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REVIEW

ADRENOMEDULLIN – WHAT DO WE KNOW 10 YEARS SINCE ITS DISCOVERY?

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Adrenomedullin – what do we know 10 years since its discovery?
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Adrenomedullin (ADM) is a 52-amino acid peptide with structural homology to calcitonin gene-related peptide (CGRP) initially isolated from human pheochromocytoma. ADM is synthesized by many mammalian tissues including the adrenal medulla, endothelial and vascular smooth muscle cells, myocardium and central nervous system. ADM binds to plasma membrane receptors composed of calcitonin receptor-like receptor (CRLR), a member of serpentine receptor superfamily, and receptor activity modifying protein (RAMP) type 2 or 3. ADM has also some affinity for $CGRP_1$ receptor composed of CRLR and RAMP1. ADM dilates blood vessels in both endothelium-dependent and independent manner and decreases systemic arterial pressure. Intrarenally administered ADM increases natriuresis by vascular and tubular mechanisms. In addition, ADM inhibits migration and proliferation of vascular smooth muscle cells and attenuates myocardial remodelling by inhibiting protein synthesis in cardiomyocytes and proliferation of cardiac fibroblasts. ADM is expressed in various tissues from early stage of embryogenesis and is also synthesized in placenta, uterus and fetal membranes. Plasma ADM level is increased in arterial hypertension, acute coronary syndromes, heart failure, renal diseases and septic shock, being involved in the pathophysiology of these disorders. Experimental ADM treatment is beneficial in arterial and pulmonary hypertension, heart failure, septic shock and ischemia/reperfusion injury. Proadrenomedullin N-terminal peptide (PAMP) is another product of ADM gene which is co-secreted by ADM-producing tissues, with some effects similar and some opposite to ADM.

Key words: *adrenomedullin, proadrenomedullin N-terminal 20-peptide*

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Abbreviations: ACE – *angiotensin-converting enzyme,* ADM – *adrenomedullin,* AMBP-1 – *adrenomedullin binding protein-1,* CGRP – *calcitonin gene-related peptide,* CRLR – *calcitonin receptor-like receptor,* IL-1 – *interleukin-1,* NEP – *neutral endopeptidase,* PAMP – *proadrenomedullin N-terminal 20-peptide,* PDGF – *platelet-derived growth factor,* RAMP – *receptor activity-modifying protein,* SNS – *sympathetic nervous system,* TGF-β – *transforming growth factor*-β, TNF-α – *tumor necrosis factor*-α

Introduction

In 1993 Kitamura et al. [88] isolated a new peptide from human pheochromocytoma. The peptide, which they named "adrenomedullin" (ADM), stimulated cAMP production in human platelets and exerted potent and long-lasting hypotensive activity in the rat. Initially, it was suggested that ADM 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 ADM functions as a circulating hormone and local paracrine mediator with multiple biological activities [51]. The purpose of this paper is to highlight the most important aspects of adrenomedullin biology and perspectives of its therapeutic applications.

Structure of ADM

Human adrenomedullin consists of 52 amino acids. Its structure is homologous to calcitonin gene-related peptide (CGRP), calcitonin and amylin, which all belong to the same peptide family. ADM molecule contains 6-amino acid ring formed by disulfide bond between residues 16 and 21 (Fig. 1). The C-terminal tyrosine residue is amidated (-$CONH_2$). Both these structural features are essential for its biological activity. ADM_{22-52}, which does not contain intramolecular ring, binds to ADM receptors but has no activity and is used as ADM receptor antagonist.

Synthesis, relase and metabolism

ADM is encoded by a gene contained in humans in chromosome 11 and consisting of 4 exons and 3 introns. The mature ADM peptide is derived from preproADM containing 185 amino acids (Fig. 2). After cleaving of 21-residue N-terminal signaling peptide, preproADM is converted to proADM, which is a precursor of mature ADM (amino acids 95-146 of preproADM) as well as of another active peptide, proadrenomedullin N-terminal 20-peptide (PAMP, amino acids 22–41 of preproADM). High level of ADM mRNA is detected in adrenal medulla, and its lower expression is observed in heart atria and ventricles, kidney, lung and several areas of the brain. ADM gene is also expressed in vascular endothelial and smooth muscle cells. Secretion of ADM was examined in isolated and/or cultured endothelial cells, vascular smooth muscle cells and cardiomyocytes. ADM production is up-regulated by oxidative stress, proinflammatory cytokines such as TNF-α and IL-1, angiotensin II and endothelin-1 [28, 202, 203]. The effect of shear stress on ADM secretion by endothelium is controversial, *viz*. both stimulation [27] and inhibition [197] have been described. Hypoxia stimulates ADM secretion both *in vitro* and *in vivo* [55]. Hyperglycemia up-regulates vascular ADM through the protein kinase C-dependent mechanism, which may contribute to elevated plasma ADM level in diabetes mellitus [48]. Infusion of atrial natriuretic peptide (ANP) increases plasma ADM concentration in humans [223]. Neither acute hypervolemia nor chronic sodium loading has any effect on plasma ADM level

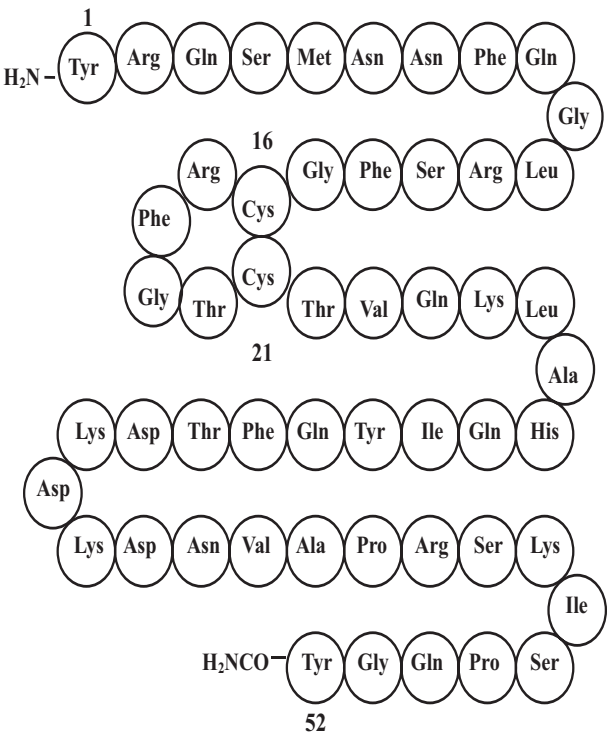

Fig. 1. Structure of human ADM molecule

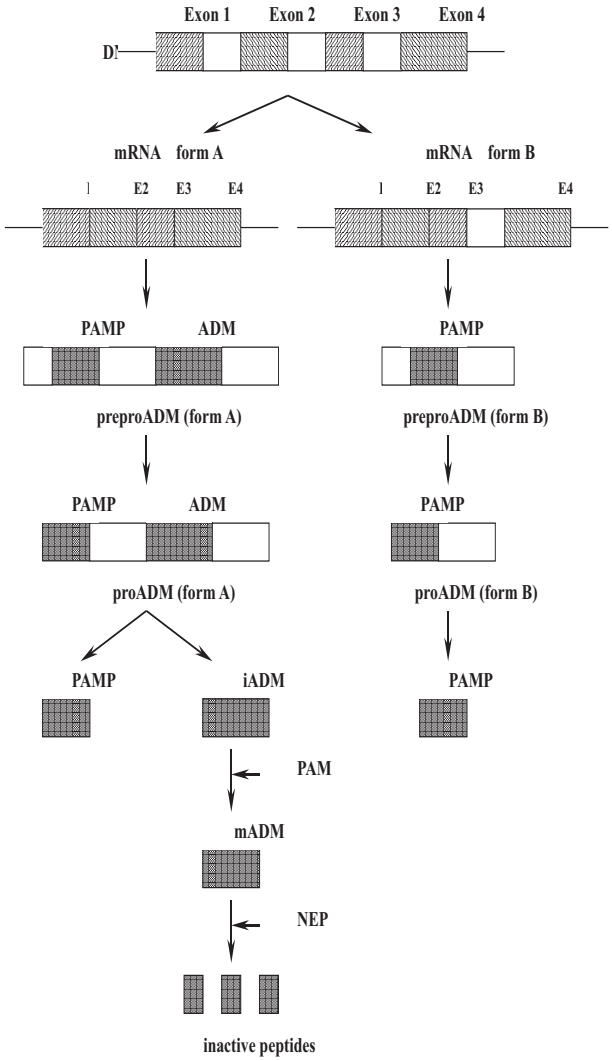

Fig. 2. Synthesis and metabolism of ADM and PAMP (PAM – *peptidylglycine α-monooxygenase*, NEP – *neutral endopeptidase*)

[62]. Aldosterone stimulates ADM production in human vascular smooth muscle cells [220]. In the adrenal medulla, ADM is co-secreted with catecholamines following cholinergic stimulation. In contrast to other peptides, ADM is not stored in secretory granules (except in pancreatic endocrine cells) but rather released immediately after synthesis.

ADM 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 ADM. Several lines of evidence indicate that this is not the case. First, in contrast to catecholamines, ADM concentration in adrenal vein is not higher than in inferior vena cava. Second, physiological stimuli which regulate adrenal catecholamine and ADM secretion, such as hypoglycemia and hypotension, have no effect on plasma ADM. In addition, plasma ADM level does not increase during an attack of pheochromocytoma when catecholamines are rapidly liberated. Now it is believed that plasma ADM is derived mainly from endothelial cells in different vascular beds.

ADM is secreted as a so-called immature, glycine-extended 53-amino acid peptide (iADM), which is subsequently converted to mature ADM (mADM) by enzymatic amidation (Fig. 2) [89]. iADM is the main circulating form of the peptide comprising 85% of total plasma ADM. Although iADM is biologically active *in vivo* [13], this depends upon amidation of the peptide in tissues because co-administration of the amidation inhibitor abrogates this activity. The amidating enzyme, peptidylglycine α-monooxygenase (PAM) is co-expressed with ADM in different tissues and contains two domains which catalyze two consecutive reactions: peptidylglycine hydroxylating monooxygenase (PHM) and peptidylhydroxyglycine α-amidating lyase (PAL) [144].

In plasma, ADM is specifically bound to adrenomedullin binding protein-1 (AMBP-1), identified as a complement factor H [165]. Binding to factor H increases its receptor-mediated effects of ADM but suppresses its receptor-independent antimicrobial activity. On the other hand, ADM binding enhances the activity of factor H, which is an inhibitor of alternative pathway of complement activation. ADM-factor H interaction has several important implications. First, protein-bound ADM cannot be detected by most of plasma ADM assays. Thus, total plasma ADM is probably higher than reported in most studies. It has been suggested that plasma ADM level is too low to act on tissue receptors and that ADM functions only as a local but not circulating hormone. This view has now to be revised. In addition, high level of plasma ADM noted in some clinical states, especially septic shock, may in fact reflect shift of the peptide from protein-bound to free pool due to a decrease in factor H concentration. Thus, it is possible that total plasma ADM in septic shock was in fact overestimated in early studies. In contrast to ADM, neither CGRP nor PAMP binds to factor H.

Circulating ADM is rapidly metabolized with $T_{1/2}$ of about 20 min. Plasma ADM level is lower in aorta than in pulmonary artery suggesting that the

lung may be a major site of peptide clearance. ADM concentration in urine is about 15-times higher than in plasma. Plasma ADM is easily filtered in glomeruli and then metabolized by neutral endopeptidase (NEP) in brush border membrane of the proximal tubule (Fig. 2). Thus, although the kidneys are involved in ADM clearance, urinary peptide originates from local intrarenal production rather than from systemic circulation.

Receptors and signal transduction mechanisms

ADM binds to and activates at least two types of G-protein-coupled receptors. Some effects of ADM are mediated by $CGRP_1$ receptors and are antagonized by specific $CGRP_1$ antagonist, $CGRP_{8-37}$. These receptors have slightly higher affinity for CGRP than for ADM [11]. Specific ADM receptors are blocked by ADM_{22-52} and have higher affinity for ADM than for CGRP. In contrast, $CGRP_2$ receptors do not bind either ADM or CGRP. At the micromolar concentrations, ADM can also bind to calcitonin and amylin receptors, however, it is unlikely that this is its physiologically significant mechanism of action.

Recently, the molecular structure of ADM and $CGRP_1$ receptors has been identified (Tab. 1). Two plasma membrane receptors for calcitonin peptide family exist: calcitonin receptor (CTR) and calcitonin receptor-like receptor (CRLR). They share about 55% homology at a primary structure level. Each of these receptors can bind an accessory protein, named receptor activity-modifying protein (RAMP) [125, 167]. 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 $CGRP_1$, ADM_1 and ADM_2 receptors, respectively. Likewise, CTR may bind one of the RAMPs forming three types of amylin receptor, AM_1, AM_2 and AM_3. In addition, CTR unbound to any RAMP functions as a calcitonin receptor. Although RAMP isoforms differ in their tissue distribution, no pharmacological differences between ADM_1 and ADM_2 or between 3 types of amylin receptor have been identified so far and the functional significance of this receptor redundancy remains unclear. The expression pattern of RAMP isoforms in a given cell may change in physiological and pathological conditions determining the responsiveness to ADM and CGRP. Although the expression of CRLR and RAMP1 in tissues well correlates with CGRP binding and CRLR and RAMP2 /RAMP3 with ADM binding [16, 45], these peptides bind to some regions of the brain which do not express CRLR, suggesting that additional ADM receptors with different molecular structure exist [51].

Table 1. Molecular structure of ADM and related peptide receptors

Receptor name	Receptor molecule	RAMP protein	Agonists
$CGRP_1$	CRLR	RAMP1	CGRP > ADM
ADM_1	CRLR	RAMP2	ADM > CGRP
ADM_2	CRLR	RAMP3	ADM > CGRP
CTR	CTR	–	Calcitonin
AM_1	CTR	RAMP1	Amylin
AM_1	CTR	RAMP2	Amylin
AM_1	CTR	RAMP3	Amylin

In many cell types, ADM and $CGRP_1$ receptors are coupled to G_s protein and adenylate cyclase. In addition, ADM may either increase [188, 204] or decrease [123] intracellular Ca^{2+} concentration independently of cAMP. ADM stimulates nitric oxide synthase (NOS) in rabbit ventricular cardiomyocytes [59] and endothelial cells [47]. ADM may also stimulate mitogen-activated protein kinases (MAPKs) in vascular smooth muscle cells [66] and inhibits MAPK activity in mesangial cells [25]. Finally, ADM activates ATP-sensitive K^+-channels in vascular smooth muscle cells independently of other signaling pathways mentioned above [174].

Biological activity

Cardiovascular system

Systemic ADM administration lowers blood pressure due to a decrease in peripheral vascular resistance [12]. This is often accompanied by an increase in cardiac output secondary to reduced afterload. Decreased peripheral resistance and BP induces reflex tachycardia, however, heart rate increases to the lower extent than after other vasodilators in-

ducing comparable hypotension. *In vitro*, ADM dilates blood vessels of different vascular beds and in different animal species. The mechanism of vasodilatory effect of ADM has been addressed in many studies and the results differ depending on the experimental model. In general, most data indicate that ADM may induce endothelium-independent relaxation by acting on $CGRP_1$ receptors and elevating cAMP level in vascular smooth muscle cells (VSMC). Because ADM is secreted by endothelial cells, it may function as one of endothelium-derived relaxing factors. ADM may also activate potassium channels in smooth muscle cells causing cell hyperpolarization [174]. In addition, ADM binds to specific receptors in endothelial cells and elicits endothelium-dependent vasorelaxation mediated by nitric oxide [47], endothelium-derived hyperpolarizing factor (EDHF) [229], and/or vasodilatory prostanoids [235]. ADM activates endothelial nitric oxide synthase (eNOS) by at least two mechanisms. First, ADM elevates intracellular calcium level which increases eNOS activity [188]. Second, ADM activates phosphatidylinositol 3-kinase (PI3K) and protein kinase B/Akt, which phosphorylates eNOS and increases its activity even at low Ca^{2+} concentration [153]. ADM may also inhibit vasoconstrictor endothelin-1 production by endothelial cells [96]. The relative contribution of different mechanisms to ADM-induced vasorelaxation differs depending on animal species and vascular preparation.

ADM inhibits migration of vascular smooth muscle cells induced by angiotensin II or platelet-derived growth factor [98]. ADM may bidirectionally regulate VSMC proliferation. It stimulates proliferation of quiescent VSM cells in the absence of other stimulating factors [66, 187], but inhibits proliferation induced by PDGF or fetal serum [80]. ADM inhibits endothelial cell apoptosis induced by serum deprivation [82, 88]. This effect is mediated by nitric oxide but is cGMP-independent [181]. In addition, ADM stimulates proliferation of endothelial cells – the process involved in angiogenesis and reendothelialization of injured vessels [127]. The effects of ADM on vascular cell cycle may be important in vascular remodeling in arterial hypertension or during the development of restenosis following angioplasty.

Although systemically infused ADM increases cardiac output, this is secondary to reduced peripheral resistance [173]. The direct effect of ADM on myocardial contractility is more controversial. ADM reduces contractility of isolated rabbit ventricular myocytes by stimulating NO production, which decreases intracellular Ca^{2+} concentration through cGMP-dependent mechanism [59]. In contrast, ADM has positive inotropic effect on isolated perfused rat heart [204] and isolated rat papillary muscles [58]. Finally, in one study [201], no effect of ADM on myocardial contractility was observed. Several studies have demonstrated that ADM inhibits protein synthesis and hypertrophy of cardiomyocytes, as well as proliferation of cardiac fibroblast and production of extracellular matrix [217]. Since ADM is synthesized and secreted by isolated cardiomyocytes and cardiac fibroblasts [56], this peptide may regulate myocardial hypertrophy and remodeling in arterial hypertension or heart failure in a paracrine/autocrine manner.

The kidney and water-electrolyte balance

Intravenous or local intrarenal infusion of ADM increases urine output and urinary sodium excretion. The effect is mainly associated with renal vasodilation, increase in renal blood flow (RBF) and glomerular filtration rate (GFR). However, ADM also inhibits tubular sodium reabsorption. In addition, low doses of ADM increase natriuresis without affecting GFR, which suggests predominantly tubular effect [73]. ADM is produced in glomeruli, distal tubules and medullary collecting duct [161]. Thus, not only circulating but also locally produced ADM may regulate renal function. Inhibition of neutral endopeptidase augments natriuretic effect of ADM by reducing peptide clearance [107]. Both specific ADM receptors and $CGRP_1$ receptors are expressed in the nephron and ADM increases cAMP generation in cortical thick ascending limb and distal convoluted tubule [34, 161]. However, it is still unclear whether ADM directly regulates tubular transport. Two *in vitro* studies revealed that ADM paradoxically stimulated osmotic water permeability in inner medullary collecting duct [161] and Na^+ uptake in apical membranes of distal tubules [105]. These effects would favor activation of tubular reabsorption. Thus, it is suggested that ADM may inhibit tubular transport indirectly, by dilating renal vasculature and increasing peritubular hydrostatic pressure. The role of ADM in the regulation of Na^+ balance is unclear since neither ADM nor its receptor gene

expression in the kidney is affected by high- or low-sodium diet [69, 171].

Another renal target for ADM are mesangial cells. ADM increases cAMP level in mesangial cells leading to their relaxation and increase in filtration coefficient, and this effect contributes to an increase in GFR [162]. In addition, ADM inhibits angiotensin II-induced migration and proliferation of mesangial cells and the generation of reactive oxygen species by these cells [97]. These effects may attenuate the progression of chronic nephropathies.

Systemic ADM administration increases plasma renin activity. This effect is not secondary to ADM-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 [70].

Central nervous system

ADM and its receptors are abundantly expressed in the brain with the highest level found in the thalamus, hypothalamus, adeno- and neurohypophysis [184]. Apart from locally produced ADM, circulating peptide may enter the central nervous system through the circumventricular organs such as area postrema, lacking a blood-brain barrier [186]. Thus, some of the effects of peripherally administered ADM may in fact be mediated *via* the CNS. Intracerebroventricularly (*icv*) administered ADM inhibits water intake induced by angiotensin II or hyperosmolality [132] and salt appetite induced by hypovolemia [177]. In addition, inactivation of endogenous ADM by antisense oligonucleotides or specific antibodies increases water and sodium intake, suggesting that ADM tonically inhibits thirst and salt appetite [176, 211]. In conscious rats, centrally injected ADM increases urine output and urinary sodium and potassium excretion [64]. Thus, some central effects of ADM correspond with its peripheral activities. However, in contrast to peripheral hypotensive effect, centrally administered ADM increases blood pressure and heart rate by stimulating sympathetic nervous system [178].

ADM and endocrine glands

Centrally administered ADM stimulates oxytocin [183] and inhibits hyperosmolality-stimulated vasopressin secretion [238] by posterior pituitary. *In vitro*, ADM inhibits both basal and CRH-stimulated ACTH secretion by anterior pituitary cells [180]. A decrease in ACTH is also observed following peripheral ADM administration. In contrast, *icv* injection of ADM stimulates hypothalamo-pituitary-adrenal axis by activating CRH-producing neurons [24, 185].

Many studies addressed the effect of ADM on the adrenal gland in which the peptide is produced at large quantities. ADM and its receptors are abundant not only in adrenal medulla but also in adrenal cortex, in particular in aldosterone-producing zona glomerulosa (ZG) [6]. ADM inhibits aldosterone production induced by angiotensin II and potassium in dispersed ZG cells [7, 8]. The inhibitory effect of ADM is mediated by $CGRP_1$ receptors and is associated with reduced intracellular Ca^{2+}. *In vivo*, ADM has no effect on plasma aldosterone despite increasing plasma renin activity in healthy sheep [23] and humans [104], consistently with its direct inhibitory effect on the adrenal cortex. ADM also prevents an increase in plasma aldosterone level induced by infusion of angiotensin II [163]. However, ADM may increase basal and ACTH-stimulated aldosterone secretion, the effect mediated by specific ADM receptors coupled to adenylate cyclase [81, 213]. In contrast to ZG, ADM and its receptors are expressed at a very low level in zona fasciculata and zona reticularis, the areas of adrenal cortex which synthesize cortisol and androgens, respectively. Consistently with this, ADM has no effect on cortisol secretion by adrenocortical cells [245].

Apart from regulating hormone secretion, ADM stimulates growth and proliferation of ZG cells in the MAPK-dependent manner [61, 182]. It seems that tonic ADM production regulates proliferation of ZG cells, since inactivation of endogenous ADM with antisense oligonucleotides or long-term ADM receptor blockade inhibits proliferation of rat ZG cells and induces their apoptosis [113].

Although large amounts of ADM are present in the adrenal medulla, this hormone has either no [120] or only very weak stimulatory effect on catecholamine production [8, 121].

ADM is synthesized in pancreatic polypeptide-producing F cells of the pancreatic islets. In addition, ADM receptors are expressed in insulin-producing β-cells but not in other cell types of the endocrine pancreas. Exogenous ADM inhibits basal and glucose-stimulated insulin secretion *in vitro* and *in vivo* but has no effect on other pancreatic hormones. Anti-ADM antibodies stimulate basal

insulin secretion [246], indicating that endogenous ADM tonically inhibits insulin secretion.

Recent studies suggest that ADM has a potent anabolic effect on bone tissue. ADM stimulates proliferation of osteoblasts and to a lesser extent of chondrocytes *in vitro*, and stimulates bone mineralization *in vivo*. The effect is antagonized by amylin(8–37), suggesting the involvement of amylin receptors. However, osteoblasts do not express CRLR, the only currently known amylin receptor. Interestingly, truncated ADM analogues such as ADM_{26-52} and ADM_{34-52} also have this activity, whereas in other tissues they rather antagonize the effect of ADM. Thus, in any case, the effect of ADM in bone tissue has to be mediated by the receptors distinct from any other known ADM receptors [29]. These truncated ADM analogues are endogenously produced (see below), suggesting that they may be involved in the regulation of bone metabolism. ADM may be potentially useful in the treatment of osteoporosis, supplementing other currently used drugs which unlike ADM inhibit bone resorption rather than stimulate bone formation.

Gastrointestinal tract

ADM mRNA and ADM immunoreactivity are detected in gastrointestinal mucosa, especially in gastric endocrine cells. Gastrointestinal ADM gene expression is up-regulated by fasting [131, 175]. Intravenously (*iv*) administered ADM inhibits basal and gastrin-stimulated HCl secretion [172], the effect mediated at least in part by the stimulation of somatostatin [54].

Reproductive system

ADM is produced by the granulosa cells of the ovarian follicle and its synthesis increases markedly during the follicular phase. ADM stimulates growth of these cells and augments the stimulatory effect of FSH [1]. Before ovulation, ADM concentration in follicular fluid is higher than in plasma [117, 130]. Plasma ADM level increases during the follicular phase and decreases during the luteal phase of the menstrual cycle [115].

Both ADM and its receptors are expressed in the uterus and their expression markedly increases during pregnancy. This is accompanied by marked increase in maternal plasma ADM concentration [94]. ADM inhibits galanin- or bradykinin-induced uterine contractility [221, 234]. ADM appears in many fetal tissues very early suggesting that it may be involved in growth and embryogenesis [39]. In addition, ADM is synthesized by syncytiotrophoblast of the placenta and the fetal membranes, especially by the amniotic epithelium, and is detected in amniotic fluid at the level higher than in plasma [116]. ADM concentration in the umbilical vein is higher that in umbilical artery, suggesting net ADM production by the placenta [124]. ADM may play multiple roles during normal pregnancy. High plasma ADM level may contribute to maternal hemodynamic changes, i.e. peripheral vasodilation and increase in cardiac output. ADM may regulate local uteroplacental circulation. The hormone contained in amniotic fluid may confer local antimicrobial defence (see below). ADM may also regulate embryogenesis, placental development, placental vasculogenesis and uterine contractility.

ADM is also produced by the epithelial cells of the mammary gland and is detected in milk [68, 164]. It is suggested that milk ADM is involved in the regulation of growth and maturation of neonatal intestinal epithelium, gastrointestinal secretion and motility, and possibly in antimicrobial defence.

Immunity and inflammation

ADM possesses potent antimicrobial properties against both Gram-positive and Gram-negative bacteria [4]. This is associated with the amphipatic design of the peptide consisting of spatially separated charged and hydrophobic domains, which permits intercalation into bacterial membranes [3]. ADM is synthesized by epithelial cells at the mucosal surface of respiratory, gastrointestinal and urinary tract, oral cavity and skin, where it may confer local antimicrobial protection. ADM concentration in saliva is 8-times higher, and in gingival cervical fluid 20 000 times higher than in plasma. ADM gene expression in oral keratinocytes is up-regulated in the presence of bacteria which are commonly found in the mouth. *Staphylococcus aureus* and other skin pathogens stimulate ADM production in skin epithelial cells whereas *Helicobacter pylori* in gastric epithelial cells. Interestingly, ADM concentration in gingival cervical fluid is 3-fold lower in patients with peridontitis than in healthy subjects, suggesting that ADM deficiency may contribute to this disease [3].

ADM gene is expressed in peripheral blood monocytes and is rapidly up-regulated during their transformation to macrophages [101]. ADM has been demonstrated to inhibit proinflammatory cy-

tokine secretion in several studies [65, 232]. Apart from acting on inflammatory cells, ADM reduces endothelial permeability increased by reactive oxygen species, endotoxins or cytokines and thus may limit the formation of inflammatory exudate [52].

Cell proliferation

ADM has different effects on proliferation depending on the cell type. ADM stimulates proliferation of fibroblasts, keratinocytes, endothelial cells, osteoblasts, and many tumor-derived cells. ADM is expressed in almost all tumor cells studied to date, suggesting that it may be an important tumor growth factor. In addition, due to its angiogenic properties ADM may promote tumor angiogenesis [51]. Experimental studies indicate that neutralization of ADM by specific antibodies inhibits, whereas ADM overexpression augments tumor growth. [247]. In contrast, in some cell types such as vascular smooth muscle cells, mesangial cells, glial cells and glial cell tumors, the inhibitory effect of ADM on growth was observed in most studies.

ADM overexpression and ADM gene knockout

The important data about physiological role of ADM were obtained using genetically modified animals. Mice which overexpress ADM gene in the cardiovascular system [195] are characterized by lower blood pressure, increased renal NOS activity and plasma cGMP concentration. Blood pressure is normalized by the administration of NOS inhibitor, suggesting that excess NO is responsible for hypotension in these animals.

Two independent groups have generated ADM knockout mice. Mice with disrupted ADM gene [14, 196] do not produce either ADM or PAMP. Homozygous mutation is lethal as the embryos are characterized by poorly developed vitelline vessels, hemorrhages, myocardial hypertrophy and, first of all, extreme hydrops fetalis, and die between embryonic day 13.5 and 14.5. These data indicate that ADM is essential for normal embryonic development, especially placental and vitelline vessels vasculogenesis. Hydrops fetalis in $ADM^{-/-}$ mice results probably from increased endothelial permeability (see above). Heterozygous $ADM^{+/-}$ mice survive until adulthood and are fertile but are characterized by arterial hypertension accounted for by NO deficiency, since these animals excrete less NO metabolites in their urine and are less sensitive to hypertensive effect of NOS inhibitors.

Another research group [194] inserted a stop mutation at the starting point of ADM coding sequence in the preproADM gene, thus generating mice which do not synthesize ADM but with intact PAMP production. Homozygosity is also lethal but homozygous embryos do not exhibit placental and vascular abnormalities or hydrops fetalis. Heterozygous animals are viable and fertile and are characterized by normal blood pressure. Administration of angiotensin II together with high-salt diet produces similar increase in BP in wild-type and $ADM^{+/-}$ mice, but induces more marked organ damage in the latter strain, as evidenced by greater hypertrophy of the left ventricle and coronary artery wall, higher vascular oxidative stress, and increased urinary excretion of lipid peroxidation products. These data suggest that ADM limits the degree of end-organ damage in the course of Na^+- induced hypertension independently of BP level. The comparison of phenotypes of $(ADM + PAMP)^{+/-}$ and $ADM^{+/-}$ mice suggests that PAMP is necessary for maintaining normal BP through the NO-dependent mechanisms, however, the differences may depend not only on PAMP/ADM peptide pattern but also on different genetic background of the two strains used to obtain knockout animals.

ADM in pathology and perspectives of its therapeutic application

Arterial hypertension

Plasma ADM 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 [95]. Experimental studies suggest that overloaded myocardium may be the source of ADM in severe but not in mild or moderate hypertension. Plasma ADM and ADM mRNA in the left ventricle are normal in spontaneously hypertensive rats (SHR) [109]. In the model of high-renin hypertension, two-kidney one-clip hypertension, plasma ADM level is initially normal but gradually increases after several weeks [63, 231]. Left ventricular ADM gene expression is increased in Dahl salt-sensitive rats fed high-Na^+ diet [190]. However, the most dramatic changes in the ADM system, i.e. marked up-regulation of ADM gene ex-

pression, ADM 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) [151] and in stroke-prone SHR [206, 228]. These abnormalities are antagonized by hypotensive therapy with either diuretic or ACE inhibitor, suggesting 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 ADM gene expression [129, 170]. In addition, mechanical stretch stimulates ADM secretion from cultured cardiomyocytes [215]. Thus, ADM gene is up-regulated by myocardial pressure overload and myocardial hypertrophy.

It is suggested that up-regulation of cardiac ADM system in hypertension is a protective mechanism decreasing myocardial overload due to vasodilatory and natriuretic properties of ADM, as well as limiting further myocardial hypertrophy and remodeling. Adenovirus-mediated ADM gene overexpression decreases blood pressure and attenuates cardiac and renal structural and functional damage in Dahl salt-sensitive rat [243], spontaneously hypertensive rat [21], DOCA-salt hypertensive rat [33], and in two-kidney one-clip hypertension [224]. Chronic infusion of ADM at doses which have no effect on blood pressure improves creatinine clearance, decreases proteinuria and renal histological changes and reduces renal expression of ACE and TGF-β in DOCA-salt SHR [128] and Dahl salt-sensitive rats [146], suggesting that renoprotective effect of ADM is independent of the normalization of blood pressure.

Heart failure

Plasma concentration of ADM is elevated in patients with congestive heart failure and correlates with disease severity [53, 242]. High plasma ADM is an independent negative prognostic indicator in these subjects [166]. Moreover, plasma ADM may help to stratify patients for therapy with β-adrenergic antagonists, since subjects with high ADM get more benefit from such treatment [169]. Several lines of evidence suggest that increased myocardial ADM production may contribute to high plasma ADM in patients with heart failure. ADM concentration in myocardial tissue obtained from heart transplant recipients with severe heart failure is higher than in donors [74]. In contrast to healthy subjects, in patients with heart failure ADM concentration in coronary sinus is higher than in aorta indicating significant contribution of myocardium to circulating ADM pool [72]. ADM concentration in pericardial fluid is elevated in heart failure patients and markedly exceeds its concentration in plasma [210]. ADM, CRLR, RAMP2 and RAMP3 gene expression is markedly up-regulated in different animal models of heart failure induced by volume or pressure overload [31, 145, 150, 214, 240]. Several mechanisms may contribute to overproduction of ADM in overloaded myocardium including angiotensin II, endothelin-1, mechanical stretch of myocytes and hypoxia. Up-regulation of ADM may be a compensatory mechanism decreasing cardiac preload and afterload. ADM may also improve contractility of the failing myocardium due to its positive inotropic properties. In addition, ADM inhibits myocardial remodeling by attenuating myocyte hypertrophy, proliferation of myocardial fibroblasts and production of extracellular matrix [36]. Finally, ADM reduces aldosterone and vasopressin secretion, which are up-regulated in heart failure Thus, ADM cooperates with natriuretic peptides in counteracting the effects of simultaneously activated vasoconstricting and sodium retaining mediators, such as rennin-angiotensin-aldosterone, sympathetic nervous system and endothelin. *Iv* infused ADM has beneficial hemodynamic and neurohormonal effects in humans with heart failure leading to an increase in cardiac output and natriuresis, reduction of peripheral resistance, pulmonary capillary wedge pressure, left ventricular end diastolic pressure and plasma aldosterone, and increase in ejection fraction [138]. Nevertheless, some [71, 140] but not all [103] studies indicate that vasodilatory and natriuretic effects of ADM are impaired in heart failure. The mechanism of this "adrenomedullin resistance" is not clear at present. Inhibitors of neutral endopeptidase which prolong the half-life of natriuretic peptides also augment the effect of ADM in experimental heart failure [30].

Atherosclerosis

ADM was detected in macrophages found within the atherosclerotic plaque [142]. Plasma ADM is increased in patients with chronic ischemic stroke and correlates with the extent of carotid artery atherosclerosis [198]. In theory, ADM could inhibit atherogenesis due to its inhibitory ef-

fect on migration and proliferation of vascular smooth muscle cells, inhibition of endothelial cell apoptosis and anti-inflammatory activity. Unfortunately, the effect of ADM on atherogenesis in classic models, such as cholesterol-fed rabbits or LDL receptor knockout mice has not been studied so far. However, overexpression of ADM gene has been reported to attenuate restenosis following balloon-induced or cuff-induced arterial injury in rodents [2, 5, 60, 233]. In addition, intimal thickening following injury was enhanced in $ADM^{+/-}$ mice. Although restenosis differs slightly from typical atherosclerotic lesions, these data indirectly suggest the atheroprotective role of ADM.

Myocardial infarction

Plasma ADM concentration increases during acute phase of myocardial infarction reaching its maximum on day 2–3 and returning to baseline after about 3 weeks [93]. Plasma ADM positively correlates with central venous pressure, left ventricular and diastolic pressure and pulmonary capillary wedge pressure, and negatively with left ventricular ejection fraction, suggesting that an increase in ADM is associated with hemodynamic impairment [126]. Consequently, high plasma ADM is an independent negative prognostic factor in these patients [136]. Myocardium may be an important source of plasma ADM in the course of acute coronary syndromes. The expression of ADM, CRLR and RAMP2 increases in ischemic and non-ischemic myocardium following coronary artery ligation in the rat [137, 160]. During the episode of vasospastic angina, ADM level in coronary circulation is higher than in peripheral blood [75]. Moreover, in patients with acute coronary syndromes, ADM concentration in pericardial fluid is markedly increased above the level found in plasma [149]. Hypoxia augments ADM gene expression in cardiomyocytes through the mechanism involving oxidative stress [239]. In addition, mechanical stretch, angiotensin II and proinflammatory cytokines synthesized in the infarcted area may stimulate ADM production in the myocardium. However, other tissues, such as the kidney may also contribute to high ADM because urinary immature and mature ADM are also increased after infarction [10]. Up-regulation of ADM system may be protective in the course of myocardial ischemia. ADM reduces oxidative stress-induced myocardial cell injury [143, 149]. ADM may also limit myocardial ischemia by causing local coronary vasodilation. Moreover, systemic hemodynamic effects of ADM decrease myocardial oxygen demand. Transgenic ADM overexpression before myocardial ischemia-reperfusion decreases superoxide anion generation in the hypoperfused myocardium, inhibits apoptosis of cardiomyocytes, decreases infarct area and reduces the episodes of ventricular fibrillation [22]. Chronic ADM infusion at low doses which do not affect blood pressure inhibits cardiac remodeling in the rat following experimental myocardial infarction [141].

Renal diseases

Plasma ADM concentration is increased, whereas urinary ADM excretion is decreased in various types of glomerulonephritis [85, 100]. In addition, plasma ADM progressively increases in patients with chronic renal failure. This is mainly accounted for by reduced peptide clearance, however, increased ADM production due to chronic volume overload cannot be excluded [61, 155]. In some studies, a decrease in plasma ADM after hemodialysis was observed, however, this occurred not earlier than 15–20 h after dialysis procedure, suggesting that reduced ADM production rather than its clearance through the dialysis membrane was involved [114]. In contrast, other authors observed an increase in plasma ADM following hemodialysis, most likely due to activation of leukocytes by bioincompatible dialysis membranes. Marked increase in plasma ADM level was observed in patients with the episodes of severe hypotension following hemodialysis, suggesting the role of ADM in this complication [15].

Hypoxia up-regulates ADM gene expression in the kidney [133]. ADM may protect the kidney against ischemia-reperfusion injury. It has been demonstrated that ADM deficiency in $ADM^{+/-}$ mice aggravates, whereas ADM overexpression attenuates histological lesions and functional impairment in experimental ischemic acute renal failure [152].

Septic shock

Plasma ADM is markedly increased in patients with sepsis and septic shock, exceeding by at least one order of magnitude the values observed in any other diseases [154]. Bacterial endotoxins and pro-inflammatory cytokines up-regulate ADM gene expression in many tissues both *in vitro* and *in vivo*

[199]. It has been suggested that ADM contributes to extreme vasodilation and hypotension associated with septic shock. Apart from directly relaxing blood vessels, ADM up-regulates cytokine-induced iNOS gene expression in the vascular wall [200]. ADM_{22-52}, an ADM receptor antagonist, prevents lipopolysaccharide-induced hypotension in the rat [122], and administration of anti-ADM antibodies attenuates hypotension in the model of polymicrobial sepsis induced by colonic ligation and puncture [226]. However, recent studies suggest a more complex role for ADM in pathophysiology of septic shock. Local vasodilatory effect of ADM may limit hypoxic injury of tissues. In addition, ADM possesses bactericidal, anti-inflammatory and positive inotropic properties, all of them being potentially beneficial in the course of sepsis. In mice overexpressing ADM in their vasculature, LPS administration induces less hemodynamic and inflammatory changes, lower degree of liver damage and lower mortality than in control animals, suggesting protective role of ADM [195]. The course of septic shock consists of two consecutive phases: early hyperdynamic phase characterized by increased cardiac output, reduced peripheral resistance and tissue hyperperfusion, and late hypodynamic phase associated with high vascular resistance and reduced cardiac output leading to tissue hypoperfusion and end-organ damage. Plasma ADM is elevated throughout the course of septic shock but ADM seems to be involved in hemodynamic adaptation only in the hyperdynamic phase. Transition to the hypodynamic phase is associated with reduced responsiveness of the vasculature to ADM [99, 227]. Thus, reduced sensitivity to ADM may contribute to the development of hypodynamic phase of septic shock. The mechanism of this ADM hyporesponsiveness may involve reduction of plasma AMBP-1 concentration in late sepsis. Administration of exogenous AMBP-1 augments vasodilatory effect of ADM *in vitro*, restores the effect of ADM on blood vessels in the late period of experimental sepsis, and delays the transition to hypodynamic phase, thus decreasing the rate of end-organ damage [236, 244]. Apart from improving hemodynamics, ADM+AMBP-1 therapy reduces plasma TNF-α concentration [237]. Thus, ADM/AMBP-1 therapy provides a novel approach for the treatment of septic patients.

Respiratory diseases

Plasma ADM is increased in the rats with experimental pulmonary hypertension induced by monocrotaline. The source of plasma ADM may be a right ventricle, since ADM mRNA in righ ventricular myocardium is simultaneously elevated [189]. Plasma ADM is also increased in humans with primary and secondary pulmonary hypertension [26, 147, 148]. Transgenic overexpression of ADM gene, chronic *iv* ADM infusion or therapy with aerosolized ADM decreases pulmonary arterial pressure and reduces hypertrophy of the right ventricle [77, 135, 241]. Inhaled ADM reduces histamine-, acetylcholine-, and antigen-induced bronchoconstriction and microvascular leakage in guinea pigs [156].

Gastrointestinal pathologies

The expression of ADM and its receptor increases in injured gastric mucosa. Exogenous ADM attenuates mucosal lesions induced by hypertonic NaCl or reserpine [38, 225]. Thus, ADM may be involved in the restitution of mucosal lesions and may be considered as a potential drug for the treatment of peptic ulcer disease. ADM prevents ethanol- or reserpine-induced gastric injury only if administered centrally but not peripherally, suggesting that vagal nerve may be involved in its gastroprotective effect [78].

Diabetes mellitus

The existence of ADM in pancreatic islets and its inhibitory effect on insulin secretion suggest that ADM may be involved in the pathogenesis of diabetes mellitus. In type 1 diabetes, plasma ADM is increased only in patients with microangiopathy. Increased ADM may result from endothelial activation and/or impaired renal clearance in subjects with diabetic nephropathy [40]. This suggests that increased ADM in type 1 diabetes is a consequence of the disease rather than a causal agent. The results concerning type 2 diabetes are more controversial. Some studies revealed increased plasma ADM independent of side complications even in recently diagnosed disease, suggesting that excess of ADM could lead to insulin deficiency [49, 218]. However, other studies do not support these data demonstrating increased ADM level only in patients with diabetic nephropathy [87]. Recent studies suggest that also deficiency of ADM may impair car-

bohydrate metabolism, because $ADM^{+/-}$ mice are characterized by insulin resistance which is corrected by low-dose ADM infusion [193].

Gestational pathologies

It has been suggested that ADM deficiency contributes to the development of pregnancy-induced hypertension. Some authors have reported reduced ADM concentration in maternal blood in comparison to normotensive pregnant women [45], and lower ADM gene expression in placenta, fetal membranes and umbilical artery in preeclamptic pregnancies [79, 91]. However, others observed no difference or even increased ADM in maternal blood, cord blood and/or amniotic fluid, suggesting that ADM is stimulated by shear stress and helps to maintain fetoplacental perfusion despite systemic vasoconstriction [32, 112]. These data suggest that the role of ADM in pregnancy-induced hypertension differs among patients. In a rat model of preeclampsia induced by administration of NOS inhibitor into pregnant dams, ADM infusion reduces blood pressure, improves intrauterine growth and decreases pup mortality [111]. Maternal plasma ADM is also increased in some, but not in all, women with gestational diabetes mellitus, suggesting the involvement of ADM in this complication in a subset of pregnancies [118].

Adrenomedullin and the eye

ADM is expressed in the outer layer of the retina, in particular in retinal pigment cells. ADM dilates retinal artery, decreases intraocular pressure, and relaxes the sphincter smooth muscle. ADM is present in the aqueous humor and vitreous fluid at a level 4–20 times higher than in plasma. ADM concentration in vitreous fluid is markedly increased in patients with proliferative vitreoretinopathy, the most common complication of retinal detachment originating from the proliferation of retinal pigment cells [219].

Proadrenomedullin N-terminal 20-peptide (PAMP)

PAMP consists of 1–20 amino acids of proADM. Similarly to ADM, N-terminal residue is amidated, however, PAMP does not contain intramolecular disulfide bond. The distribution of PAMP in mammalian tissues is similar to ADM consistently with their origin from a common precursor. PAMP/ADM 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. PreproADM mRNA is alternatively spliced to generate either so called form A or form B (Fig. 2). Form A originates when all introns are removed and contains both PAMP and ADM coding sequences. Form B arises when 3rd intron is retained and creates truncated preprohormone, because this intron contains a premature stop codon. Consequently, translation of form B generates PAMP but not ADM. The regulation of splicing process is not clear at present. However, it has been demonstrated that hypoxia increases, whereas TNF-α decreases form B/form A ratio [119]. Also, whereas some factors such as cholinergic stimulation in pheochromocytes [84] and angiotensin II in cardiac myocytes [216] enhance the secretion of both peptides, other, such as aldosterone in vascular smooth muscle cells, augment the secretion of ADM but not PAMP [220]. Thus, secretion of both proADM-derived peptides may be, at least to some extent, separately regulated. PAMP levels in plasma (<1 pM) and urine (~25 pM) are severalfold lower than that of ADM, due to more rapid clearance of PAMP by neutral endopeptidase [134].

Specific PAMP binding sites, distinct from ADM receptors, have been identified in blood vessels, adrenals, kidneys, heart, brain and spleen [50, 67]. However, molecular structure of PAMP receptors has not been identified.

Iv administered PAMP decreases arterial pressure due to systemic vasodilation, although this peptide is less potent than adrenomedullin [35, 139]. PAMP dilates blood vessels mainly by decreasing norepinephrine release from vasomotor sympathetic fibers [191, 192]. PAMP decreases norepinephrine release by inhibiting voltage-gated N-type Ca^{2+} channels. Some studies have reported that PAMP can also induce direct SNS-independent cAMP-mediated vasorelaxation [17, 20]. Recently, it has been reported that PAMP may antagonize the stimulatory effect of ADM on endothelial NO production, suggesting more complex role of this peptide in the regulation of vascular tone [106].

PAMP inhibits release of catecholamines from adrenal medullary cells [84, 207, 208]. PAMP selectively antagonizes catecholamine secretion stimulated by nicotinic cholinergic receptors [92, 202]. It is suggested that PAMP may directly interact with nicotinic receptors [110]. Apart from reducing secretion, PAMP decreases catecholamine synthe-

sis in adrenal medulla by reducing the activity and gene expression of a rate-limiting enzyme, tyrosine hydroxylase [209]. PAMP inhibits angiotensin II- or potassium-stimulated aldosterone secretion from adrenal zona glomerulosa more potently than ADM [7, 9]. However, PAMP may also augment aldosterone production through the cAMP-protein kinase A-dependent mechanism [50, 212]. In addition, in contrast to ADM, PAMP stimulates dehydroepiandrosterone secretion from human adrenocarcinoma cells [213]. Apart from the regulation of hormone secretion, PAMP stimulates proliferation of rat ZG cells through the MAPK-dependent pathway [168].

In contrast to ADM, PAMP infused into the renal artery has no effect on either renal hemodynamics or natriuresis [222]. However, PAMP attenuates a decrease in natriuresis and diuresis induced by stimulation of renal sympathetic nerves, suggesting that this peptide inhibits norepinephrine release not only from vasomotor but also from renal sympathetic fibers [205]. PAMP was detected in renin-containing secretory granules of juxtaglomerular cells, however, its role in the regulation of renin secretion has not been studied [108].

Centrally administered PAMP increases blood pressure and heart rate by stimulating SNS activity [178]. PAMP inhibits basal but not CRH-stimulated ACTH secretion from dispersed anterior pituitary cells [179]. Both centrally and peripherally injected PAMP increases blood glucose level in fasted mice. Central effect results from sympathetic stimulation of glucagon secretion. This effect is blocked by gastrin-releasing peptide (GRP) receptor antagonist. GRP, together with neuromedin B and neuromedin C, belongs to bombesin–like peptide family, the peptides homologous to amphibian peptide, bombesin. PAMP at physiological concentrations has high affinity for GRP and neuromedin B receptors. In addition to affecting plasma glucose, centrally administered PAMP inhibits food intake and gastric emptying as well as decreases body temperature and oxygen consumption [157–159]. In addition, subcutaneously administered PAMP reduces plasma TSH in the rat [41]. These data suggest an important role of PAMP in the control of metabolism. In contrast to effect on blood glucose, other central actions of PAMP are not mediated by the bombesin receptors. Similarly to ADM, inhaled PAMP attenuates histamine or acetylcholine-induced bronchoconstriction [76]. In contrast to ADM, PAMP has no effect on protein synthesis in cardiomyocytes or on the proliferation of cardiac fibroblasts [83]. Plasma PAMP concentration is increased in arterial hypertension [102], heart failure [37] and renal failure [86]. The most important similarities and differences between ADM and PAMP are listed in Table 2.

Table 2. Comparison of biological activities of ADM and PAMP

Biological activity	ADM	PAMP
Mechanism of vasodilation		
inhibition of norepinephrine release from sympathetic endings	–	+
increase in cAMP in smooth muscle cells	+	+
NO-cGMP pathway	+	–
Catecholamine release from the adrenal medulla	inhibition	no effect
Aldosterone secretion from the adrenal cortex		
angiotensin II-stimulated	inhibition	inhibition
potassium stimulated	inhibition	inhibition
ACTH-stimulated	stimulation	stimulation
DHEA secretion from the adrenal cortex	no effect	inhibition
Natriuretic activity	+	–
Central stimulation of the SNS	+	+
Effect on blood glucose	increases through the inhibition of insulin secretion	increases by activating bombesin receptors and stimulating glucagon
Bronchodilation	+	+
Inhibitory effect on myocardial remodelling	+	–

Other proadrenomedullin-derived peptides

Apart from ADM and PAMP, several other peptides derived from proADM have been isolated from mammalian tissues. ADM fragments containing N-terminal ADM residues and intramolecular ring but lacking C-terminal chain, including ADM_{1-25} [230], ADM_{15-22} [19], ADM_{11-26} [90] and ADM_{16-31} [18] have pressor activity due to stimulatory effect on catecholamine release. C-terminal fragments of ADM lacking intermolecular ring, ADM_{26-52} and its truncated form, ADM_{34-52}, have been isolated from the pig intestine [57]. ADM_{26-52} antagonizes vasodilatory effect of ADM on pulmonary vessels in a competitive manner [44]. Thus, ADM_{26-52} may be a naturally occurring ADM receptor antagonists. The existence of ADM_{1-25} and ADM_{26-52} as well as of their truncated forms in mammalian tissues suggests that ADM may be enzymatically cleaved to its N-terminal and C-terminal fragments, both having vasopressor effect albeit exerted *via* different mechanisms. Finally, adrenotensin (ADT) consisting of amino acids 153–185 of preproADM has been isolated from some tissues. This peptide induces endothelium-dependent vasoconstriction [42, 43]. ADT antagonizes the stimulatory effect of ADM on endothelial NO generation, however, this peptide also stimulates cAMP in vascular tissue suggesting that it could have vasodilating effect under some circumstances [106].

Conclusions and future directions

Over 1200 papers about ADM have been published during the last 10 years. Initially recognized only as a vasodilatory and natriuretic peptide, ADM now appears as an extremely multifunctional mediator involved in processes so diverse as embryogenesis, normal and malignant growth, inflammation and immunity. Plasma ADM level is increased in various diseases, such as arterial hypertension, heart failure, acute coronary syndromes, renal failure and septic shock. ADM plays an important role in the pathophysiology of many of these disorders and in some of them measurement of ADM level may provide important prognostic information. Experimental ADM treatment is beneficial in systemic and pulmonary arterial hypertension, myocardial and renal ischemia-reperfusion injury, peptic ulcer disease, septic shock, and in preventing restenosis following angioplasty. Although due to its peptide nature, ADM has to be administered parenterally which limits its potential application to the acute states, other therapies such as local ADM gene delivery, NEP inhibition and AMBP-1 administration are tested under experimental conditions. The possible future approaches include modification of ADM amidating enzyme activity and ADM receptor system. On the other hand, inactivation of ADM system may also be useful, e.g. in the treatment of cancer and proliferative retinopathies. Research in ADM field opened many new interesting issues such as the regulation of ADM /PAMP ratio by alternative mRNA splicing, peptide maturation by enzymatic amidation, bidirectional interactions of ADM with the complement system and cross-reactivity of PAMP with the bombesin receptors. The discovery of RAMP proteins unravels a novel mechanism regulating ligand specificity of plasma membrane receptors. Molecular identification of PAMP receptors and functional interactions between different proADM-derived peptides with parallel and opposite activities are an important challenge for future research.

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