The Role of Adrenomedullin in the Cardiovascular System

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WONG ET AL.: ***The Role of Adrenomedullin in the Cardiovascular System.*** *Adrenomedullin (AM) is a 52 amino acid peptide that was first isolated from human pheochromocytoma. Subsequently, AM and its receptors are found to be distributed widely in the body, including the cardiovascular system. It belongs to a family of peptides that include calcitonin gene-related peptide. In blood vessels, AM causes vasodilation and regulates proliferation. It interacts closely with nitric oxide and has a role in the pathophysiology of hypertension, ischaemic heart disease, cardiac and renal failure. A non-peptide analogue of AM or gene therapy may be of potential therapeutic use. The role of AM in septicaemic shock also merits further investigation. (J HK Coll Cardiol 2004;12:75-81)*

Adrenomedullin, cardiovascular system, nitric oxide, peptide, vasodilation

摘要

腎上腺髓質激素是一種52 氨基酸的多肽，它最早是在人的嗜鉻細胞瘤中分離而得的。隨後發現人體內廣泛存在腎上腺髓質激素和它的受體。它是屬於多肽類家族，其中包括有降鈣素基因相關多肽。腎上腺髓質激素將引起血管的擴張，並調節其增殖。它與一氧化氮相互作用，在高血壓、缺血性心臟病、心衰和腎衰的病理生理中起著重要作用。腎上腺髓質激素非多肽類相似物或基因治療可能會有潛在的治療作用。腎上腺髓質激素在敗血症性休克中的作用值得作進一步的研究。

關鍵詞：腎上腺髓質激素　心血管系統　氧化氮　多肽　血管擴張

Introduction

Adrenomedullin (AM) was first discovered in 1993 by Kitmura and co-workers as a new peptide from human pheochromocytoma with its ability to raise cyclic adenosine 3'-5'-monophosphate (cAMP) levels in platelets.[1] Subsequently, AM had aroused much interest because of its potent and long lasting depressor effect when injected intravenously into the rat.[2] Human AM consists of 52 amino acids and has a ring structure formed by one intramolecular disulfide bond and an amidated carboxyl terminal, structures that are essential for its bioactivity. It belongs to a family of peptides that include calcitonin gene-related peptide (CGRP) and amylin.[3] These peptides bind to either the calcitonin or the calcitonin receptor-like (CL) receptor.

The human AM gene is located at the single locus of chromosome 11 and consists of 4 exons and 3 introns.[4] In the 5'-upstream sequence of the AM gene, several binding sites for activator protein-1 and activator protein-2, cAMP-regulated enhancer and a shear stress responsive element "GAGACC" are present, which play roles in the transcriptional regulation of AM gene.[3] The circulating form of AM is formed from successive enzymatic cleavage of a 185-amino-acid preproadrenomedullin (preproAM) sequence (Figure 1). Cleavage of a 20-amino acid sequence in the N-terminal region of proadrenomedullin yields the proadrenomedullin N-terminal 20-peptide (PAMP),[4] which is also a potent vasodilator. Recently, two other peptides in the family have been identified, namely long- and short-form intermedin which consist of a 47-

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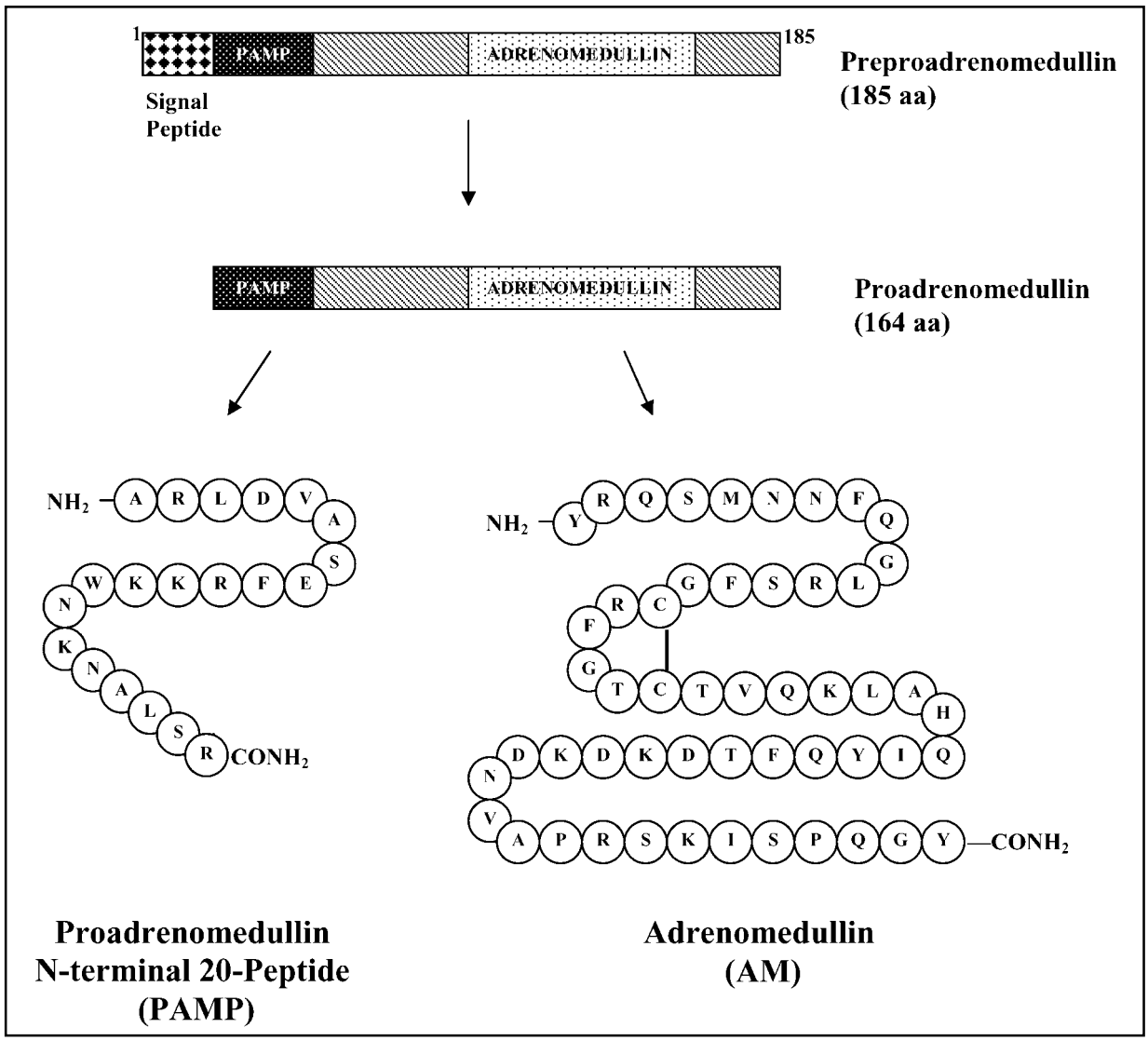

Figure 1. *Schematic presentation of post-translational processing of the human preproadrenomedullin gene product.*

amino acid and a 40-amino acid peptide respectively.[5] The long form intermedin is also known as AM2. They also have vasodilatory and hypotensive actions.

Tissue Distribution and Synthesis

AM was originally identified in pheochromocytoma, however, in the process of identifying AM-secreting cells, it has been shown to be widely distributed in many tissues and fluids. Immunoreactive AM is found in the human cardiovascular, renal, respiratory, gastrointestinal, reproductive, neurological, endocrine and immune systems.[6] It circulates in plasma in the low fmol/ml range, and is also present in urine, cerebrospinal and amniotic fluid. The plasma half-life of AM in man is 22 minutes and the volume of distribution is 880 ml/kg.[7]

Secretion of AM is influenced by physical and hormonal factors such as shear stress, ventricular wall stress, hypoxia, cytokines and endocrine and paracrine hormones. Inflammatory cytokines including tumour necrosis factor (TNF)-α, TNF-β, interleukin (IL)-1α, IL-1β, and lipopolysacccharide (LPS) strongly stimulate

the synthesis and secretion of AM in endothelial cells and vascular smooth muscle cells (VSMCs).[8] Activation of NF-IL6 mediates the induction of AM expression by cytokines.[8] In rat, LPS increases AM expression in various tissues including lung, heart, liver, and kidney.[9] In VSMCs, AM production is also stimulated by glucocorticoids, thyroxine, angiotensin II, bradykinin and adrenaline.[10]

Receptors and Signal Transduction

Specific binding sites for AM are present in rat heart, lung, spleen, liver, diaphragm, spinal cord and different parts of the brain, with the greatest density of binding sites in the heart and lung.[11] Initially, AM was thought to bind to a CGRP receptor, as these related peptides have overlapping binding sites and vascular effects. It is now known that a functional AM or CGRP receptor consists of at least three proteins: the CL receptor, receptor-activity-modifying proteins (RAMPs) and a receptor component protein (RCP).[12] Three subtypes of RAMPs, namely RAMP1, RAMP2, and RAMP3, have been identified. CL receptor can function either as a CGRP receptor or an AM receptor depending on the co-expression of different subtypes of RAMPs (Figure 2). Co-expression of CL receptor with RAMP1 forms a CGRP receptor while co-expression of CL

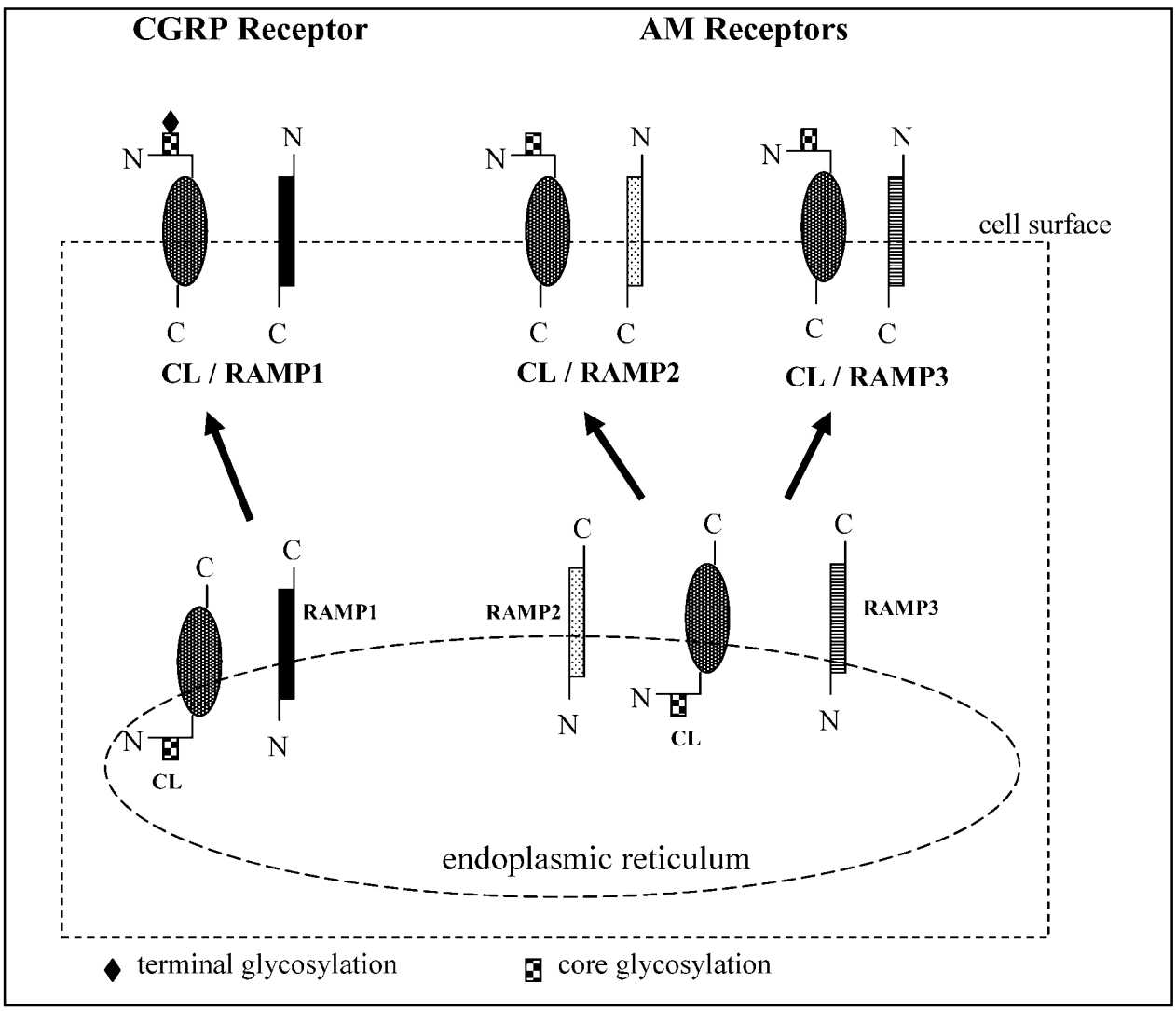

Figure 2. *CGRP and AM receptors.*

receptor with RAMP2 or RAMP3 results in binding of AM. These RAMPs may control the transport and glycosylation of the CL receptor to define the specificity of receptor. RAMP1 presents CL receptor as a mature glycoprotein at the cell surface to form a CGRP receptor, whereas the RAMP2 or RAMP3-transported receptors are core glycosylated AM receptors.[12]

The vasodilatory and hypotensive responses elicited by AM are mediated via at least two mechanisms: a direct action on VSMCs to activate the adenylyl cyclase-PKA pathway resulting in increase of intracellular cAMP, and an action on endothelial cells to stimulate nitric oxide (NO) release.[13] Furthermore, potassium ion-ATP channel may also be involved in AM-induced vasodilation in isolated perfused rat kidney in which the endothelium-derived hyperpolarizing factor (EDHF) opens the potassium ion channels.[14]

Cardiovascular Effects

AM plays a major role in the maintenance of cardiovascular and renal homeostasis.[14,15] In addition, it modulates the hypertrophy of cardiomyocytes and the growth of fibroblasts, and also have antimicrobial effects.[4] In transgenic mice with one AM allele deleted, there was increase in blood pressure and decrease in NO expression,[15] suggesting that AM is involved not only in blood pressure regulation but also in endothelial function. Infusion of AM into human brachial artery significantly increases forearm blood flow via a NO-dependent pathway that can be blocked by L-NMMA.[16] Infusion of AM in man also lowers pulse wave velocity.[17] In sheep, intracoronary AM causes vasodilation, also through the release of NO.[18] In Dahl salt-sensitive rats, AM restores NO-dependent vasodilatation, through upregulation of NO production and reduction in superoxide formation.[19]

Intravenous infusion of AM lowers blood pressure, and increases heart rate and cardiac output.[20] A direct cardiostimulatory effect can also be demonstrated in the isolated perfused rat heart.[21] However, AM is also negatively inotropic under other circumstances, such as in isolated rabbit cardiac ventricular myocytes, rat papillary muscle and human left ventricular myocytes.[22] This negative inotropic effect may be mediated by NO.

Intravenous infusion of AM increases renal blood flow, glomerular filtration, urine flow and fractional urinary sodium excretion, and decreases distal tubular sodium reabsorption independent of blood pressure.[23] The natriuresis and diuresis may be mediated via renal prostaglandin[24] and NO.[25]

As well as regulating vascular tone, AM may also regulate vascular proliferation and remodelling. It dose-dependently inhibits thymidine incorporation and proliferation induced by platelet-derived growth factor in VSMCs.[26] AM may also be an angiogenic factor. Vascular density and endothelial cell proliferation are stimulated by AM. Many tumours express AM; an antibody against AM or an AM antagonist suppresses tumour growth in animal models.[27] Whether AM can be used to increase angiogenesis in ischaemia remains to be explored.

Neuroendocrine Effects

High levels of AM are found in the adrenal medulla and zona glomerulosa. Intravenous AM infusion lowers circulating cortisol and adreno-corticotrophic hormone (ACTH) levels in sheep.[28] There appears to be a complex interaction between AM and the renin-angiotensin system; AM stimulates the release of renin,[29] but inhibits aldosterone secretion.[30]

AM immunoreactivity is widely distributed in the central nervous system (CNS).[31] In the supraoptic nucleus (SON) and in the magnocellular parts of the paraventricular nucleus (PVN), AM is expressed. As a neuropeptide, AM may regulate body fluid homeostasis by inhibiting water drinking and salt appetite. Blockade of the action of endogenous AM by passive immuno-neutralization results in exaggerated sodium appetite.[32] By restraining salt and water intake, AM's central actions complement its renal actions. AM expression is increased in the ischemic cerebral cortex in the rat after middle cerebral artery occlusion,[33] where it may increase cerebral blood flow and promote collateral perfusion.

Plasma AM Levels in Diseases

Plasma AM levels are increased in a variety of diseases: congestive heart failure,[34] myocardial infarction,[35] renal diseases,[36] hypertension,[37] diabetes mellitus,[38] the acute phase of stroke[39] and septic shock.[40] Plasma AM levels are elevated in patients with essential hypertension in proportion to the severity of hypertension,[36] especially in those with left ventricular hypertrophy. However, neither acute nor chronic salt loading, nor anti-hypertensive therapy changes the circulating level of AM in patients with essential hypertension. Plasma levels of AM are increased in patients with congestive heart failure, whether systolic[34] or diastolic.[41] The failing human heart secretes increased amounts of AM. Plasma AM concentration correlates with pulmonary capillary wedge pressure and inversely with ejection fraction. It decreases after treatment of the heart failure. Plasma AM concentration is elevated in relation to the degree of renal failure.[36]

Injection of LPS in the rat produces a marked increase in plasma AM, suggesting AM may be involved in sepsis.[42] Circulating AM level is also increased in sepsis in man.[40] In the early, hyperdynamic phase of septicaemia, there is upregulation of AM, followed by a reduction in vascular responsiveness to AM in the late, hypodynamic response.[43] Administration of AM and its binding protein (AMBP-1) maintains cardiovascular stability and reduces sepsis-induced mortality.[44] Transgenic mice overexpressing AM are resistant to septicaemic shock.[45] Therefore, AM may be responsible for many of the changes in the circulatory system in septic shock.

AM as Therapeutic Agent

Infusion of AM lowers blood pressure in hypertension[46] and lowers ventricular end-diastolic pressure and increases cardiac output in heart failure.[47] Therapy based on increased stimulation of AM receptors may have potential application in hypertension and heart failure. In AM-knockout mice, there is accelerated cardiac hypertrophy and renal damage induced by angiotensin II,[48] suggesting that endogenous AM protects against cardiac and renal damages. In the rat model of myocardial infarction induced by coronary artery ligation, a one-off early intravenous administration of AM prevents subsequent left ventricular remodelling.[49] As AM is a peptide, it cannot be given orally. In patients with pulmonary hypertension, AM administered by inhalation reduced pulmonary vascular resistance.[50] A non-peptide analogue is eagerly awaited for clinical studies. Neutral endopeptidase inhibitors increase the level of AM as well as other vasoactive peptides, but their use is limited by the increased likelihood of angiooedema. Expression of AM is known to be increased after angioplasty and stenting;[51] AM gene delivery successfully inhibited neointimal formation after balloon angioplasty in a rat model.[52] Gene therapy targeting the AM system is a novel and promising modality of treatment for the future.

Conclusions

In summary, AM and its receptor are distributed widely in the body, including the cardiovascular system. In blood vessels, AM causes vasodilation and regulates proliferation. It interacts closely with NO and has a role in the pathophysiology of hypertension, ischaemic heart disease, cardiac and renal failure. A non-peptide analogue of AM or gene therapy may be of potential use in these diseases. The role of AM in septicaemic shock, a condition characterised by high mortality, also merits further investigation.

Acknowledgements

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