

THE APELIN-APJ SYSTEM IN THE EVOLUTION OF HEART FAILURE

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Abstract

Heart failure is a chronic, progressive disease in which the overexpression of biologically active molecules and neurohormonal activation are the key factors of the evolution and natural history. The apelin-APJ system is a newly discovered molecular pathway and the RAAS counterbalance is its principal effect. The apelin is a potent inotrope, vasodilator and diuretic with crucial cardioprotective effects against angiotensin and aldosterone injuries. Intense and prolonged RAAS induces the downregulation of the apelin and its receptor at myocardial level and cancels their protection. Compared to the vasoactive agents used in the treatment of acute heart failure, exogen apelin has unique inotropic and vasodilatory effects without deleterious consequences, being a promising therapeutic option.

Keywords: apelin, APJ receptor, heart failure, biomarker.

Introduction

Heart failure is a chronic, progressive disease affecting approximately 1-2% of all the adult population, increasing to more than 10% in the population segment over 70 years; its incidence rose with the extension of life-span and the aging of the population [1-3]. Its prognosis is poor, the mortality is higher than that of some cancers and the morbidity is marked by a low quality of life and frequent rehospitalizations. Death occurs in months to years from the onset, but in an unpredictable manner [4-10].

Its evolution is the result of the overexpression of a number of biologically active molecules with negative influence on the cardiovascular system, with chronic activation of signaling pathways secondary to inadequate tissue perfusion. The compensatory mechanisms described until now are the activation of the adrenergic and renin-angiotensin systems (RAAS) with the purpose of maintaining the cardiac output but with long-term deleterious effects [11]. Balancing these systems, there are other molecular pathways with key roles in the physiology of the cardiovascular system and which are now the principal subject of research in this field.

Physiological effect of apelin/APJ receptor pathway on the cardiovascular system

The gene of apelin is located on the long arm of X chromosome; the apelin is synthesized as preproapelin (77 aminoacids) which is then cleaved by an angiotensin-converting enzyme to shorter active fragments (C-terminal peptides: apelin-13, -16, -17, -19, -36). It was discovered in 1998 from bovine stomach extracts and named apelin: "APJ endogenous ligand". The most active of all is apelin-13, the shorter peptide, with its pyroglutamated form representing the principally biological active ligand; its activity is 8 and 60 folds higher than apelin-17 and apelin-36, respectively [12].

Apelin's receptor, APJ, identified in 1993 by homologous cloning in the Human Genome Project, was initially considered an orphan until the discovery of apelin in 1998 [13]. APJ has 31% sequence homology with angiotensin II type 1 receptor (AT1R) and shares its tissue distribution, but it cannot bind angiotensin II (Ang II) [14]. It is coupled with G_i protein, and probably G_q protein too [15,16], and it is desensitized differently by different fragments of apelin. The shorter fragments activate the receptor for a short period of time because it is rapidly internalized in the cellular membrane, returning to the surface within an hour and becoming available for

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activation again. In contrast, the longer fragments of apelin dissociate slowly from the receptor, blocking it in an active state for a longer period of time and lowering the recycling rate. At cellular level, the apelin activates the protein kinase B (Akt), phosphatidyl-inositol-3 kinase (PI3K) and the extracellular signal-regulated kinases (Erk) pathway, which are involved in controlling apoptosis and cellular migration and proliferation [17].

Apelin has complex functions as a potent inotrope, vasodilator and diuretic. The apelin and its receptor can be found in a variety of tissues (central nervous system, enterochromaffin-like gastric cells, pancreatic islet cells, osteoblasts, T-lymphocytes, adipose tissue), with higher concentrations in the lungs, cardiovascular system and spleen. It is synthesized locally in the endothelium and it is found in the cellular organelles as endoplasmic reticulum, Golgi apparatus and secretory vesicles [18,19]. Later studies showed that the most important site of apelin-APJ expression and action is the cardiovascular system, especially the vascular endothelium, vascular smooth muscle cells, endocardial endothelium, and in a lesser extent, the myocardium. This explains the higher concentration in the lungs and spleen, paralleling the level of vascularization [20-25].

In the atrial tissue the apelin concentration is 200 folds higher than in the ventricular tissue and it appears to be correlated to the plasma level. Although we do not know the exact source of the plasma apelin, it may be generated from the atrium [26].

In some other tissues as the kidney and adrenal gland it is expressed only in the blood vessels, although its effects on these tissues are complex and cover multiple sites [27]. Apelin is a diuretic, but its effects on the kidneys are not limited only to the renal tissue. Apelin is synthesized in the brain and inhibits the secretion of vasopressin from the neurons, favoring water excretion; also it acts on the renal microcirculation and probably on the tubular function to validate its diuretic effect [28-30].

The apelin/APJ system counter-regulates the RAAS by antagonizing the activity of Ang II, its major effector. Siddique et al. describe this mechanism as a result of direct, physical interaction between the APJ and ATR1 receptors, dimerization and sending ATR1 into a low affinity state, thus decreasing its interaction with Ang II. Apelin induces this physical interaction and increases the density of APJ as compared to ATR1 (by increasing its expression and its availability at the membrane level), but the density of both receptors were not modified by the exogenous Ang II in this study [31]. This may be the explanation for the development of cardiovascular diseases in the presence of dysfunctional apelin/APJ system that cannot balance the overactivated, injurious RAAS. Also, recent studies demonstrated a relationship between apelin/APJ system and the angiotensin converting enzyme type 2 (ACE2) which has a key role in counterbalancing the RAAS by

catalyzing the transformation of Ang II into angiotensin 1-7 (Ang 1-7), a molecule with opposing effects to Ang II [32,33]. These findings stand for the theory that apelin/APJ is a beneficial molecular pathway in the physiology of cardiovascular system.

The apelin/APJ system has many physiologic effects on water balance, glycemic control, nutritional behavior, immunity, but its principal target is the cardiovascular system. Latest studies have showed that apelin/APJ system has a key role in its normal function and in the development of vascular and heart diseases such as atherosclerosis, coronary heart disease, heart failure, systemic and pulmonary arterial hypertension and ischemic-reperfusion lesion [14].

Its action seems to be mainly autocrine and paracrine, the ligand expression paralleling the receptor expression, but its plasma concentration also corresponds to a circulating hormone. Plasma concentration is about 10^{-10} g/ml with a half time of less than 5 minutes; its slow, sustained, inotropic effect is validated in a subnanomolar concentration ($EC_{50}=33$ pmol/l), being the most potent endogenous inotropic molecule, overcoming the adrenomedulin and endothelin [34,35]. In the experimental studies conducted so far the effect reached its maximum in 20-30 min - completely different from β mimetics which act within seconds - and last longer; it was valid even when the NO production was blocked, and the endothelin, the Ang II and β receptors were inhibited [35].

The underlying mechanism for apelin's inotropy is the activation of phospholipase C, protein kinase C, Na^+H^+ sarcolemmal exchange (NHE) and Na^+Ca^{++} exchange (NCX) pathways, without involving the L-type Ca^{++} -channels and voltage-activated K^+ -channels [35] and without inducing myocardial hypertrophy [11]. Szokodi et al. describe that even after inhibition of both NHE and NCX, the inotropic effect of apelin continues in a proportion of about 40%; this fact suggests the presence of additional, unclear, mechanism for inotropism [35]. In the study of Dai et al. results showed that the inotropic effect of apelin is due mostly to an increase in Ca^{++} availability in the myocardial cell and not to a sensitization of myofilaments; the mechanism is not yet completely understood [36,37].

Apelin-APJ receptor interaction at the vascular level induces vasodilation through NO release from endothelial cells (in arteries and veins) with consequent decrease in systemic vascular resistance, lowering the pre- and the afterload of the left ventricle and improving its filling pattern [38]. The APJ receptor is present in the vascular smooth muscle and it stimulates its contraction as was demonstrated in experimental condition on endothelial denuded vein [39]. The vasoconstriction induced by apelin on denuded vessels and the vasodilation produced in the presence of normal functioning endothelium suggest the complexity of apelin's role on the circulatory system, the fine tuning of the vascular tone and the easily disturbed

apelin's effects in the presence of injured endothelium.

The role of apelin/APJ system in heart failure

Experimental animal studies

The studies conducted so far showed the importance of apelin/APJ pathway in the development of heart failure and the fact that its downregulation in the myocardial cell contributes to the structural and functional alteration of the heart [40,41].

In experimental models of Iwanaga and colleagues (Dahl salt-sensitive rats with left ventricular hypertrophy and secondary heart failure), the cardiac apelin and APJ receptor were down-regulated in the LV dysfunction stage, associated with an up-regulation of ACE2 (the breakdown enzyme for apelin peptides), which may contribute to further lowering the plasma apelin, which is already downregulated. In their study, the rats were treated with angiotensin receptor blocker (ARB), metalloproteinase (MMP) inhibitor and a betablocker, and although there was functional improvement in all groups, only in the ARB group the apelin and APJ expression increased; this is a strong proof of the fact that the beneficial effects of ACEI/ARBs are based on the modulation of apelin. Further, the expression of apelin was downregulated after infusion of Ang II, both in the pressor and the subpressor dose, effect that was prevented with the AT1R blockade, sustaining furthermore the interrelation between the RAAS and the apelin/APJ system [42].

Similar results about the tissue expression of apelin and its mRNA were obtained by Wang and colleagues on dogs with heart failure, but levels of APJ (and its mRNA) were equivalent to normal dogs. This difference may be due to the characteristics of the species used in the experiments, but further investigation is needed [43]. Used as an exogenous vasoactive agent, apelin showed a unique combination of inotropic and vasodilatory properties of apelin, which leads to a rise of cardiac output and to a decrease of systemic vascular resistance without significant arterial hypotension and tachycardia, regarding the dose. This study brings additional proof about the importance of apelin and its receptor in the development of heart failure and the potential favorable effect of treatment with exogenous apelin in patients with systolic dysfunction, and it is backed-up by other, recent studies [44].

In a study on genetic apelin deficiency in mice (Apelin^{-/-}) the results led to the conclusions that apelin is a crucial peptide for maintaining the systolic function of the aging heart in the conditions of chronic pressure overload [45]. Szokodi et al also found low levels of apelin mRNA in rats' myocardium exposed to mechanical stretch and chronic pressure overload [35]. Wang and colleagues used similar experimental models in 2013 (apelin^{-y} and apelin^{+y}) and observed an aggravated postinfarction remodeling,

neovascularization and impaired functional recovery of apelin deficient mice. The lack of apelin compromises the activation of pro-survival Akt/PI3K and Erk pathway, both in vitro and in vivo, thus amplifying the myocardial damage and lowering the global cardiac performance [46]. More recent studies that used apelin analogues confirmed these results and demonstrated their capacity of lowering the myocardial ischemic/reperfusion injury in vivo [47].

Zhang et al. in 2013 experimented the effects of exogenous apelin on rats with myocardial infarction induced through left anterior artery (LAD) ligation; their results showed exquisite effects of apelin on microcirculation and angiogenesis by stimulating the migration of endothelial cells to ischemic regions, enhanced healing and new vessel formation. Also, apelin decreased the vascular permeability in the ischemic region with possible effects on postischemic edema and inflammation. These findings can be extrapolated to ischemic heart disease with consequent heart failure, suggesting the potential benefit of apelin treatment, both short and long term [48].

In 2012 a study using exogenous apelin on Dahl salt-sensitive rats with end-stage heart failure showed an improvement of LV dysfunction and remodeling, a lowering of the oxidative stress (through inhibition of NADPH oxidase), a regulation of the Akt/eNOS pathways and apelin/APJ expression in the rat hearts after apelin treatment. This study showed the correlation of apelin with the oxidative damage and gave the premise for the potential benefit of the apelin treatment in the end-stage heart failure [49].

Exogenous apelin showed benefit also upon the myocardial hypertrophy and fibrosis (secondary to increased afterload) through inhibition of fibroblasts TGF- β -induced differentiation into myofibroblasts and diminishing the fibrosis process. The already formed myocardial hypertrophy and fibrosis were reduced and the LV dysfunction was prevented [50].

The studies published so far bring evidence for down-regulation of apelin and its receptor during the process of myocardial remodeling (hypertensive or ischemic) with consequent reduction of its inotropic, antifibrotic and cytoprotective effects. The treatment with apelin restores the position of APJ within the membrane and revives the mechanism of inotropy, both in failing and normal hearts [41]. The exogenous apelin has benefic effects on the cardiac dysfunction secondary to other causes too, besides hypertensive, ischemic or idiopathic cardiomyopathy [51,52].

Human studies

In humans the studies conducted so far led to conflicting results: although some research showed no significant difference of apelin plasma level between IDC patients and normal controls, larger studies have showed that apelin rises in early, mild to moderate heart failure, and

lowers significantly in severe, end-stage heart failure [53]. Plasma apelin increases in early heart failure and lowers in the final stage but did not correlate with NT-proBNP and, with the information we have at this moment, it cannot be used as a diagnostic tool, nor as a prognostic marker [54-56]. Also, plasma apelin appears not to be correlated with age, sex, body mass index, etiology of heart failure and renal function [55].

In patients with heart failure plasma levels of apelin (from peripheral venous puncture) is reduced compared to controls, regarding of NYHA class and the severity of systolic dysfunction [55]. Chandrasekaran and colleagues measured the level of apelin in three different sites (coronary sinus, aorta and renal vein) in patients with heart failure (of different NYHA class) and controls. They found a significant reduction of apelin in the coronary sinus of heart failure patients as compared to controls and a reduction of apelin in the aorta of controls in comparison with the coronary sinus that was not seen in heart failure patients. Also, the heart failure patients had no apelin gradient between the three sites as was seen in control patients [57]. These findings stand for lowering of the apelin production in dysfunctional myocardium.

Left ventricular apelin mRNA increases in chronic heart failure secondary to ischemic heart disease or idiopathic dilated cardiomyopathy (IDC). Also, in IDC the APJ receptor mRNA is significantly decreased [27], although the plasma apelin was no different from the healthy controls as was demonstrated by another study [53].

In 2010 Piktin et al analyzed samples of human myocardial tissue collected from cardiac transplant recipients (for dilated cardiomyopathy and ischemic heart disease) and found extreme down-regulation of APJ in all samples with unchanged apelin levels, which may be the explanation for the progression of the cardiac failure, the apelin having no receptors to manifest its inotropic effect [26].

It appears that the mechanical stretch is one of the stimuli for down-regulation of APJ receptor, with secondary up-regulation after mechanical offloading of the left ventricle (after left ventricle assisting device implantation) in Chen et al. study in 2003 [54]. Cardiac resynchronization, another method of improving the mechanic and electric properties of a failing heart, had a good influence over the plasmatic apelin at nine months after implantation of the device [58]. It is already well-known that wall stress and activation of RAAS augment in heart failure and these pathways are feasible of altering the apelin-APJ expression. Also, the Ang II negatively influence the level of APJ in the cardiomyocytes [42].

Conclusions

Apelin/APJ is a crucial molecular pathway in the protection of the heart from the injuries caused by hemodynamic overload or structural damage, regardless of

the cause, and thus temporary preventing the development of heart failure.

Apelin is a counter-regulator of the RAAS and keeps its activation in a balance.

Intense and prolonged RAAS activation disable the apelin cardioprotection and puts the system into withdrawal. Ang II is a strong mediator of apelin downregulation in myocardial tissue.

Experimental administration of apelin in heart failure showed unique combination of inotropism and vasodilation effects with no short-term deleterious effects. This is the premise for the potential benefit of apelin in heart failure, but further studies are needed.

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