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Adrenomedullin: A novel peptide hormone: A review

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Abstract

Adrenomedullin is a peptide hormone extracted from pheochromocytoma in humans has 52 amino acid held together by a single disulphide bond. The name adrenomedullin was given as it is abundantly found in adrenal medulla, comes under calcitonin gene-related peptide family. Production of adrenomedullin is up-regulated by several factors such as oxidative stress, pro-inflammatory cytokines, angiotensin II, hypoxia, hyperglycemia, natriuretic peptide and aldosterone. It is synthesized as part of a larger precursor molecule, termed proadrenomedullin. Adrenomedullin receptor consists of seven transmembrane domains and belongs to G-protein dependent receptor family. This peptide has not only a potent hypotensive and vasorelaxing effect but it performs various biological functions. It has potent effects on cell migration, growth, and apoptosis. So with this overview adrenomedullin is a novel peptide hormone that plays significant physiological functions in different systems of the body.

Keywords: Adrenomedullin, calcitonin gene-related peptide, hypotension, proadrenomedullin

1. Introduction

Adrenomedullin (AM), a novel peptide hormone was initially discovered by ^[1], extracted from pheochromocytoma in humans by monitoring the elevated 3' 5' cyclic adenosine monophosphate (cAMP) production in platelets. It forms a ring structure by 52 amino acid residues held by one intra-molecular disulfide bond. Since the peptide was abundantly found in the adrenal medulla, therefore this accounts for the name adrenomedullin. The peptide is classified as a member of the calcitonin gene-related peptide (CGRP) superfamily, which includes calcitonin, calcitonin gene related peptides (CGRP), amylin and intermedin. High level of AM is present in the adrenal medulla and circulating AM is most abundant in vascular wall ^[2]. AM is produced in several tissues (kidney, lung, and heart) and its production is up-regulated by several factors such as oxidative stress, pro-inflammatory cytokines, angiotensin II, hypoxia, hyperglycemia, natriuretic peptide and aldosterone.

AM have a hypotensive, vasodilator, paracrine/ apocrine effect, in addition it inhibit myocyte protein synthesis and cardiac fibroblast proliferation. AM is also an angiogenic factor that is induced by hypoxia and can alter the permeability of vascular endothelial cells. Also the potent effects of AM on cell migration, growth, and apoptosis (programmed cell death) have led to the hypothesis that AM may be a key player in tumor growth and metastasis ^[3]. AM may also contribute to blood volume regulation through its natriuretic and diuretic functions in the kidney and its effects on central nervous system control of thirst and salt appetite ^[4]. AM is highly expressed in the skin and oral mucosa, and this expression pattern has been linked to its potent antimicrobial effect ^[5]. This ubiquitous hormone enhances during implantation ^[6]. According to what mentioned above AM is a multifunctional peptide that can exert many important and interrelated biological functions under both normal and disease conditions.

2. Structure and synthesis of adrenomedullin

Adrenomedullin is a 52 amino acid peptide with a single disulfide bridge between residues 16 and 21 and with an amidated tyrosine at the carboxy terminus (fig 1A and 1B). It has a homology with calcitonin gene-related peptide (CGRP) and has therefore been added to the calcitonin/CGRP/ amylin peptide family. Rat adrenomedullin has 50 amino acids, with 2 deletions and 6 substitutions compared with the human peptide. Porcine adrenomedullin is nearly identical to the human peptide, with only a single substitution (Gly for Asn) at position 40 ^[7].

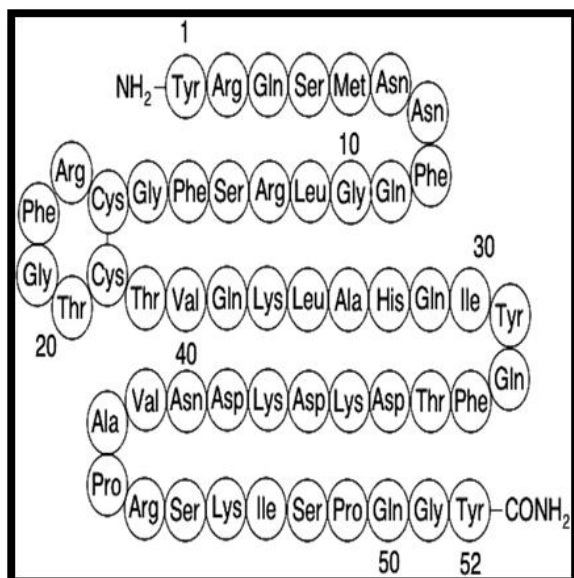


Fig 1A: Structure showing 52 amino acid sequence with a single disulphide bond

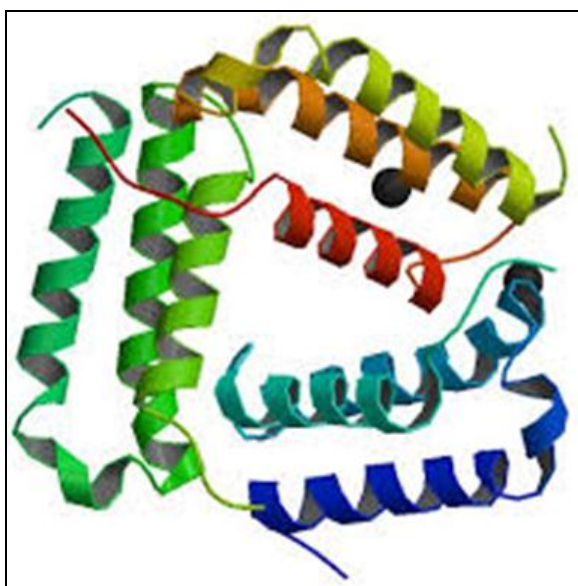


Fig 1B: Structure of adrenomedullin

Adrenomedullin is synthesized as part of a larger precursor molecule, termed preproadrenomedullin. In rat and human this precursor consists of 185 amino acids, while the porcine precursor has 188 residues. Preproadrenomedullin contains a 21-amino acid N-terminal signal peptide that immediately precedes a 20-amino acid amidated peptide, designated preadrenomedullin N-terminal 20 peptide or PAMP. The gene encoding preproadrenomedullin is termed the adrenomedullin gene and has been mapped and localized to a single locus of chromosome 11. The adrenomedullin gene is expressed in a wide range of tissues. The initial report on the distribution of adrenomedullin mRNA suggested that the highest levels of expression were seen in the adrenal medulla, ventricle, kidney and lung. Since the discovery that the adrenomedullin gene is more highly expressed in endothelial cells than in the adrenal medulla, this peptide has come to be regarded as a secretory product of the vascular endothelium, together with nitric oxide (NO) and endothelin. Therefore, Adrenomedullin expression is seen in all tissues of the body and comparisons between tissues may simply reflect varying degrees of tissue vascularity [7].

3. Receptors

Adrenomedullin receptors have close association with receptors of the related peptide CGRP (fig 2). AM and CGRP receptors are functionally correlated because of their cross-reactivity and their similar biological action. AM-receptor consists of seven transmembrane domains and belongs to G-protein dependent receptor superfamily. The AM receptor was initially isolated from rat vascular smooth muscle cells (VSMC), but has successively been identified in human endothelial cells, in rat astrocyte cells, heart and lungs. This receptor is also present in human lungs, breast, brain, ovary, colon and prostate tumoral cell lines. Interaction of AM with AM-receptor determines vasodilatation through direct and indirect mechanisms. The direct mechanism activates cellular adenylyl-cyclase and increases intracellular cyclic AMP levels, while the indirect mechanism acts through an increase of intracellular calcium that determines a rise in target cell production of nitric oxide. The increase of intracellular calcium is biphasic and consists of a first phase of release of intracellular calcium deposits and of a second phase of increased membrane calcium channel permeability. Release of intracellular calcium deposits is preceded by activation of phospholipase C and inositol-triphosphate synthesis. Elevation of inositol-triphosphate concentration activates nitric oxide-synthetase. How Am activates and regulates target cell genes is still question of debate? A rapid but transitory expression of C-fos mRNA in VSMC and fibroblasts has been described. This increased expression of C-fos mRNA in cardiac myocytes and fibroblasts could suggest that these cells are the genomic target of AM. Adrenomedullin seems to have little affinity for receptors for the other two members of the peptide family, (1) calcitonin and (2) amylin [7].

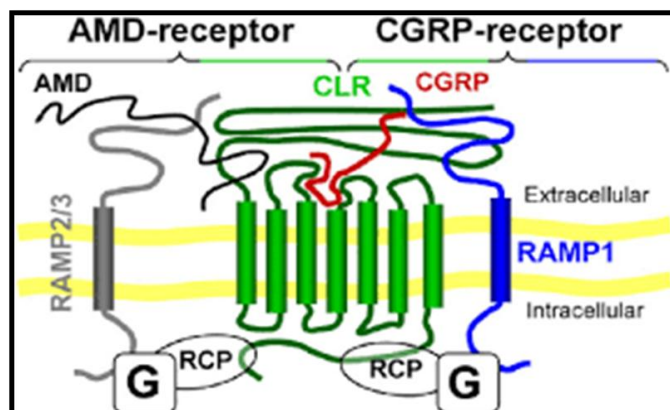


Fig 2: Schematic diagram of postulated interactions of adrenomedullin (black) and calcitonin gene-related peptide (red) with their receptor components. Co-expression of the calcitonin receptor-like receptor (green) with the receptor activity-modifying protein 1 (blue) forms the "mature" CGRP receptor. In contrast, coexpression of CLR with RAMP2/RAMP3 molecules forms the AM receptor. Both receptors are interacting with the receptor component protein (RCP, ovoid) to form heteromers that are associated with downstream effectors, e.g., G-proteins (squares).

Adrenomedullin acts through calcitonin receptor-like receptor (CRLR) associated with one of the three receptor-activity-modifying proteins (fig 3): RAMP1, RAMP2 or RAMP3 [4]. Co-expression of RAMP1 with CRLR produces a CGRP receptor, whereas co-expression of RAMP2 or RAMP3 with CRLR produces an ADM receptor [8].

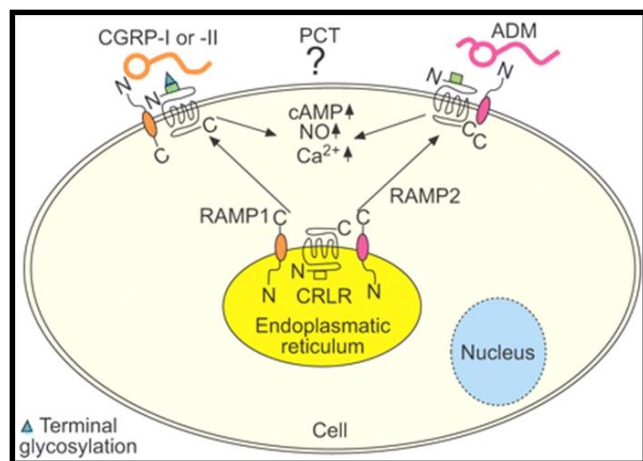


Fig 3: Receptors for calcitonin (CT) peptides CGRP: calcitonin gene-related peptide; ADM: adrenomedullin; NO: nitric oxide; N: amino terminal; C: carboxyl terminal.

Based on the structural homologies, different CT peptides have overlapping bioactivities, which they exert by binding to the same family of receptors. There are two subgroups of these G protein-coupled receptors with seven transmembrane domains: CT receptors and CT receptor-like receptors (CRLR). Three accessory proteins, which are called receptor activity-modifying proteins (RAMP-1, 2 and 3), act upon these receptors, thus altering their specific responsiveness and ligand affinity, and hence modifying the physiological profile of the CT-peptide superfamily. Depending on which of the different RAMPs is associated with the receptor, each member of the CT-gene family of peptides binds with differing affinities. Whether procalcitonin (PCT) and other CT precursor peptides from the other CALC genes are also ligand for these receptors is currently unknown.

4. Biological actions of adrenomedullin

Adrenomedullin can act as both a hormone and a cytokine to regulate the regional blood flow, vascular tone, leukocyte migration and differentiation, electrolyte balance, cardiac function, glucose uptake and hormone secretion. Adrenomedullin has been shown to have a remarkable range of actions, from regulating cellular growth and differentiation, through modulating hormone secretion to antimicrobial effects. It plays an important role in cardiovascular system. AM imposes a potent vasodilatory effect in humans and increases blood flow to various organs. For instance, increased AM expression could enhance hepatic and renal circulation. In systemic circulation, vasodilation could be resulted from either endothelium-dependent or endothelium-independent mechanisms, through AM and CGRP receptors. In addition, the endothelium-derived vasodilation could be mediated by cAMP and nitric oxide [2].

4.1 Vascular actions

Systemic administration of AM elicits a potent hypotensive effect due to its vasodilatory action. Vasodilator action of AM is mainly mediated by endothelium-derived nitric oxide. AM increases endothelial NO synthase (eNOS) activity by elevating intracellular free calcium concentration or by activating phosphatidylinositol 3-kinase and protein kinase B/Akt. It has also been shown that AM increases interleukin-1 (IL-1) - induced NO synthesis by enhancing the expression of inducible NO synthase (iNOS) in vascular smooth muscle cells.

Endothelium-independent vasodilatation to CGRP, activation

of CGRP receptors on smooth muscle cells is coupled to production of cAMP by adenylyl cyclase. The increase in intracellular cAMP concentration (cAMP) then stimulates protein kinase A (PKA), which opens K^+ channels and activates Ca^{2+} sequestration mechanisms to cause smooth muscle relaxation. Endothelium-dependent vasodilatation to CGRP, CGRP interacts with receptors on endothelial cells and stimulates production of nitric oxide (NO). This is mediated via cAMP accumulation, although a direct effect of PKA on endothelial NO synthase (eNOS) is yet to be fully characterized. Diffusion of NO into adjacent smooth muscle cells, activating guanylate cyclase, then leads to relaxation.

Adrenomedullin increases intracellular cyclic adenosine monophosphate (cAMP) by stimulation of adenylyl cyclase and activation of protein kinase A (cAMP/PKA signaling pathway) as well as increases cyclic guanosine 3',5'-monophosphate (cGMP) by stimulation of NO activated guanylyl cyclases and activation of cGMP-dependent protein kinase (PKG) (NO/cGMP/PKG signaling pathway). It has been shown that AM induced increases in NO are mediated by activation of protein kinase A and phosphatidylinositol 3-kinase. The vasodilatory action of AM may be mediated by endothelium-derived NO and/or vasoactive prostanoids which is endothelium-dependent vasodilation as well as by an increase in intracellular cAMP which is endothelium-independent vasodilation [8].

4.2 Angiogenesis

AM has angiogenic potential. Angiogenesis is a multistep process that involves migration and proliferation of endothelial cells, functional maturation of the newly assembled vessels, and remodeling of the extracellular matrix [9]. Akt, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase 1/2 (ERK1/2), and focal adhesion kinase (p125FAK) play an important role in angiogenesis in endothelial cells. [10] Demonstrated that AM activated Akt, MAPK/ERK1/2, and p125FAK in human umbilical vein endothelial cells (HUVECs), and produced increases in their DNA synthesis and migration. AM induced tube formation in HUVECs, and its effect was inhibited by pretreatment with a phosphatidylinositol 3-kinase (PI3K) inhibitor or mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK) 1/2 inhibitor. These findings suggest that AM exerts angiogenic activities through activation of Akt, MAPK and p125 FAK in endothelial cells. *In vivo*, overexpression of AM augments collateral flow in ischemic tissues partly through activation of endothelial nitric oxide synthase (eNOS) [11]. Earlier studies have shown that the vasodilatory effects of AM are mediated by cAMP/protein kinase in smooth muscle cells (SMCs) and by the eNOS/NO pathway in endothelial cells. Thus AM-induced angiogenesis and vasodilation may synergistically improve blood perfusion in ischemic tissues. AM activates the PI3K/Akt-dependent pathway in vascular endothelial cells, which is considered to regulate multiple critical steps in angiogenesis, including endothelial cell survival, proliferation, migration, and capillary-like structure formation [12]. Both AM and calcitonin-receptor-like receptor (CRLR) are upregulated through a hypoxia-inducible factor-1 (HIF-1)-dependent pathway under hypoxic conditions. AM binds to CRLR modified by receptor-activity-modifying protein 2 (RAMP2) and RAMP3. AM induces angiogenesis through activation of Akt, MAPK, and p125FAK in endothelial cells. AM also induces Smooth muscle cell migration and vasodilation. These activities synergistically improve tissue

ischemia.

4.3 Growth and development

Adrenomedullin was originally purified from a human adrenal tumor. Cuttitta's group extended their initial observation of adrenomedullin and L1 receptors in pulmonary tumors to study the expression of Adrenomedullin in human tumor cell lines in general [13]. This opened up the possibility of adrenomedullin being an autocrine/ paracrine growth factor in tumors and possibly normal cells. A neutralizing antiadrenomedullin monoclonal antibody was growth inhibitory to these cells, which also showed both 125I-adrenomedullin binding (L1 receptor mRNA was also present) and adrenomedullin-stimulated cAMP. In Swiss 3T3 cells adrenomedullin increased DNA synthesis in a dose-dependent manner by a mechanism involving specific adrenomedullin receptors and increased cAMP/PKA [14]. These findings have been confirmed, and Swiss 3T3 cells were shown to produce correctly processed adrenomedullin, which is regulated by cytokines and growth factors. In normal and malignant skin, adrenomedullin and the L1 receptor were detected and adrenomedullin increased 3H-thymidine uptake. Adrenomedullin also stimulated DNA and cAMP synthesis in human oral keratinocytes. The effect on DNA synthesis was inhibited by an adenylyl cyclase inhibitor and mimicked by cAMP. In quiescent rat VSMCs, Adrenomedullin and CGRP stimulated DNA synthesis and cell proliferation. These facts provide strong evidence for a growth promoting effect of adrenomedullin, possibly mediated via cAMP. [15] Reported that adrenomedullin exerted mitogenic effects on these cells that correlated with increases in cAMP and c-fos expression.

4.4 Reproductive effect

AM is produced by granulosa cells of the ovarian follicle [16]. AM causes a dose-dependent increase in the intracellular levels of cAMP and enhance the effects of FSH, acting additionally to produce cAMP in the granulosa cells [17]. Since, FSH is known to induce granulosa cell differentiation through cAMP-mechanism. AM may play a supportive role in the process of granulosa cell differentiation. In patients undergoing ovarian stimulation, follicular fluid AM levels correlated with serum 17-beta-estradiol concentration, suggesting a possible regulatory effect of the sexual hormones on AM production by the ovary during the ovulatory process [17]. AM levels in follicular fluid collected just before ovulation were significantly higher than those in the plasma. Furthermore, addition of AM to cultured granulosa lutein cells augmented progesterone secretion in a dose-dependent manner, suggesting AM may be a local factor to enhance progesterone production by granulosa lutein cells [18]. Plasma AM levels increase during the follicular phase and decrease during the luteal phase and the changes in plasma AM are related to changes in LH and 17betaestradiol [19].

4.5 Kidney

AM and its gene expression have been reported to be observed in the glomerulus, distal tubules, and medullary collecting duct cells of the kidney [20]. [21] Demonstrated that the calcitonin receptor-like receptor (CRLR), a receptor with 7 transmembrane domains, can function as either a CGRP receptor or an AM receptor, depending on which members of a new family of single-transmembrane domain proteins, which are called receptor-activity-modifying proteins 87 (RAMPs), are expressed. RAMP1 presents the receptor at the cell surface as a CGRP receptor, whereas RAMP2- or

RAMP3-transported receptors are AM. CRLR, RAMP2, and RAMP3 mRNAs were expressed in the rat renal cortex and medulla [22]. Immunohistochemical analysis of the human kidney revealed CRLR-like immunoreactivity in the juxtaglomerular arteries, the glomerular capillaries and chief cells of the collecting duct [23]. These results suggest that AM and its receptor system in the kidney may be involved in the regulation of renal hemodynamics, glomerular filtration and tubular Na⁺ homeostasis *in vivo*. There is also evidence for a role for AM in mesangial cell biology. Mesangial cells grown in primary culture synthesize AM which is stimulated by TNF alpha and IL-1 beta and under hypoxic conditions. AM increases cAMP levels in mesangial cells leading to inhibited proliferation, reactive oxygen generation and macrophage infiltration. In addition, AM has been reported to stimulate hyalurnoic acid, an important extracellular matrix component, release from mesangial cells through p38 kinase and PI3-kinase pathways [24]. These data suggest that there may be a role for AM not only in the pathophysiology of mesangial cell proliferation and matrix biology, but also in protecting the renal glomeruli from inflammatory reactions or immune injuries. Although AM mRNA expression has been reported to exist in glomerulus, cortical collecting duct, outer medullary collecting duct, and inner medullary collecting duct, it is still unclear whether AM directly regulates tubular transport. AM increases cAMP levels in renal tubular basolateral membranes and the cortical thick ascending limb, and in the distal convoluted tubule. These data suggest that AM may have an autocrine/paracrine role in renal tubular function. AM stimulates osmotic water permeability in the inner medullary collecting duct and Na⁺ uptake in apical membranes of the distal tubules [25]. These effects would favor activation of tubular reabsorption, although AM is known to be a natriuretic hormone probably through its vasodilating action. Intravenous infusion of AM in healthy volunteers [26] resulted in an increase in plasma renin activity associated with a decrease in arterial blood pressure. [27] Reported that AM is expressed in juxtaglomerular structures and that it has a direct stimulatory effect on renin secretion and renin mRNA abundance by receptors on juxtaglomerular cells, possibly through increases in cAMP. AM may act as an autocrine / paracrine stimulatory factor in the control of renin secretion and renin gene expression. AM administration to experimental animals has been reported to increase urine output and urinary sodium excretion in a dose-dependent manner in association with renal vasodilation, increased in renal blood flow, and glomerular filtration rate (GFR). However, as low doses of AM increase natriuresis without affecting GFR, AM may inhibit tubular sodium reabsorption. AM-induced renal vasodilator, diuretic and natriuretic responses may be partially mediated by the release of endogenous nitric oxide and renal prostaglandins. Interestingly, neutral endopeptidase (NEP) inhibition potentiates an increase in sodium excretion in the absence of an increase in GFR or further increases in renal blood flow in response to exogenous AM, indicating not only that AM may be a substrate for NEP, but also that a decrease in tubular sodium reabsorption may be the mechanism for natriuresis [28].

4.6 Lung

In addition to pulmonary vasodilatory effects, AM inhibits histamine- or acetylcholine-induced bronchoconstriction in anesthetized guinea pigs. Since plasma levels of AM are reported to rise during an acute asthma attack, AM may play

an important role in airway function. Furthermore, AM may have an anti-inflammatory role in the lung, because a previous study showed that AM significantly inhibited the secretion of cytokine-induced neutrophil chemo-attractant, a member of the 94 interleukin-8 family, from lipopolysaccharide-stimulated rat alveolar macrophages [29].

4.7 Digestive apparatus

AM mRNA and AM immunoreactivity are detected in gastrointestinal mucosa and gastrointestinal AM gene expression is up-regulated by fasting. Intravenous administration of AM inhibits both basal and gastrin-stimulated gastric acid secretion in conscious rats. In addition, AM prevents damage of gastric mucosa in either reserpine-treated or pylorus-ligated rats [30]. Taken together with the central actions of AM on gastric function (described above), AM is thought to have an anti-ulcer effect through the inhibition of gastric acid secretion. AM and its specific binding sites are also localized in the pancreas and liver. AM inhibits stimulated amylase secretion by reducing the calcium sensitivity of the exocytotic machinery of the pancreatic acini. In addition, AM contributes to the relaxation of hepatic stellate cells and the regulation of sinusoidal microcirculation [31].

TRPA1 activators have 3 potential target cells: intestinal epithelial (IE) cells, enterochromaffin (EC) cells, and TRPA1-positive sensory neurons. As a result of TRPA1 stimulation, TRPA1 agonists stimulate IE cells to release ADM, EC cells to release 5-HT, and sensory neurons to release neuropeptides/neurotransmitters, respectively, resulting in physiological and biodefensive responses in vasodilatation, motility, secretion, and pain signaling.

4.8 Bone

Expression of AM and its receptor are seen in osteoblasts during the later stages of rodent embryogenesis and in maturing chondrocytes of fetal mice. AM stimulates the proliferation of fetal and adult rat osteoblasts. Also AM increases protein synthesis *in vitro* and mineralized bone area *in vivo*. Another study demonstrated that human osteoblasts secrete immunoreactive AM and that AM stimulates intracellular cAMP production in these cells, suggesting the role of endogenous AM as an autocrine or paracrine regulator of bone formation [32].

4.9 Immunity and inflammation

The epithelium provides a first line of defense against potentially pathogenic microorganisms. AM is produced by epithelial cells at mucosal surfaces, such as skin, oral cavity, and respiratory and gastrointestinal tract, and AM has antimicrobial properties against both Gram-positive and Gram-negative bacteria in these spots. AM has also been shown to stimulate proliferation and inhibit apoptosis of cultured immature rat thymocytes, suggesting AM may play a role in the development of immunity [33]. AM gene is expressed in peripheral blood monocytes and is rapidly upregulated during their transformation to macrophages. Some studies showed that AM suppresses the secretion of proinflammatory cytokines such as tumor necrosis factor- α in Swiss 3T3 cells, macrophage cell line, and rat Kupffer cells [34]. The anti-inflammatory effect of AM was also demonstrated in different *in vivo* models of inflammation [35].

5. Conclusion

Adrenomedullin has been shown to be virtually ubiquitous,

being produced by a great number of different cell types, in all tissues of the body, with the possible exception of the thyroid and thymus. Comparison of plasma adrenomedullin concentrations with its receptor affinity clearly suggests that adrenomedullin is not a conventional hormone. Plasma adrenomedullin is increased in a variety of pathological conditions, usually; it appears, as a compensatory response to cardiovascular changes. The one situation where plasma adrenomedullin concentrations may reach those levels required for receptor activation is in septic shock, where adrenomedullin may cause cardiovascular change. It may be predicted that the application of knockout technology to the adrenomedullin gene may influence every tissue in the body. It may also be necessary to apply modifications of the technology to distinguish adrenomedullin from other Adrenomedullin gene products, such as proadrenomedullin peptide.

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