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Adrenomedullin assay and its clinical significance

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Abstract. – Adrenomedullin (Am) is a recently discovered peptide, first purified from pheochromocytoma specimens, with a chemical structure similar to that of CGRP and amylin. Adrenomedullin is present in numerous human body tissues and its powerful vasodilatatory activity is thought to play an essential role in cardiovascular and renal homeostasis..

Key Words:

Adrenomedullin, Diabetes, Arterial hypertension, Shock, Bronchial asthma, Liver cirrhosis.

Introduction

In 1993, Kitamura and Kangawa[1] isolated from human pheochromocytoma tissue a new peptide with potent vasodilatatory and natriuretic activity. The isolated substance presented a strong hypotensive action and was successively demonstrated to be present also in normal adrenal medullary cells. The isolated peptide was thus given the name of adrenomedullin (Am).

Biochemistry

The gene that codifies for adrenomedullin is located on chromosome 11 and consists of 4 exons and 3 introns. The fourth exon codifies for adrenomedullin, while the second and the third exons codify for a substance named PAMP[9]. Adrenomedullin messanger RNA (mRNA) generates a 185 aminoacid precursor named *preproadrenomedullin*. The first 21 aminoacid residuum forms a peptide signal, while the other 164 residuum represents a non active precursor named *proadrenomedullin*. A proteolytic reaction gives place to two peptides: PAMP (*proadrenomedullin N-terminal 20 peptide*) corresponding to the 20 aminoacid residue of the N-terminal region and adrenomedullin corresponding to the 52 aminoacid residue of the C-terminal region. Structural analogy and biological activity have lead to classification of adrenomedullin as a member of the peptide superfamily of calcitonin, *calcitonin gene-related peptide* (CGRP) I and II, and amylin[2,3]. All these peptides share 6 to 7 aminoacid residues that form a cyclic structure closed by a disulfuric bond and a C-terminal residue which is essential for receptor recognition. The cyclic structure is responsible for the biological activity of the single peptides. The elimination of the cyclic part of the chemical structure converts these peptides into receptor antagonists[4-8].

Receptors

Am and CGRP receptors are functionally correlated because of their cross-reactivity and their similar biological action.

Membrane Am receptor has been recently clonated. Am-receptor consists of seven transmembrane domains and belongs to G-protein dependent receptor superfamily[11]. The Am receptor was initially isolated from rat vascular smooth muscle cells (VSMC)[12], but has successively been identified in human endothelial cells[13], in rat's astrocyte cells[14], in rat's heart and lungs[15]. This receptor is also present in many human lung, breast, brain, ovary, colon and prostate tumoral cell lines[16].

Interaction of Am with Am-receptor determines vasodilatation through direct and indirect mechanisms. The direct mechanism acti-

vates cellullar adenyl-cyclase and increases intracellular cyclic AMP levels[17,18], 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 preceeded 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[20]. This increased espression of C-fos mRNA in cardiac myocytes and fibroblasts could suggest that these cells are the genomic target of Am.

Production sites and localization

Immunofluorescence assays utilizing radioactively labeled antibodies directed against the 3-12 portion of the 1-52 N-terminal segment of Am[21] have allowed to localize Am in human tissues.

Immunofluorescence assay has demonstrated that adrenomedullin is present in elevated concentrations in human pheochromocytoma tissue but also in normal adrenomedullary tissue[22]. Adrenomedullin has been identified also in the cardiac atrium and ventriculum, aorta, kidney, bowel, pancreas, spleen, cerebral cortex, thyroid gland[22] and in small concentrations in the hypothalamus, thalamus and pituitary gland[22-25].

High concentrations of Am have also been identified in different lung cells lines: epithelial bronchial cells lining, parasympathetic neurons, endothelial cells, chondrocytes, alveolar macrophages and smooth muscular cells[26].

Eventhough Am is present in numerous tissues, few cells seem to be able to synthetize the substance. The only cells in which, up to date, it has been possible to identify Am mRNA expression are endothelial[27], VSMC[28], renal tubular[29], myocardial ventricular[30] and adrenomedullary cells.

It seems that endothelial cells are the most important source of Am production. The production rate of Am in endothelial cells has been documented to exceed twentyfold that of adrenomedullary cells[27,31].

Eventhough, VSMC have a lower secretory capacity (about fivefold less) compared to that of endothelial cells, VSMC express Am gene fourfold more frequently than adrenomedullary cells[28].

Cardiac ventricular cells, lung and kidney cells[8] express the same quantity of Am mRNA as adrenal medullary cells, but have a lower concentration of the peptide. Probably these tissues release and metabolize the peptide at a faster rate[32].

Normal plasma levels of Am are 18.2 pg/mL[33], 7.8 pmol/L[34]. The peptide has also been measured in sweat and urine.

Adrenomedullin urinary concentration is sixfold plasma concetration, and this is easily explained by the considerable kidney production of the peptide[35].

Factors that stimulate and inhibit adrenomedullin synthesis and release

The important influence of Am on vascular tone has been studied by identifying stimulatory and inhibitory stimuli of Am secretion in tissue cultures of rat endothelial and smooth muscular cells. There is proof that the inflammatory cytokines Il-1, TNF-alfa and beta and LPS, chief mediators of septic shock, are potent inhibitors of Am synthesis and secretion by VSMC[28,36], and less by endothelial cells[37]. Thrombin instead is strong activator of Am and also of ET-1.

Several substances such as norepinephrine, isoproterenol, glucocorticoid and mineralocorticoid hormones, sexual and thyroid hormones elevate, though to a slight degree, Am secretion by endothelial cells[37]. Activation of the sympathetic nervous system undoubtly stimulate Am secretion as demonstrated by a positive correlation between norepinephrine and Am[34].

Glucocorticoid and thyroid hormones seem to act as stimulatory factors on Am secretion by VSMC[38].

Experiments conducted on endothelial cell cultures have shown that TGF-beta (beta tumor growth factor), known as the most important stimulatory factor for endothelin-1 (ET-1), behaves as a potent inhibitor of Am secretion[37]. CGRP and ET-1, to a lesser degree, have an inhibitory effect on Am secretion by endothelial cells[37].

Adrenomedullin is probably eliminated by the kidney. Infact, patients with renal failure present elevated plasma Am levels[39]. No evidence of Am hepatic clearance exists up to date.

Role of Am in cellular proliferation

Results of researches on the possible role of Am in cellular proliferation are controversial. Numerous studies have shown that Am can inhibit the growth[40] and the migration of VSMC[41], and on the other hand, well known studies have shown that Am behaves as a growth factor in numerous mammalian cells lines[42]. The recent discovery of the presence of Am and Am receptor in rat and mouse embryos and in placental trophoblasts opened a whole new field of investigation on the substances involved in development control and embryonal differentiation[43].

Activity of adrenomedullin

Cardiovascular system

Adrenomedullin determines vasodilatation of numerous vascular districts accompanied by elevation of cardiac output[1,44-47]. The administration of endovenous Am in normotensive rats causes a dose-dependent fall of arterial blood pressure and of peripheral resistances[1,48,49]. In humans with normal pressure intravenous administration of Am induces a significant fall of systolic and diastolic arterial blood pressure without a compensatory neurohumoral response[50].

Endothelial and VSM cells production and secretion of both Am and Am receptor has lead researchers to suggest that Am modulates vascular tone by an autocrine/paracrine mechanism[27].

Adrenomedullin acts as a local mediator of vascular homeostasis inducing vasodilatation by binding to Am receptor and inhibiting syntesis and secretion of the vasoconstrictor peptide endothelin-1[51]. This action is thus independent from the activation of adrenergic and/or cholinergic receptors[12]. Adrenomedullin would then represent a competitive factor for ET-1 in the regulation of vascular tone.

The function of Am on the heart has still to be understood. Am determines complex and apparently contradictory effects on the myocardium.

Animal studies demonstrate that the fall in vascular peripheral resistances induced by Am is accompanied by a rise in heart rate and a slight fall of sistolic ejection fraction. In this way cardiac output actually rises[46].

In the dog the intravasal injection of Am in the coronary arteries causes a reduction of the vascular resistance of these vessels[52].

Kidney

Numerous papers have demonstrated kidney production of Am[29]. The production of Am in the kidney suggests that this substance is one of the vasoactive peptide autocrine/paracrine family locally acting in the kidney essential for the intrarenal and general hemodynamic regulation[29].

Adrenomedullin determines a rise in glomerular filtration rate (GFR). This effect is thought to depend from the rise in glomerular hydrostatic pressure, due to the nitric oxide-dependent reduction of the afferent and efferent arteriolar resistances[53]. Some Authors suggest that Am also exercises a direct action of GFR[54,55].

Adrenomedullin acts on renal sodium metabolism elevating the sodium secretion and this effect is similar to that exercised on glomerular filtration rate and on renal plasma flow[56]. It has been demonstrated that Am also determines a reduction of fractional sodium readsorption in the distal tubules[56], and this may be partially explained by the vasodilatation of the post-glomerular artery[54].

Recent data have shown that acute and cronic sodium load in both hypertensive and normotensive subjects, reduces plasma renin levels and elevates ANP, but does not induce a variation of plasma Am levels[57].

Respiratory system

Animal studies have shown that Am plays an important role in respiratory function. When Am is administered by aerosol, it causes a significant dose-dependent inhibition of acetylcholine and histamine induced bronchial constriction and sustained bronchial dilatation. The mechanism underlying this bronchodilatatory effect must still be identified, but a smooth muscle cell receptor mediated response might be implicated[58].

Gastrointestinal tract

A subpopulation of enterochromaffin gastrointestinal tract cells containing serotonin coexpress Am[59].

Immunohistochemical investigations that identify Am have shown controversial results in the pancreatic tissue. The reason for these confusing data is the great structural homology among CGRP and YY peptide.

Initially Am was identified in peripherally located pancreatic cells[23], however later studies have not been able to confirm the results. In tissue culture pancreatic beta cells express Am and Am-receptor[59].

Endocrine system

We have limited knowledge of the precise physiologic function of Am in the hypothalamic-pituitary system. Adrenomedullin has been shown to inhibit ACTG release[60] and to stimulate the thirst response[61]. No action on aldosterone secretion has been identified[64]. In vitro researches on adrenal cells have shown a suppressive effect of this hormone[62,63].

Adrenomedullin in disease

Arterial hypertension

In these recent years many studies have been carried out to identify the relationship between Am and arterial hypertension. However, the data available from patients with essential hypertension are still controversial. While some Authors have not shown a significant difference of Am levels between hypertensive and normotensive patients[33], others report an elevation of Am levels in patients with hypertension[34].

Reduction of pressure values with antihypertensive therapy does not modify Am levels. Am levels seem to correlate directly, however, with creatinine levels[33].

Atherosclerosis

In the atherosclerotic plaque, macrophages produce TNF-alfa, which is a known stimulus for Am production. Adrenomedullin's action on vascular tone and its inhibitory effects on smooth muscle vascular cells may indicate a future use of this substance as an anti-atherosclerotic treatment[65].

Septic shock

Acute cardiocirculatory failure during septic shock could, among its many factors, also imply adrenomedullin. High Am levels have been measured in the lipopolisaccharide endotoxic shock animal model, and it has been suggested that the potent vasodilatory effect of the substance could contribute to shock pathogenesis[28].

Cardiac failure

Patients with cardiac failure show elevated adrenomedullin plasma levels that are directly correlated with severity of the disease[67]. In acute myocardial infarction, Am levels rise to a fivefold compared to normal values. In patients with MI complicated by cardiac failure, Am levels may rise even more significantly.

Elevation of Am levels, like that of ANP, in myocardial infarction and cardiac failure, could represent a compensatory mechanism to the excessive vasoconstriction that follows these pathological events. In this way cardiac function could be ameliorated through a modulation of vascular tone characterized particularly by reduction of the preload and the postload[68].

Renal failure

In renal failure Am levels are significantly elevated, probably due to a reduced renal clearance of the substance similarly to what takes place for other low weight polypeptides like insulin and parathormone[69-71].

Adrenomedullin clearance by dialytic treatment is irrelevant, and while hypertension is reduced after dialysis, Am levels remain elevated[72]. This elevation, however could be partially due to an higher post-dialysis cathecolamine release, causing elevation of Am levels in presence of reduced post-dialysis blood pressure[73,74].

Bronchial asthma

In hypoxic chronic obstructive pulmonary disease and bronchial asthma high plasma Am levels have been reported[68]. Adrenomedullin plasma levels are also higher during acute asthma attacks compared to stable asthma disease[75]. The cause of this elevation is not known. It seems possible that Am's bronchodilatatory action is a reaction to elevated cathecolamine levels present during asthma crisis.

Hepatic cirrhosis

We hold interest in focalizing on the possible relationship between the severe hemodynamic modifications induced by liver failure and Am. Adrenomedullin plasma levels rise

during hepatic cirrhosis and may be they could play a role in the pathogenesis of the altered haemodynamic and electrolyte conditions present in this disease[76,77].

Patient with chronic liver failure, and in particular those with ascites, present elevated values of Am but also of other vasodilatatory peptides such as ANP, CGRP, substance P and glucagon[78,79]. However, the insight into the responsability of each of these factors and their reciprocal relationship in liver cirrhosis has yet to be clarified.

Hyperthyroidism

Recent studies have shown a rise of plasma Am levels in thyrotoxicosis[80]. Plasma Am levels also show a direct correlation with serum levels of free fraction of T4[80]. Patients with Graves disease present circulating Am levels twofold those of normal subjects, similar to those of patients with severe cardiocirculatory failure[81]. Correction of the hyperthyroidism rapidly returns Am levels to normal[80]

The relationship between thyroid hormone and Am, however still needs an explanation. Adrenomedullin elevation during thyrotoxic state could be a consequence of the circulatory hyperdynamic state and of the high output cardiac failure[80]. The reduction of peripheral resistances and the relative reduction of diastolic pressure, typical of hyperthyroidism, could recognize in Am elevation one of their pathogenetic factors[80].

Diabetes

Adrenomedullin interferes with insulin secretion, but the mechanism of this interaction is still controversial. Some studies document that the supplementation of Am to rat pancreatic islets causes a dose-dependent reduction of insulin secretion[82]. Other studies that utilize the same experimental protocol, instead, document a significant stimulation of insulin secretion[60].

Vascular smooth muscle cells grown in culture medium containing elevated glucose levels show high quantities of Am mRNA[82].

The protocols of in vivo studies in animals subjected to OGTT demonstrated that intravenous administration of Am induces a fall in plasma insulin levels and a consequent rise in glycemia[83]. The same authors report diabetic patients to have above normal Am levels[82]. These data await to be confermed and interpreted in the light of further experiments.

Neoplasia

There are strong evidences of a role for Am in growth control of human neoplastic cells. Research conducted on lung, breast, ovary, colon and prostate neoplastic cells lines show cellular expression of Am and Am-receptor[16].

Also, human microcitoma, adenocarcinoma, bronchoalveolar carcinoma, squamous cell carcinoma, lung carcinoid, ganglioblastoma and neuroblastoma express Am but not Am-receptor[84-86].

The coexpression of Am and Am-receptor in numerous neoplastic cell populations sugests a role for Am and Am-receptor as an autocrine growth factor that promotes uncontrolled cellular replication in neoplastic cells[16]. This hypothesis is validated by the observation that tumoral growth may be blocked by anti-Am monoclonal antibodies.

A new hypotensive adrenomedullin precursor hormone: PAMP

Proadrenomedullin N-terminal 20 peptide (PAMP) presents in humans a distribution similar to that of Am. The hormone is present in adrenal medulla and cortex, in blood and in elevated quantities inside pheochromocytoma tissue[87,88]. Endovenous injection of PAMP in the anaesthetized rats shows a strong dose-dependent hypotensive effect that is consistently bigger than that of Am[89]. It seems that this hypotensive effect may be due not to direct vasodilatation, as in the case of Am, but to inhibition of cathecolamine secretion from sympathetic nervous terminations[90].

Proadrenomedullin N-terminal 20 peptide may be elevated in arterial hypertension and in cardiac failure[91]. Studies in the hypertensive rats show an higher concentration in the cardiac atria compared to that of normal animals. These data suggest that PAMP could play a role in myocardial protection consequent to arterial hypertension[91].

Similarly to Am, PAMP does not influence either basal or ACTH-dependent aldosterone secretion. However, PAMP can suppress angiotensin II and potassium stimulated aldosterone production. This last mechanism of action is much more strong than that seen with Am[64].

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