The Apelinergic System: The Role Played in Human Physiology and Pathology and Potential Therapeutic Applications

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Apelin is a recently discovered peptide, identified as an endogenous ligand of receptor APJ. Apelin and receptor APJ are expressed in a wide variety of tissues including heart, brain, kidneys and lungs. Their interaction may have relevant pathophysiologic effects in those tissues. In fact, the last decade has been rich in illustrating the possible roles played by apelin in human physiology, namely as a regulating peptide of cardiovascular, hypothalamus-hypophysis, gastrointestinal, and immune systems. The possible involvement of apelin in the pathogenesis of high prevalence conditions and comorbidities – such as hypertension, heart failure, and Diabetes Mellitus Type 2 (T2DM) – rank it as a likely therapeutic target to be investigated in the future. The present paper is an overview of apelin physiologic effects and presents the possible role played by this peptide in the pathogenesis of a number of conditions as well as the therapeutic implications that might, therefore, be investigated.

Introduction

In 1993, O'Dowd et al[1] identified a gene with closest identity to the angiotensin II type 1 receptor (AT-1). This receptor – APJ – was kept "orphan" until 1998, when Tatemoto et al[2] identified a selective endogenous ligand, named apelin. The first studies have demonstrated that apelin and its receptor are expressed practically ubiquitously, thus acquiring special status at organs such as heart, brain, kidneys, and lungs[3-6].

The apelinergic system distribution over such variety of tissues has suggested it might play relevant roles in human physiology. Indeed, apelin is involved in the regulation of cardiovascular, gastrointestinal, and immune functions, as well as in bone physiology, fluid homeostasis and cardiovascular system embryonal development[7-9]. Recently, the apelinergic system has been demonstrated to be involved in the pathogenesis of a number of high prevalence conditions – such as hypertension, heart failure (HF), obesity, glucose intolerance and diabetes mellitus type 2 (T2DM), as well as HIV infections, diabetes insipidus, gastric ulcer and osteoporosis[7-9]. Therefore, it is a growing consensus that the modulation of the apelin/APJ binomial may be a therapeutic target to be investigated in the future[10,11].

With that in mind, the purpose of the present paper is to review the apelinergic system and provide an overview of its physiologic and pathologic involvement, so that a better understanding of the apelin/APJ binomial may lead to new investigations as well as to possible future clinical applications.

Key words

Peptides/Physiology; peptides/drug effects; apeline; hypertension; cardiac output, low; diabetes mellitus.

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APJ receptor

In 1993, O'Dowd et al[1] characterized a 700 base pair fragment by using polymerase chain reaction (PCR). A detailed analysis revealed a number of similarities with transmembrane domains gene sequences of GPCRs (G protein-coupled membrane receptors). The codified protein had 380 aminoacids and was named APJ.

High homology between APJ receptor and AT-1 receptor was soon identified: 115 aminoacids (AA) (30%) of the total sequence and 86 AA (54%) in transmembrane regions[1], as well as high similarity in the tissue expression of both receptors[3]. However, apelin, an endogenous ligand of the APJ receptor, does not bind to AT-1 receptor[12], nor does angiotensin II (AngII) binds to APJ[1,2].

Although predominantly located in the membrane, APJ Receptor was also found in the nucleus of cerebellum and human hypothalamus cells – a nuclear localization signal was identified in the third intracellular loop[13]. These data suggest that this receptor may play a role in gene transcription regulation, as previously shown for AngII[10] – an aspect that should be a topic for investigation in the future.

After studying APJ receptor distribution in rats, mice, and humans, the conclusion was that it is abundantly expressed in the central nervous system (CNS), as well as in a number of peripheral tissues, especially in lung and heart[1,3-5,14]. A number of studies have shown that in the cardiovascular system the APJ receptor is found in the endothelial cells of small intramyocardial, renal, pulmonary and adrenal vessels, in coronary arteries, in endocardial endothelium cells, and in vascular smooth muscle cells[5]. Interestingly enough, APJ immunoreactivity follows a transversally estriated pattern in cardiomyocytes, thus indicating the co-localization of receptor with T-tubules[5].

Apelinergic peptides

APJ receptor was kept orphan until 1998, when Tatemoto et al[2] isolated a 36-AA peptide from bovine stomach extracts,

which they named apelin. The peptide induced extracellular acidification and inhibited cAMP formation in a cell line of Chinese hamster ovaries that expressed the APJ receptor. The localization of the pre-proapelin codifying gene - a 77 AA pre-peptide – in humans is Xq25-26.1, with 1,726 base pairs that include 3 exons. Curiously enough, high homology was shown between the different species in pre-proapelin protein sequence, namely in the 23 AA in terminal C[12]. Indeed, 65-77 C-terminal fragment of apelinergic peptides is key to bind to APJ receptor and to its biological activity, whereas N-terminal plays a key role in modulating ligand-receptor interaction[15].

The first studies on apelin identified different isoforms which are thought to exist *in vivo*: apelin-36 (apelin42-77), apelin-17 (apelin61-77), apelin-13 (apelin65-77) and apelin-13 in its pyroglutaminated form [(Pyr1)apelin-13], which means to say, with N-terminal glutamate residue[2]. While shorter isoforms seem to be more powerful in their cardiovascular action, apelin-36 is more efficient in blocking HIV infection in APJ-positive cells. It is currently thought that Apelin-36 acts as a precursor, with limited biological activity, up to the moment it is under proteolysis and post-translational changes, to then produce biologically more active peptides, predominantly (Pyr1)Apelin-13, whose pyroglutamination preserves biological function and prevents enzyme degradation[8]. Lee et al[16] have described a possible APJ receptor antagonist: by substituting terminal C phenylalanine in Apelin-13 with alanine residue, a peptide is created. In a dose-dependent mode, that peptide annihilates the hypotensive action of IV Apelin-13 in rats.

The early studies that characterized apelin tissue distribution were conducted in rats' tissues, and evidenced the presence of its pre-propeptide in a wide variety of tissues[3,4,6,12,17]. In humans, pre-proapelin RNAm is abundant in the CNS and in the placenta, as well as in more moderate volume in kidneys, heart, lungs and mammary gland. It should be pointed out that apelin is biologically active in the myocardium, in cardiac endothelium, and in the endothelium of large vessels and small veins and arteries[7]. In endothelial cells, apelin is not found in Weibel-Palade bodies, vesicles resulting from induced endothelial peptide secretion, thus suggesting apelin consitutive release[5].

An immunoenzyme assay estimated the concentration of circulating apelin in humans to be between 3 and 4 ng/mL[18]. This is a low concentration if compared to that of other circulating hormones. However, it is comparable to endothelium-derived vasoactive mediators, which suggests endothelial release of apelin.

It is currently thought that carboxypeptidase ACE2 (Angiotensin Converting Enzyme 2) plays the role of apelin degradation through highly efficacious cleavage of the terminal C phenylalanine from the apelinergic peptides[19]. Such correlation therefore suggests the high relevance of apelin in cardiovascular regulation since ACE2 is involved in the renin-angiotensin-aldosterone system[8]. Indeed, ACE2 is also involved in the degradation of Ang II to angiotensin 1-7, which, opposedly to Ang II, seems to play relevant functions in cardiac and renal protection[20]. Therefore, the induction of that enzyme may, in the future, be a therapeutic target in major cardiovascular pathologies.

Interaction apelin/APJ

One of the major transduction pathways for apelin signal depends on the interaction with a Gi-protein coupled to the APJ receptor, independently of Ras protein; although dependent on Protein Kinase C (PKC)[7]. Indeed, if PKC, phospholipase C (PLC), the Na^+-H^+ (NHE) exchanger and the Na^+-Ca^{2+}(NCX) exchanger are inhibited, apelin effects will be significantly reduced, namely in regard to its positive inotropic effect[21]. It should be pointed out that PKC activation results in NHE phosphorylation, which promotes inner cell alkalinization and sensitization of myofilaments to Ca^{2+}, as well as NCX activation, whose action is then reversed to promote Ca^{2+} entry into the cell (Figure 1). Hashimoto et al[22] have recently demonstrated that apelin induces phosphorylation of myosin light chain in vascular smooth muscle cells, which is of major relevance in initiating smooth muscle contraction. The inhibition of PKC, NHE and NCX is translated into a significant reduction of that effect.

In addition to adenyl cyclase inhibition pathway, apelin activates ERKs (extracellular regulated kinases) pathways through a PTX (pertussis toxin) sensitive G protein, in a

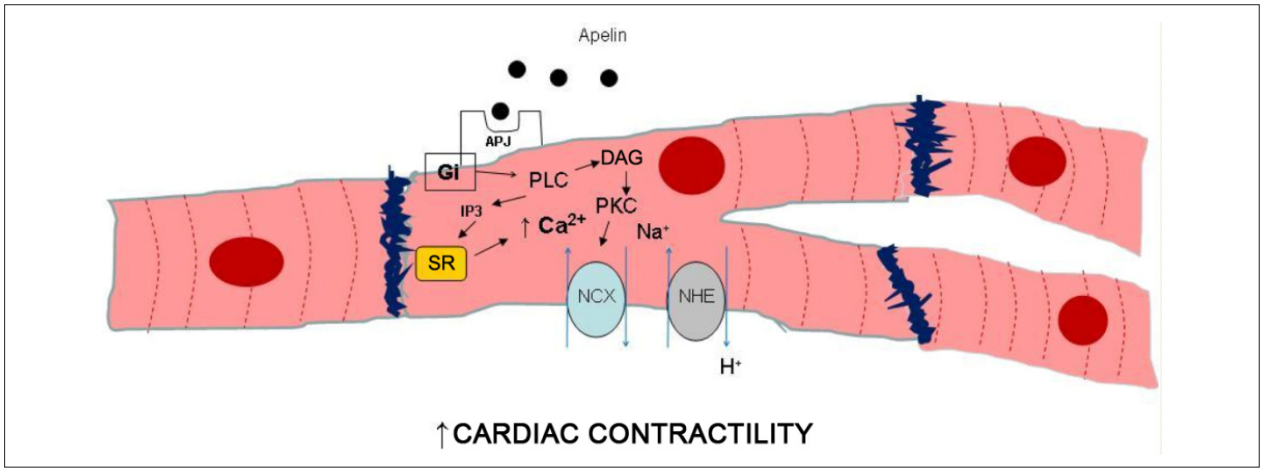

Figure 1 - Intracellular pathways responsible for positive inotropic effect of the apelin/APJ interaction. DAG - Diacylglycerol; Gi - Inhibitory G protein; IP3 - Inositol Triphosphate; NCX - Na+/Ca2+ Exchanger; NHE - Na+/H+ Exchanger; PKC - Protein Kinase C; PLC - Phospholipase C; SR - Sarcoplasmatic Reticulum.

PKC-dependent process[23]. PI3K-p70S6K endothelial cells proliferation control is also activated by apelin through two mechanisms: one is ERK-dependent and the other is PI3K-dependent[24]. It is important to point out that PI3K/Akt seems to be responsible for the phosphorilation and resulting activation of endothelial nitric oxide (eNOS) synthase, which is indispensable for most of apelin vasoactive effects[25].

Apelin/APJ: the role played in human physiology and pathology and potential therapeutic applications

Cardiovascular development and homeostasis

Apelin and its receptor are expressed in embryonal and adult tissues. As a result, they perform a wide action spectrum, starting at very early stages of cardiovascular development which goes on to adult life. Their contribution is increasingly evident in a number of pathophysiologic processes.

The high expression of APJ receptor in the endothelium of embryonal vessels and in the tunica intima of retinal vessels has already been demonstrated[14], as well as increased and reduced expression of that receptor in the formation and stabilization of retinal vessels, respectively[26].

Those data give stronger support to the idea that apelin plays a major role in endothelial cell proliferation – whether at embryonal development and physiologic states, or at pathological states (for instance, malignant neoplasms and diabetic retinopathy). Therefore, possible therapeutic applications may be envisaged for the future, such as the use of agonists to promote therapeutic angiogenesis (for instance, for ischemic conditions), as well as angiogenic antagonists, as a strategy to control tumoral growth and diabetic retinopathy.

Early studies on apelin and APJ receptor tissue distribution pointed towards high expression of their RNAm in the heart and in blood vessels of rats and humans[4,6,14,17,18]. APJ expression was especially high in rat's heart[15], while apelin was abundantly expressed in human endothelial cells of large conduction vessels[3]. More recently, APJ receptor RNAm was detected in the tunica media of large vessels[27], as well as immunoreactivity to apelin in endothelial cells of small resistance vessels[28].

In 2000, Lee et al[12] demonstrated for the first time that after IV infusion of apelin-13 in anesthetized rats, systolic and diastolic pressures reported temporary decrease (approximately 10mmHg). In the follow-up, another group of investigators demonstrated that the hypotensive effect was inversely correlated to apelin isoform molecular weight. That effect was absent after the administration of an NO synthase inhibitor (NOS)[25], which suggests that apelin vasoactive effects occur through an NO-dependent mechanism. Indeed, apelin causes endothelium-dependent vasodilation through the Akt activation that phosphorylates eNOS and promotes NO release as well as increased GMPc levels[25]. However, it may trigger a vasoconstrictive response if encountering a dysfunctional endothelium, in which case it will act on the vascular smooth muscle cells, which also express APJ receptor[27] (Figure 2).

In reference to cardiac effects, Szokodi et al[21] have demonstrated that in intact rat heart preparations, apelin-16 had a positive inotropic effect, increasing contractility with an approximately 30 pM EC_{50}, and maximum effect of approximately 70% response to isoprenalin (β-adrenergic agonist). *In vivo* experiments have confirmed those results through increased maximum pressure developed (P_{max}) and intraventricular pressure increase velocity (dP/dt_{max}), both in normal rats as in heart failure rats[29]. Hemodynamic studies in mice have demonstrated that the administration of apelin

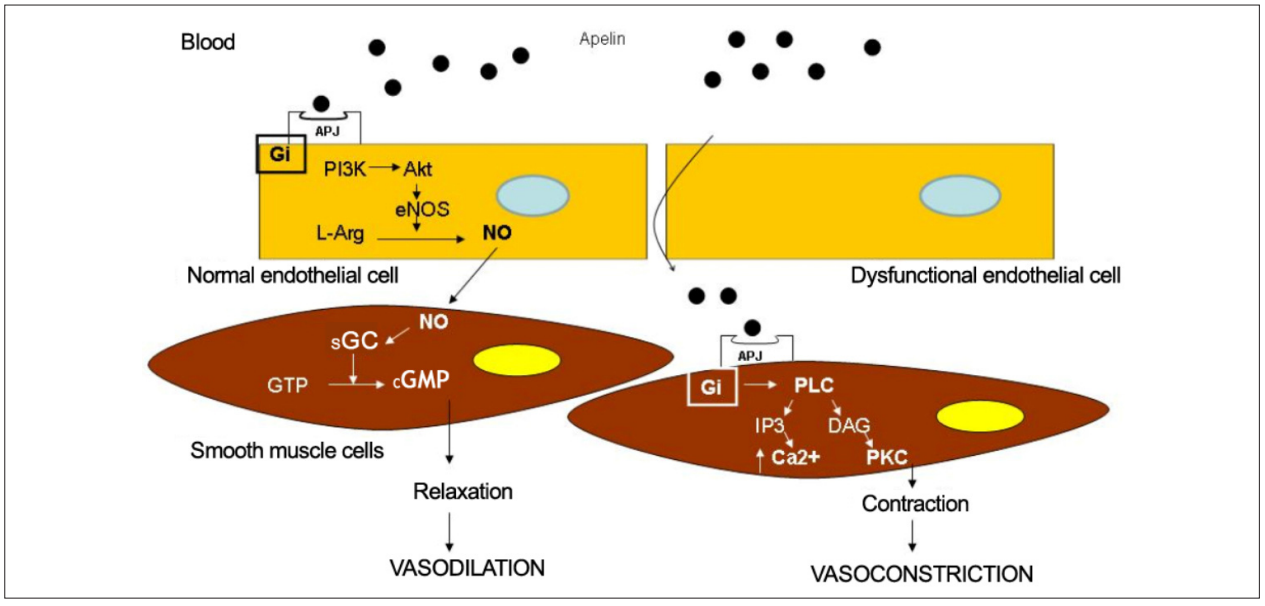

Figure 2 - Intracellular pathways responsible for the vasomotor effects in the apelin/APJ interaction in the absence and in the presence of endothelial dysfunction. DAG - Diacylglycerol; eNOS - Endothelial Nitric Oxide Synthase; Gi - Inhibitory G protein; sGC - Soluble Guanylate Cyclase; GTP - Guanosine triphosphate; cGMP - Cyclic Guanosine Monophosphate; IP3 - Inositol Triphosphate; L-Arg - L-Arginine; NCX - Na+/Ca2+ Exchanger; NHE - Na+/H+ Exchanger; NO - Nitric Oxide; PI3K - Phosphoinositide 3-kinase; PKC - Protein Kinase C; PLC - Phospholipase C.

reduces left ventricle preload and afterload and chronically increases cardiac output, with no evidence of hypertrophy[30].

In addition to confirming the *in vivo* positive inotropic effect, a study in our group demonstrated apelin negative inotropic effect in isolated cardiac muscle, suggesting other cells may be required – in addition to myocardial cells – so that positive inotropic effect is revealed. We have also demonstrated that apelin does not perform any evident function on myocardial diastolic properties[7].

The apelin/APJ pair is intrinsically related to the AngII/AT-1 pair in regulating the pathophysiology of cardiovascular function. *Knock-out* mice for APJ receptor present increased vasopressive response to Ang II. However, they present normal blood pressure levels[31]. In a major study by Iwanaga et al[32] involving HF rats (with reduced expression of apelin and APJ receptor), treatment with telmisartan – an antagonist of AT-1 receptors – made apelin/APJ levels reverse back to normal, thus suggesting that the efficacy of AT1 receptors inhibition in HF may also result from restoring normal values of apelin, a powerful positive inotropic endogenous agent. Therefore, direct regulation of the apelin/APJ pair by AngII/AT1 is left as a suggestion.

The demonstration that physical exercise promotes increased apelin and APJ receptor expression in the myocardium of hypertensive rats, as well as the change from a pathologic, ethiologically hypertensive hypertrophy into a physiologic hypertrophy from exercising[33] leads to the thought that physical exercise beneficial effect in reducing blood pressure levels in hypertensive individuals may be partially due to increased apelin/APJ expression at cardiovascular level.

In addition to its physiologic effects, apelin was also involved in the pathogenesis of HF. In the early studies, apelin circulating levels showed to be increased in recent onset HF patients, with progressive reduction accompanying worsening HF[34]. A wide-reaching populational study demonstrated that apelin serum concentration was reduced in patients presenting systolic HF due to left ventricular dysfunction[35]. Such decrease may result from the endothelial dysfunction associated to HF (thus affecting apelin production)[34] or from the exhaustion of a possible compensatory mechanism for proper cardiac output maintenance carried out by apelinergic system in HF patients. In 2006, Dai et al[36] demonstrated that apelin has a significant positive inotropic action on failing myocardium as a result of a transient increase of Ca^{2+}.

Recent studies have demonstrated that: 1) apelin therapy in rats with isoprenalin-induced lesions promotes the restoration of cardiac function, and is also cardioprotective, against lipidic peroxidation, for instance[37]; 2) apelin levels are reduced in coronary disease patients submitted to hemodialysis; it is speculated that apelin may be involved in the physiopathology of cardiovascular disease secondary to chronic renal failure, and that it may be utilized in the future as a treatment approach for uremic cardiomyopathy[38]; 3) ventricular resinchronization therapy promotes a long-term increase of apelin plasma levels[39], which may be associated to cardiac function improvement; 4) although it has been considered a possible marker for cardiac hypoxy (acute and chronic)[40, 41] apelin circulating levels do not seem to be of help for the clinical evaluation and prognosis of acute or chronic HF patients[35,42,43]; 5) apelin may be utilized as a marker to assess the development of isolated atrial fibrillation[44] as well as a diagnostic marker to distinguish the dyspnea from pulmonary causes from that of cardiovascular cause[45].

In regard to the last aspect, Ellinor et al[44] have demonstrated that apelin levels are reduced in patients with isolated atrial fibrillation, which suggests serum concentration of that peptide may be utilized as a risk index for the onset of that kind of arrhythmia in individuals with no manifestation. Interestingly, apelin-36 plasma levels are shown to be decreased in patients with pulmonary parenchyma chronic disease and preserved cardiac function. The determination of apelin-36 and proBNP (type B natriuretic peptide precursor) levels may, therefore, act as a new diagnostic method to distinguish dyspnea from pulmonary causes from that from cardiovascular cause[45].

Hypothalamus-hypophysis axis and fluid homeostasis

As described earlier, APJ receptor and apelin are expressed in different areas at the CNS, particularly intensely in hypothalamic supra-optical (SON) and paraventricular (PVN) nuclei[46,47]. Given the expression pattern, it was soon suggested that the apelinergic system could be involved in the modulation of the hypothalamus-hypophysis axis activity as well as in fluid homeostasis.

Indeed, as demonstrated earlier, apelin administration increases ACTH (adrenocorticotrophic hormone) and cortisone serum levels while decreasing prolactin, FSH (follicle stimulating hormone), and LH (luteinizing hormone) levels within 30 minutes after infusion. A recent study[48] has demonstrated that apelin is typically localized in corticotropic cells, promoting autocrine/paracrine ACTH release, which points towards the presence of an apelinergic system in adult rats' hypophysis.

Although APJ receptor and apelin high expression in vasopressinergic neurons has suggested a relevant role played in fluid homeostasis, great discrepancy has been shown in corresponding results since the administration of apelin to rats was associated to both water consumption reduction[46], and increase[12,49]. The absence of dipsic behavior effect in rats has also been described[50].

The unmasking of the association between apelin and body hydric homeostasis may allow the application of new therapeutic proposals associated to the apelin/APJ binomial for pathologies such as hypertension and diabetes insipidus.

Correlation with insulin, obesity and food intake

In 2005, Boucher et al[51] demonstrated that apelin was a new endocrine adipokine produced at mature adipocytes of mice and humans. Additionally, relevant correlations with insulin and obesity have been established. Plasma and adipocyte apelin levels increase was evident in obese mice with hyperinsulinemia, as well as reduced apelin secretion by the adipocytes of insulin-dependent rats reporting low insulin levels and when fasting. That secretion was quickly replaced after food intake, thus leading to the conclusion that insulin increases apelin expression. This relevant correlation with insulin became more interesting when APJ receptor was shown

to have expression in pancreatic islets, and when apelin was shown to inhibit glucose-dependent insulin secretion through direct action on mice β- cells[52].

Interestingly, a positive correlation could be observed between apelin serum levels and body mass index (BMI)[53]. It was also observed that apelin serum levels are increased in diabetic or glucose intolerance[54] patients, and that tumor necrosis factor-α (TNF-α) increases apelin levels in human adipocytes[55].

A most recent study on the pathophysiologic role played by apelin showed that its intraperitoneal administration in normal and obese mice for 14 days reduced body adiposity without affecting food intake, reduced insulin, leptin and triglycerides level, increased adiponectin levels, increased the expression of uncoupling proteins (UCP), and reduced respiratory quocient[56].

Apelin may also play a relevant role in regulating cardiovascular function in diabetes condition: diabetic mice treated with apelin reported increased vasodilating response to acetylcholine through PI3K/Akt/eNOS pathway[57].

All results seem to lead to the belief that apelin is a key regulator in a normal glucolipidic metabolism, also playing a possible role at pathologic scenarios such as obesity, insulin resistance and T2DM. Therefore, the use of apelin may be investigated as a potential therapeutic target for these pathologies.

As shown for fluid homeostasis, the role played by apelin in eating behavior is not yet well established, since data available are still scarce and contradictory[56,58,59]. Further studies are, therefore, required so that new conclusions on the role played by apelin in food intake can be reached.

Apelinergic system and new pathophysiologic implications

The apelinergic system actions described previously were the ones to draw closest attention on the part of medical community. However, there is an ongoing strong investigation task with the purpose of demonstrating new pathophysiologic implications of this newly found system.

Indeed, in regard to gastrointestinal function, the apelin/APJ binomial seems to perform relevant functions in gastric acid and cholecystokinin secretion, as well as epithelial cell proliferation in stomach mucosa[60], with possible involvement in the pathogenesis of peptic ulcer, ulcerous colitis[61] and Crohn's disease[61]. Apelin stimulates the proliferation of osteoblasts[62,63] and inhibits apoptosis[62,63]. In the future, it may be used as therapeutic support for some bone diseases. Finally, the APJ receptor is a co-receptor for the entry of HIV in host's cells[18,64]. Apelin was shown to inhibit the infection of CD4+ and APJ+ cells, with efficacy being higher in heavier isoforms[65-67].

Conclusion

The new roles played by the apelinergic system in human physiology and pathology are under continuing expansion. In fact, apelin is involved in the homeostasis regulation of major systems in our body, such as in the pathogenesis of a number of diseases, many of which accounting for extremely high levels of morbiditiy and mortality worldwide. There is a long way ahead, though. Further studies are required so that apelin and APJ receptor secrets can be revealed, thus expanding our knowledge on the functions performed by the apelinergic system in human physiology and pathology. Then, and only then, will the apelin/APJ system be properly considered as a future therapeutic target.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any graduation program.

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