Adrenomedullin and The Kidney

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Adrenomedullin (AM) is a multi-functional peptide discovered in human pheochromocytoma. Initially, it was suggested that AM was synthesized only by tumor cells, however, subsequent studies revealed that it was produced also by normal adrenal medulla as well as by many other tissues. Now it is well established that AM functions as a circulating hormone and local paracrine mediator with multiple biological activities. AM stimulated cAMP production in human platelets and exerted potent and long-lasting hypotensive activity in the rat. AM is a physiologically relevant regulator in fluid and electrolyte homeostasis; inhibition both water and salt intake, increase renal blood flow, and cause diuresis and natriuresis. The up-regulation of cardiac AM system in hypertension may be a protective mechanism decreasing myocardial overload due to vasodilatory and natriuretic properties of AM, as well as limiting further myocardial hypertrophy and remodeling. AM may protect the kidney against ischemia-reperfusion injury. AM is also suggested as antiapoptotic, anti-inflammatory and angiogenic factor. In this review, I offer a review of our current knowledge on AM and give the putative role of AM in water-electrolyte balance, hypertension and kidney disease.

Key Words : Adrenomedullin, Water-electrolyte balance, Hypertension, Kidney

Introduction

Mammalian circulation is regulated by subtle mechanisms involving several neural and hormonal factors. Vasoactive peptides, including atrial natriuretic polypeptide, endothelin, and angiotensin, are especially important regulators in the cardiovascular system. To clarify the intricacies of circulation, it is important to discover still unidentified vasoactive peptides. Human adrenomedullin (AM) is a 52-amino acids peptide that was discovered in pheochromocytoma cells[1]. Now it is well established that AM functions as a circulating hormone and local paracrine mediator with multiple biological activities[2]. The purpose of this paper is to review the most important aspects of AM biology and perspectives of its therapeutic applications in water-electrolyte balance, hypertension and kidney disease.

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Structure of AM

Human AM consists of 52 amino acids, has one intramolecular disulfide bond. AM molecule contains 6-amino acid ring formed by disulfide bond between residues 16 and 21 (Fig. 1). The C-terminal tyrosine residue is amidated (-CONH). Both these structural features are essential for its biological activity. Its structure is homologous to calcitonin gene-related peptide (CGRP), calcitonin and amylin, which all belong to the same peptide family[3].

It should be noted that the 14 residue amino terminal extension in AM is not found in CGRP and amylin. AM may belong to the CGRP superfamily based on its slight sequence homology and similar pharmacological activities to CGRP[2]. In addition to human AM, the amino acid sequence of AM has been elucidated in rat, canine, mouse, porcine, and bovine species. Among these species, the ring structure and carboxy terminal amide structure, both of which are essential for biological activity seem to be

well preserved[4].

Synthesis, relase and metabolism

AM is encoded by a gene contained in humans in chromosome 11 and consisting of 4 exons and 3 introns. The mature AM peptide is derived from preproAM containing 185 amino acids (Fig. 1). After cleaving of 21-residue N-terminal signaling peptide, preproAM is converted to proAM, which is a precursor of mature AM (amino acids 95-146 of preproAM) as well as of another active peptide, proadrenomedullin N-terminal 20-peptide (PAMP, amino acids 22-41 of preproAM)[2].

Although AM was discovered in malignant tissue arising from adrenal medulla, AM mRNA has been shown to be highly expressed in normal tissues such as cardiac atrium and ventricle, aorta, kidney, lung, and uterus as well as in adrenal gland[2, 5]. AM gene is also expressed in vascular endothelial and smooth muscle cells[2]. AM protein is also highly expressed in adrenal gland, atrium, and lung[2]. However, the concentration of immunoreactive AM in aorta and ventricle was less than 5% of that in adrenal gland, even though a high level of AM mRNA was found in these circulatory system[2]. It was suggested that AM synthesized in these tissues may be rapidly and constitutively secreted into the blood or function as an autocrine or paracrine regulator[2, 4]. Indeed, it has been demonstrated that vascular endothelial cells and vascular smooth muscle cells actively synthesize and secrete AM[7, 8]. Recently, a new member of the AM family has been discovered (AM2) in mice, rats, and humans[9], and although the peptide has yet to be detected, mRNA is expressed in submaxillary gland, kidney, stomach, ovary, lymphoid tissues, and pan-

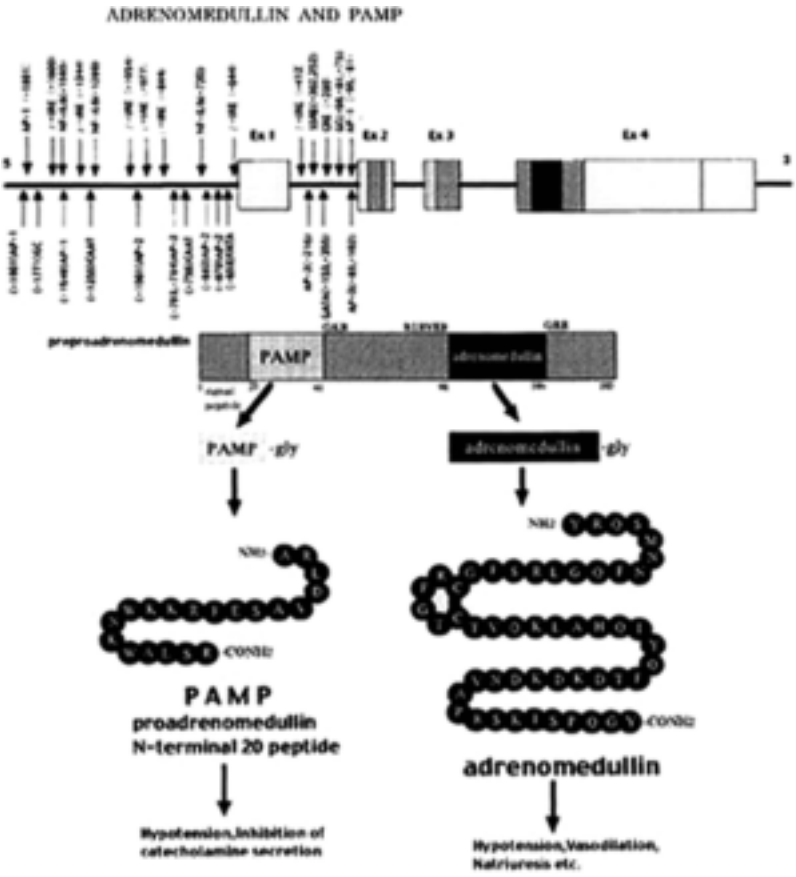

Fig. 1. The schematic presentation of adrenomedullin gene and precusor, stuctue and biosynthesis of adrenomedullin and proadrenomedullin.

creas of mice, but not in the adrenal glands or testes.

AM was secreted in isolated and/or cultured endothelial cells, vascular smooth muscle cells and cardiomyocytes. AM production is up-regulated by oxidative stress, proinflammatory cytokines such as TNF-α, IL-1β, angiotensin II and endothelin-1[10-12]. Hypoxia stimulates AM secretion both *in vitro* and *in vivo*[13]. Hyperglycemia upregulates vascular AM through the protein kinase C-dependent mechanism [14]. Infusion of atrial natriuretic peptide (ANP) increases plasma AM concentration in humans[15]. Neither acute hypervolemia nor chronic sodium loading has any effect on plasma AM level[16]. Aldosterone stimulates AM production in human vascular smooth muscle cells[17].

AM is detected in plasma at a concentration of 2-10 pM. High level of the peptide in adrenal medulla suggested that it could be the source of plasma AM. AM concentration in urine is about 15-times higher than in plasma. Plasma AM is easily filtered in glomeruli and then metabolized by neutral endopeptidase in brush border membrane of the proximal tubule[18]. Thus, although the kidneys are involved in AM clearance, urinary peptide originates from local intrarenal production rather than from systemic circulation.

Receptors and signal transduction mechanisms

AM binds to and activates at least two types of G-protein-coupled receptors. Some effects of AM are mediated by $CGRP_1$ receptors and are antagonized by specific $CGRP_1$ antagonist, $CGRP_{8-37}$. Specific AM receptors are blocked by AM_{22-52} and have higher affinity for AM than for CGRP. However, CGRP1 receptors do not bind either AM or CGRP. AM can also bind to calcitonin and amylin receptors, however, it is unlikely that this is its physiologically significant mechanism of action.

Two plasma membrane receptors for calcitonin peptide family exist: calcitonin receptor (CTR) and calcitonin receptor-like receptor (CRLR). They share about 55% homology. Each of these receptors can bind an accessory protein, named receptor activity-modifying protein (RAMP)[19, 20]. Three RAMP isoforms have been identified so far: RAMP1, RAMP2 and RAMP3. RAMPs bind to the receptor molecule in endoplasmic reticulum and facilitate their transport to the plasma membrane. In addition, RAMPs regulate the degree of receptor glycosylation and may form a part of ligand binding site determining receptor specificity. CRLR may bind RAMP1, 2 or 3, forming $CGRP_1$, AM_1 and AM_2 receptors, respectively. In addition, the functional AM receptor, composed of CRLR and the RAMP 2 or RAMP 3 is found in vascular endothelial cells[21, 22]. Thus, AM is actively synthesized and secreted from vascular endothelial and smooth muscle cells and has an autocrine and paracrine role in vascular system.

Since AM was discovered by monitoring the elevated activity of cAMP in rat platelets, cAMP has been considered the major second messenger of AM. Dose-dependent intracellular production of cAMP induced by AM has been confirmed in various tissues and cells. Moreover, information on the role of nitric oxide (NO) in alternative signal transduction pathways for AM is available[21]. The vasodilating effect of AM is reduced by the blockade of NO synthase activity with NG-nitro-L-arginine methylester (L-NAME), indicative of a partial contribution of NO to AM-induced vasodilation[22]. NO, therefore, may be an important AM mediator despite regional and interspecies variation. In bovine aortic endothelial cells, AM increases intracellular ionic calcium (Ca^{++}) and causes the accumulation of cAMP[21]. This increase in intracellular Ca^{++} may be involved in the activation of phospholipase C, thereby producing inducible NO synthase and subsequently NO. AM may also stimulate mitogen-activated protein kinases (MAPKs) in vascular smooth muscle cells[23] and inhibits MAPK activity in mesangial cells[24].

Hypotensive Action of AM

It has been known that AM has multiple functions. AM, CGRP, and amylin all have potent hypotensive and vasorelaxant effects *in vivo* and *in vitro.* Intravenous injection of AM causes a potent and long-lasting hypotensive effect in anesthetized rats *in vivo*[1]. AM causes both vasodilatation in experimental animals[25] and also increases blood flow to the lungs, heart, kidneys, and adrenal glands[26]. Peripheral infusion of AM into anesthetized rats resulted in increased RBF and urine flow that were maintained in the face of profound lowering of mean arterial blood pressure[27]. The exact regional effects of this novel peptide in experimental animals are uncertain. However, most data indicate that AM may induce endothelium-independent relaxation by acting on $CGRP_1$ receptors and elevating cAMP level in vascular smooth muscle cells. AM binds to specific receptors in endothelial cells and elicits endothelium-dependent vasorelaxation mediated by NO[28], endothelium-derived hyperpolarizing factor[29], and/or vasodilatory prostanoids[30]. AM also activates endothelial NOsynthase (eNOS).

Intravenous injection of synthetic mature AM2 decreased arterial pressure more potently than AM and also induced antidiuresis and antinatriuresis in mice (119). The significance of this molecule in humans is as yet unclear.

Water-electrolyte balance

Rats treated with AM centrally during a PEG challenge ingested significantly less salt compared to controls, without altering total fluid intake[31], suggesting that AM has a satiation function in salt ingestion. It might be predicted that the actions of AM to inhibit water and salt intake would be matched by an inhibition of AVP secretion. However, intracerebroventricular injections of AM into normal sheep did not alter basal AVP levels[32].

AM exerts direct renal and adrenal actions leading to diuresis and natriuresis. Intravenous or local intrarenal infusion of AM increases urine output and urinary sodium excretion. The effect is associated with renal vasodilation, increase in renal blood flow and glomerular filtration rate (GFR). AM also inhibits tubular sodium reabsorption. In addition, low doses of AM increase natriuresis without affecting GFR, which suggests predominantly tubular effect[33].

It is believed that AM acts as a local, paracrine, or autocrine factor in the kidney and not as a circulating hormone under normal conditions. Indeed, AM mRNA has been localized in glomerulus, cortical collecting ducts, and outer and inner medullary collecting ducts[34], a finding that supports the hypothesis that locally generated AM is important in water and sodium handling. Thus, not only circulating but also locally produced AM may regulate renal function.

Both AM receptors expressed in the nephron and AM increases cAMP generation in cortical thick ascending limb and distal convoluted tubule[34, 35]. It can be suggested that AM may inhibit tubular transport indirectly, by dilating renal vasculature and increasing peritubular hydrostatic pressure. Systemic AM administration increases plasma renin activity. This effect is not secondary to AM-induced hypotension because it is also observed after nonhypotensive doses of the peptide as well as in isolated perfused kidney and in isolated juxtaglomerular cells [36]. Thus, endogenous AM plays a physiologically relevant role in the control of fluid and electrolyte balance.

Arterial hypertension

Plasma AM concentration is increased in patients with primary arterial hypertension and is higher in individuals with complications of hypertension, such as left ventricular hypertrophy and nephrosclerosis [37]. The most dramatic up-regulation of AM gene expression, AM amidating activity, CRLR receptors and

RAMP2/RAMP3 proteins are observed in two models of malignant hypertension: SHR treated with deoxycorticosterone acetate and high-Na^{++} diet (DOCA-salt SHR)[38] and in stroke-prone SHR[39]. These data suggested that they result from hemodynamic stress and/or myocardial hypertrophy. Experimental pressure overload induced by the infusion of angiotensin II, arginine vasopressin, or by surgical aortic banding increases myocardial AM gene expression[40, 41]. Thus, AM is up-regulated by myocardial pressure overload and myocardial hypertrophy. It is suggested that up-regulation of cardiac AM system in hypertension may be a protective mechanism decreasing myocardial overload due to vasodilatory and natriuretic properties of AM, as well as limiting further myocardial hypertrophy and remodeling.

Chronic infusion of AM at doses which have no effect on blood pressure improves creatinine clearance, decreases proteinuria and renal histological changes and reduces renal expression of angiotensin converting enzyme and TGF-β in DOCA-salt SHR [42] and Dahl salt-sensitive rats[43], suggesting that renoprotective effect of AM is independent of the normalization of blood pressure.

Renal diseases

Plasma AM increases in patients with glomerulonephritis and chronic renal failure[44, 45]. This is mainly explained by reduced peptide clearance, however, increased AM production due to chronic volume overload cannot be excluded[45]. Marked increase or decrease in plasma AM level was observed in patients following hemodialysis, suggesting the role of AM during hemodialysis is not well known.

AM may protect the kidney against ischemia-reperfusion injury. After ischemic/reperfusion injury, AM heterozygote knockout mice (homozygote is lethal) had more severe renal damage with higher blood urea nitrogen levels and renal damage scores. On the other hand, AM transgenic mice, which express a higher level of AM, are protected from ischemic/reperfusion with a higher renal NOS activity. Therefore, AM plays a role in regulation of vascular tone and in renoprotection against ischemia through its NO releasing activity[46]. Taking the results from iNOS and AM KO mice together, it appears that eNOS, which is stimulated by AM, may play a protective role in renal I/R injury, while the role of iNOS remains to be proven.

Other effect : antiapoptosis, antiinflammation and angiogenesis

AM also decreases vascular endothelial cell apoptosis and acts as an antiproliferative factor for vascular smooth muscle cells. AM inhibited smooth muscle cell migration. Thus, AM had functions as a regulator in cell differentiation, survival, and growth in the vascular system. It is well known that endothelial cell survival is an essential prerequisite for DNA synthesis and migration. Previously, Kato et al [47] reported that AM is an endothelial cell survival factor. It was also demonstrated that the anti-apoptotic effect of AM is associated with Akt pathway activation[48].

In the initial phase of inflammation, vascular endothelial growth factor can act as a proinflammatory cytokine by inducing adhesion molecules that bind leukocytes to endothelial cells. AM is known to act as either a proinflammatory or an anti-inflammatory agent. AM inhibits vascular endothelial growth factor-stimulated ICAM-1 and VCAM-1 expression through a phosphatidylinositol 3'-kinase/Akt pathway[49]. AM reduced vascular endothelial growth factor-induced endothelial adhesiveness for leukocytes. These results suggest that AM may have an anti-inflammatory role.

In tumor cells, inflammation and hypoxia increased AM expression. The elevated expression of AM is associated with tumor neovascularization in xenografted endometrial tumors and renal cell carcinoma.

AM is also a tumor cell survival factor underlying human carcinogenesis. Thus, AM may have a significant role in tumor angiogenesis. In physiologic condition, AM induced angiogenesis in an *in vivo* experiment, and that it induces sprouting with endothelial cell proliferation, migration and tube formation in cultured endothelial cells through phosphatidylinositol 3'-kinase (PI 3'-kinase)/Akt, extracellular signal-regulated kinase (ERK), and tyrosine phosphorylation of focal adhesion kinase (p125FAK)[50]. These results can suggest that AM can be a potential candidate for therapeutic angiogenesis or vasculogenesis in ischemic disease.

Conclusion

In the future, further studies about the pathogenetic role of AM in water-electrolyte balance, hypertension, kidney and vascular diseases, therapeutic endothelial survival are needed.

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