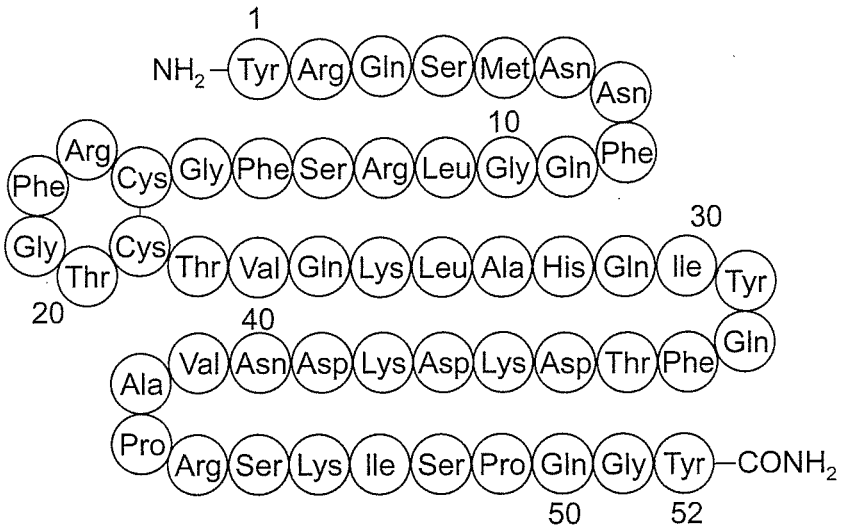

Fig. (1). Amino-acid sequence of human AM.

tective effect and long-term blood pressure-modulating effect of AM are discussed later in this review.

Blood Vessels

AM was initially characterized as having a potent, long-lasting blood pressure-lowering effect with reduced peripheral resistance following intravenous bolus injection or infusion in a relatively short period of time [1, 7, 8]. The hypotensive effect of AM observed in the short-term was shown to be largely secondary to direct vasodilatation [7, 8], which was further demonstrated by studies *ex vivo* with isolated rat aorta and with perfused rat mesenteric artery [9, 10]. Endothelium denudation abolished the vasodilator action of AM in a rat aortic ring preparation, indicating an endothelium-dependent vasodilatation [10]. Consistent with this, AM was found to dilate blood vessels *via* the nitric oxide and cyclic GMP (NO-cGMP) pathway; but there has been some inconsistency regarding the role of this intracellular pathway [10-14]. For example, Nossaman *et al.* reported that pulmonary vasodilator responses were reduced by N-nitro-L-arginine methyl ester (L-NAME) in rats, but not in cats [14]. The mechanism by which AM dilates blood vessels may differ depending on species or possibly on the regions in which the vessels are isolated from [10-14].

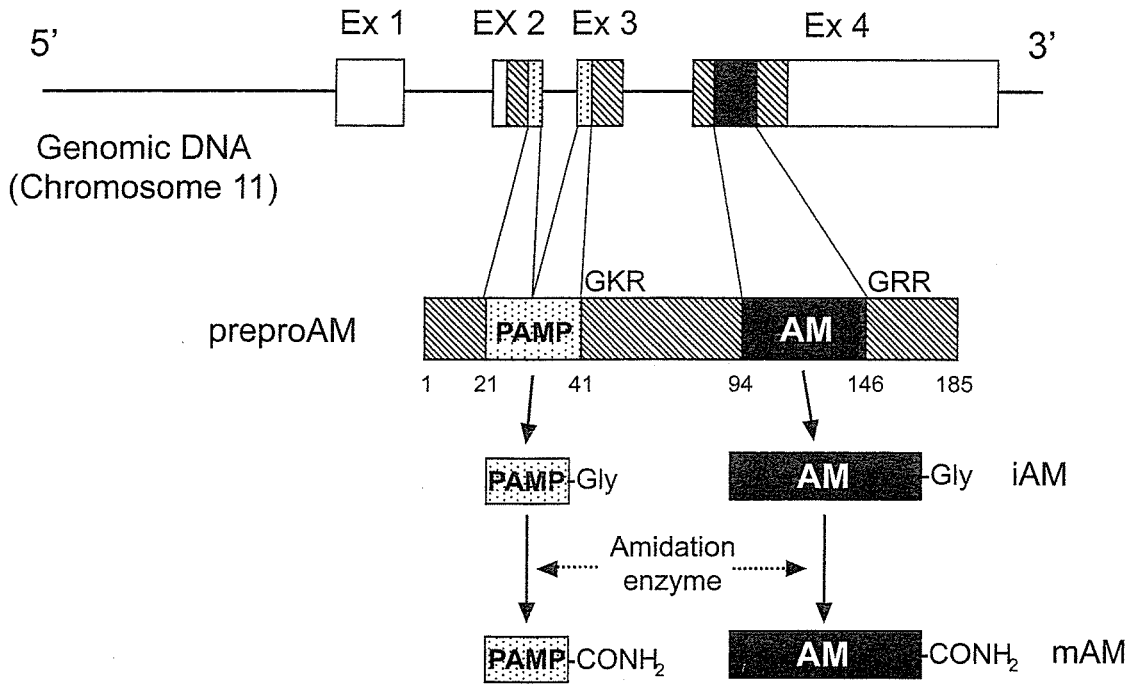

Fig. (2). Schematic representations of the AM genc and of processing of AM and PAMP from preproAM. Ex, exon: PAMP, proadrenomedullin N-terminal 20 peptide; iAM and iPAMP, intermediate forms of AM and PAMP; mAM and mPAMP, mature forms of AM and PAMP.

Table 1. Biological Actions of AM

Blood vessels	Vasodilatation Stimulation of nitric oxide (NO) production Inhibition of proliferation and migration of smooth muscle cells Inhibition of apoptosis of vascular endothelial cells Inhibition of endothelin production
Heart	Inhibition of myocardial hypertrophy and fibrosis
Kidney	Augmentation of urinary output and sodium excretion
Endocrine system	Inhibition of aldosterone production Inhibition of ACTH and vasopressin secretion Inhibition of insulin secretion
Central nervous system	Elevation of blood pressure Inhibition of dringking and salt intake

Heart

When injected intravenously, AM increased heart rate and cardiac output through the sympathetic nerve system activated by a reduction of blood pressure [15, 16]. In addition, AM has been reported to have direct actions on cardiac muscle [17, 18]. Parkes *et al.* showed a possible, positive inotropic action of AM in sheep [17], and this was supported by experiments *ex vivo* using rat myocardium [18]. In contrast, Ikenouchi reported that AM exerted a negative inotropic action toward cultured cardiac myocytes isolated from rabbit hearts [19]. These inconsistencies may have resulted from differences in the experimental conditions or species used.

Kidneys

When infused into experimental animals, AM has been shown to increase urinary volume and sodium excretion, increasing renal blood flow, with little or no change in the glomerular filtration rate [20, 21]. Ebara *et al.* reported a natriuretic action of AM resulting from inhibition of tubular sodium reabsorption in rats presumably at a level of AM in plasma within the physiological range [22]. According to an experiment *ex vivo* by Hirata *et al.*, AM reduces renal vascular resistance, dilating both afferent and efferent arterioles, and this renal action appears to be mediated by the cAMP and NO-cGMP pathways [23]. Consistent with these findings are the diuresis and natriuresis observed in a clinical setting following intravenous infusion of AM [24].

Endocrine System

Samson *et al.* showed that AM inhibited the basal and corticotropin releasing hormone-stimulated secretion of adrenocorticotropic hormone (ACTH) from cultured rat anterior pituitary cells [25]. *In vivo*, AM inhibited the release of arginine vasopressin release induced by hyperosmolality and by hypovolemia in conscious rats [26]. Yamaguchi *et al.* reported an inhibitory effect of AM *in vitro* on the secretion of aldosterone induced by angiotensin II or potassium from rat adrenal zona glomerulosa cells [27]. The inhibition of aldosterone secretion by AM was confirmed with experiments *in vivo* by the same group [28]. In isolated pancreatic islets of rats, AM had an inhibitory effect on insulin secretion, which was reversed by blocking endogenous AM with a monoclonal antibody, suggesting an autocrine or paracrine role in islet cells [29].

Central Nervous System

In contrast to peripheral infusion, the intracerebroventricular administration of AM produced a pressor response with an increased heart rate in rats [30-32]. The responses were attenuated by pretreatment with an α-adrenergic blocker, suggesting an action mediated by activation of the sympathetic nerve system [31]. AM has also been shown to centrally modulate water and salt intake [33-35]. Samson *et al.* found an inhibitory effect of centrally administered AM on the drinking of water induced by angiotensin II or dehydration in rats [33]. According to this group, the appetite for salt of volume-depleted rats was inhibited by AM, and conversely, augmented following the central injection of an anti-AM antibody or an antisense oligonucleotide of AM mRNA [34, 35], a finding indicative of the role of endogenous AM. The mechanisms of the central actions of AM remain to be clarified, though these findings imply a role for AM in central modulation of blood pressure or the water-electrolyte balance.

AM Actions in Human Studies

Blood pressure-lowering effects accompanied by an increased heart rate and cardiac output have been observed following intravenous infusion of AM in a relatively short period of time in humans [16, 36]. In those studies, plasma aldosterone levels remained unchanged despite increased plasma renin activity [16], a finding consistent with the *in vitro* and animal experiments showing an inhibitory effect of AM on aldosterone secretion [27, 28]. Troughton *et al.* found that the blood pressure-lowering effect of AM was greater in patients with essential hypertension than in normotensive controls [37]. Conversely in patients with congestive heart failure, both the hypotensive and renal responses to AM were found to be small, compared with those in control subjects [24]. Meanwhile, McGregor *et al.* reported that the hypotensive and renal responses to AM were not necessarily attenuated in patients with chronic renal failure secondary to IgA nephropathy, despite the increased plasma levels of AM and reduced renal function, as compared with those of healthy