

Original Article



Apelin: A promising therapeutic target? (Part 1)

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Abstract

Apelin is a recently discovered bioactive peptide, known to be an endogenous high-affinity ligand for the previously orphan G protein-coupled receptor APJ. Apelin/APJ as a novel signaling pathway has been shown to play many crucial roles in cardiovascular function, blood pressure regulation, fluid homeostasis, feeding behavior, obesity, type 2 diabetes mellitus, adipoinular axis regulation, cell proliferation, angiogenesis, neuroprotection and thermoregulation. This ubiquitous peptide opens a new field of research in biology and medicine. In this regard, the aim of this short review is to compile the evidence for the apelin involvement in modulation of cardiovascular system and introduction of this new peptidic pathway as a useful drug target in the treatment of cardiovascular diseases in future.

Keywords: Apelin, APJ, G-protein Coupled Receptor, Heart Failure, Nitric Oxide, Hypertension, Atherosclerosis

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Introduction

Angiotensin receptor-like 1 (APJ) is a member of G protein-coupled receptors, identified in 1993 (O'Dowd et al., 1993). APJ remained an orphan receptor until its endogenous multifunctional ligand apelin (APJ endogenous ligand) was purified from bovine stomach extracts in 1998 (Tatemoto et al., 1998). Apelin/APJ system is a novel signaling pathway with different physiological functions. This signaling system also provide a potential drug target for developing new therapies.

Synthesis

Apelin

The apelin gene is located on X chromosome at Xq25-q26.3, encoding a 77 amino acid prepropeptide, followed by an enzymatic cleavage (unknown endopeptidases). The peptide is broken to fragments of different sizes e.g. apelin-36, apelin-17, apelin-13 and apelin-12. The shorter isoforms possess greater binding affinity and biological potency (Habata et al., 1999; Hosoya et al., 2000; Kawamata et al., 2001). The only apelin degradation pathway identified to date is angiotensin-converting enzyme type 2 pathway (Japp & Newby, 2008; Masri et al., 2005).

APJ receptor

The APJ gene is mapped to chromosome 11 at 11q12, encoding a 377 amino acid G protein-coupled receptor, with seven transmembrane domains. Apelin is the only identified ligand of this receptor. Despite sharing a considerable sequence similarity with AT1 (angiotensin II type 1), this novel receptor did not display a significant binding affinity to angiotensin II, thereby has remained as an orphan receptor until 1998, when apelin was identified (Calebiro et al., 2010; Castan-Laurell et al., 2011; O'Dowd et al., 1993; Tatemoto et al., 1998). APJ couples through Gi/o and probably Gq (Klein & Davenport, 2005; Wettschureck &

Offermanns, 2005).

Apelin/APJ tissue localization

Apelin/APJ system is widely represented in both central nervous system (e.g. cerebral cortex, hypothalamus, hippocampus and pituitary gland) and peripheral tissues including heart, liver, kidney, testis, ovary, mammary glands, lung, gastric mucosa, human vasculature (endothelial cells and vascular smooth muscle cells of large conduit vessels, small arteries and veins), pancreatic islet cells, osteoblasts, T-lymphocytes and adipose tissue. Apelin is present in the endoplasmic reticulum, Golgi apparatus and secretory vesicles (intracellular localization), (Falcão-Pires & Leite-Moreira, 2005; Klein & Davenport, 2005; Klein & Davenport, 2004).

Apelin system as a possible drug target

Cardiovascular system

Vascular effects

Apelin and APJ are expressed in the vessels (Carpéné et al., 2007). The first evidence for this notion is a rapid and transient reduction in mean arterial pressure after apelin injection in rats. While this result was observed in many other studies on rodents, in an ovine model, a biphasic haemodynamic response was observed at equivalent doses used in rodents (Charles et al., 2006; El Messari et al., 2004; Lee et al., 2000). The hypotensive effect was abolished by co-administration of a non-selective NOS (nitric oxide synthase) inhibitor L-NAME (L-NG-Nitroarginine methyl ester) which suggests a nitric oxide (NO)-mediated arterial vasodilatation (Tatemoto et al., 2001). Apelin also promotes activation of endothelial NOS (eNOS) and increases the release of NO which results in an increased level of cGMP (cyclic guanosin monophosphate), ultimately resulting in relaxation of vascular musculature (Falcão-Pires & Leite-Moreira, 2005;

Ishida et al., 2004). Apelin directly activates L-arginine/NOS/NO pathway in vascular system, which may be an important mechanism in regulation of vascular function (Jia et al., 2007). Apelin can function as an arterial and venous dilator. Some studies have shown that apelin is a more effective venodilator than either hydralazine or nitrates are (Ashley et al., 2005; Japp & Newby, 2008). Apelin vascular functions extend beyond activation of eNOS.

Data indicate that apelin peptides may act directly on vascular smooth muscle APJ receptors and induce vasoconstriction. However, in the presence of a functioning endothelium NO production through endothelial APJ receptor outweighs this effect. This notion implies that apelin functions as both an endothelium-dependent vasodilator and an endothelium-independent vasoconstrictor (Japp & Newby, 2008; Maguire et al., 2009), (Fig. 1).

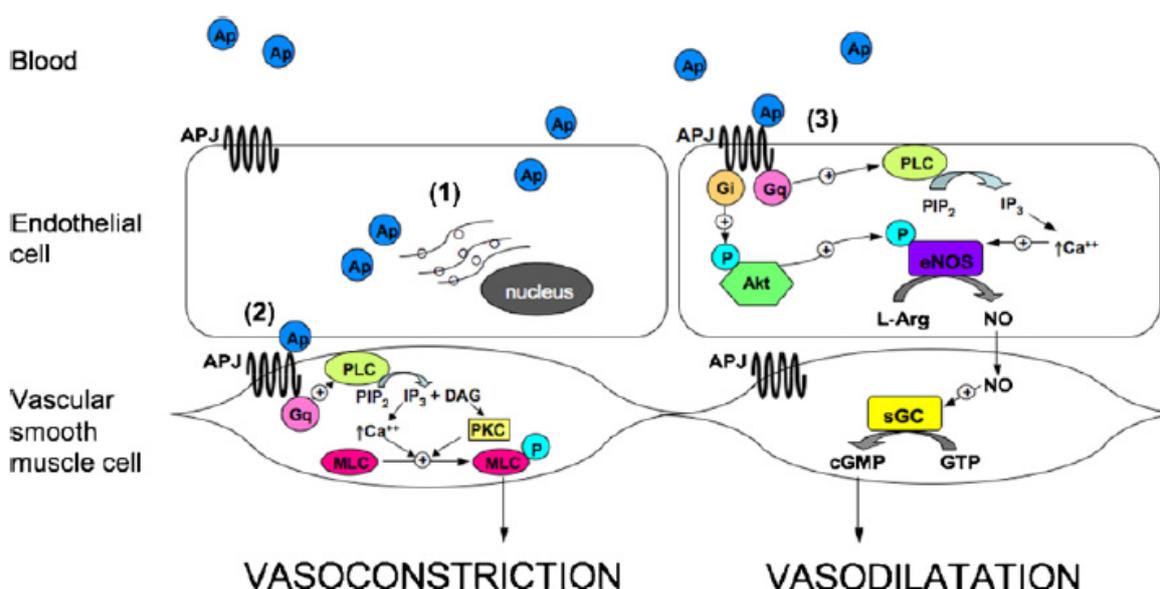


Figure 1: Hypothesised mechanisms of apelin in vascular tone regulation.

[1] Apelin peptide is synthesised in endothelial cells then transported to luminal and basolateral cell membranes. [2] Apelin directly activates APJ receptors on vascular smooth muscle cells leading to vasoconstriction. [3] Apelin activates endothelial APJ receptors and results in NO release; NO then diffuses into smooth muscle cells and results in vasodilatation. In the presence of intact endothelium the net effect is vasodilatation. Ap, apelin; P, phosphate; Gi, inhibitory G protein; Gq, Gq protein; PLC, phospholipase C; PKC, protein kinase C; IP₃, inositol-3,4,5-trisphosphate; DAG, diacylglycerol; NO, nitric oxide; sGC, soluble guanylate cyclase; eNOS, endothelial nitric oxide synthase; PIP₂, phosphatidylinositol bisphosphate; MLC, myosin light chain; L-Arg, L-Arginine; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate (Japp & Newby, 2008).

Cardiac effects

Apelin peptide is one of the most important regulators of cardiac function, exhibiting direct myocardial effects. Apelin has been shown to have a role in cardiac contractility modulation (Maguire et al., 2009); apelin increases contractility in isolated rat hearts (Szokodi et al., 2002). The studies showed a positive inotropic

effect for apelin peptide (Szokodi et al., 2002). A possible mechanism for apelin-mediated inotropy includes the involvement of sacrolemmal Na⁺/H⁺ exchanger, probably via a phospholipase C and protein kinase C dependent pathway, leading to an increased level of intracellular Ca²⁺ as well as sensitization of myo-

filaments to Ca^{2+} (resembles levosimendan). These events ultimately leads to an increased intracellular pH and stimulation of the reverse $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Farkasfalvi et al., 2007; Japp & Newby, 2008; Kentish, 1999), (Fig. 2).

These data indicate the potential involvement of apelin/APJ system in some cardiovascular diseases, which opens a promising avenue for discovering new therapeutic agents.

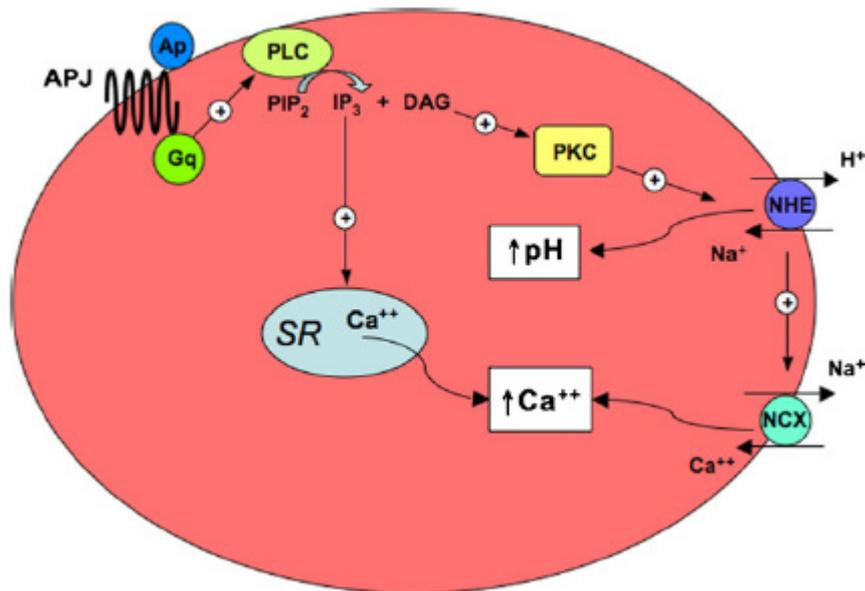


Figure 2: The possible mechanisms in apelin-mediated positive inotropy.

Ap, apelin; Gq, Gq protein; PLC, phospholipase C; SR, sacroplasmic reticulum; NHE, Na^+/H^+ exchanger; NCX, reverse $\text{Na}^+/\text{Ca}^{2+}$ exchanger; APJ, APJ receptor; PKC, protein kinase C; PIP_2 , phosphatidylinositol bisphosphate; IP_3 , inositol 1,4,5-trisphosphate; DAG, diacylglycerol (Japp & Newby, 2008).

Cardiovascular diseases and apelin/APJ axis

Apelinergetic system is considered as a novel target in heart failure (HF), (Japp & Newby, 2008). Studies suggest that apelin may help prevention of left ventricular systolic dysfunction (LVSD) and the onset of HF. Secretion of this peptide will slow down the adverse left ventricular remodeling process via maintaining the cardiac output and afterload reduction. In addition, apelin may prevent excessive fluid retention due to an effect on central vasopressin release (Chandrasekaran et al., 2010). It is evident that apelin peptide improves cardiac function and lowers the blood pressure in the chronic two-kidney-one clip (2K1C) hypertension model in rats. This promises novel treatment strategies for cardiovascular com-

plications of chronic reno-vascular conditions (Najafipour et al., 2012). Studies demonstrated that apelin level increases significantly in human atherosclerotic coronary artery and atherosclerotic plaque which may exert both beneficial and detrimental effects (Hashimoto et al., 2007; Kojima et al., 2010; Pitkin et al., 2010). On the one hand, apelin promotes smooth muscle cell proliferation and migration in to the neointima, mediates oxidative stress in vascular tissues, and when present in high levels may contribute to atherogenesis (Hashimoto et al., 2007; Li et al., 2008; Pitkin et al., 2010). On the other hand, apelin limits atherosclerotic progression by inhibiting the effects of angiotensin II on the vasculature and

also decreases macrophage infiltration (Chun et al., 2008; Leeper et al., 2009; Pitkin et al., 2010). In addition, apelin/APJ system shows protective effects in ischemic heart disease. Evidence show that apelin improves cardiac dysfunction after myocardial ischemia/reperfusion injury by suppressing the apoptosis and resisting oxidation effects. These protective effects are mediated by up-regulation of eNOS and phosphorylating Phosphatidylinositol 3-kinases (PI3K)-Akt and ERK1/2 (extracellular-signal-regulated kinases) pathways, indicating the system as an important therapy target (Simpkin et al., 2007; Zeng et al., 2009). Furthermore, apelin may be considered as a novel diagnostic plasma marker to distinguish pulmonary causes of dyspnea from its cardiovascular causes (Goetze et al., 2006).

Conclusion

In summary, apelin as a mediator peptide possessing many regulatory roles throughout the body. Apelin wide distribution suggests both autocrine and paracrine functions for this peptide. Studies have shown the potential value of this axis in the treatment of several conditions. The pathway of apelin/APJ is an interesting target in designing therapeutic agents. However, more clinical investigations are required to gain insight into the safety of apelin administration in human and clarify the contribution of this peptide to different diseases.

References

- Ashley EA, Powers J, Chen M, et al. The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. *Cardiovasc Res.* 2005; 65(1):73-82.
- Calebiro D, Nikolaev VO, Persani L, Lohse MJ. Signaling by internalized G-protein-coupled receptors. *Trends Pharmacol Sci.* 2010; 31(5):221-8.
- Carpéné C, Dray C, Attané C, et al. Expanding role for the apelin/APJ system in physiopathology. *J Physiol Biochem.* 2007; 63(4):359-73.
- Castan-Laurell I, Dray C, Attané C, Duparc T, Knauf C, Valet P. Apelin, diabetes, and obesity. *Endocrine.* 2011; 40(1):1-9.
- Chandrasekaran B, Kalra PR, Donovan J, Hooper J, Clague JR, McDonagh TA. Myocardial apelin production is reduced in humans with left ventricular systolic dysfunction. *J Card Fail.* 2010; 16(7):556-61.
- Charles CJ, Rademaker MT, Richards AM. Apelin-13 induces a biphasic haemodynamic response and hormonal activation in normal conscious sheep. *J Endocrinol.* 2006; 189(3):701-10.
- Chun HJ, Ali ZA, Kojima Y, et al. Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. *J Clin Invest.* 2008; 118(10):3343-54.
- El Messari S, Iturrioz X, Fassot C, De Mota N, Roesch D, Llorens-Cortes C. Functional dissociation of apelin receptor signaling and endocytosis: implications for the effects of apelin on arterial blood pressure. *J Neurochem.* 2004; 90(6):1290-301.
- Falcão-Pires I, Leite-Moreira AF. Apelin: a novel neurohumoral modulator of the cardiovascular system. Pathophysiologic importance and potential use as a therapeutic target. *Rev Port Cardiol.* 2005; 24(10):1263-76.
- Farkasfalvi K, Stagg MA, Coppens SR, et al. Direct effects of apelin on cardiomyocyte contractility and electrophysiology. *Biochem Biophys Res Commun.* 2007; 357(4):889-95.
- Goetze JP, Rehfeld JF, Carlsen J, et al. Apelin: a new plasma marker of cardiopulmonary disease. *Regul Pept.* 2006; 133(1-3):134-8.
- Habata Y, Fujii R, Hosoya M, et al. Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. *Biochim Biophys Acta.* 1999; 1452(1):25-35.
- Hashimoto T, Kihara M, Imai N, et al. Requirement of apelin-apelin receptor system for oxidative stress-linked atherosclerosis. *Am J Pathol.* 2007; 171(5):1705-12.
- Hosoya M, Kawamata Y, Fukusumi S, et al. Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J Biol Chem.* 2000; 275(28):21061-7.

- Ishida J, Hashimoto T, Hashimoto Y, et al. Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. *J Biol Chem.* 2004; 279(25):26274-9.
- Japp AG, Newby DE. The apelin-APJ system in heart failure: pathophysiologic relevance and therapeutic potential. *Biochem Pharmacol.* 2008; 75(10):1882-92.
- Jia YX, Lu ZF, Zhang J, et al. Apelin activates L-arginine/nitric oxide synthase/nitric oxide pathway in rat aortas. *Peptides.* 2007; 28(10):2023-9.
- Kawamata Y, Habata Y, Fukusumi S, et al. Molecular properties of apelin: tissue distribution and receptor binding. *Biochim Biophys Acta.* 2001; 1538(2-3):162-71.
- Kentish JC. A role for the sarcolemmal Na(+)/H(+) exchanger in the slow force response to myocardial stretch. *Circ Res.* 1999; 85(8):658-60.
- Kleinz MJ, Davenport AP. Emerging roles of apelin in biology and medicine. *Pharmacol Ther.* 2005; 107(2):198-211.
- Kleinz MJ, Davenport AP. Immunocytochemical localization of the endogenous vasoactive peptide apelin to human vascular and endocardial endothelial cells. *Regul Pept.* 2004; 118(3):119-25.
- Kojima Y, Kundu RK, Cox CM, et al. Upregulation of the apelin-APJ pathway promotes neointima formation in the carotid ligation model in mouse. *Cardiovasc Res.* 2010; 87(1):156-65.
- Lee DK, Cheng R, Nguyen T, et al. Characterization of apelin, the ligand for the APJ receptor. *J Neurochem.* 2000; 74(1):34-41.
- Leeper NJ, Tedesco MM, Kojima Y, et al. Apelin prevents aortic aneurysm formation by inhibiting macrophage inflammation. *Am J Physiol Heart Circ Physiol.* 2009; 296(5):H1329-35.
- Li F, Li L, Qin X, et al. Apelin-induced vascular smooth muscle cell proliferation: the regulation of cyclin D1. *Front Biosci.* 2008; 13:3786-92.
- Maguire JJ, Kleinz MJ, Pitkin SL, Davenport AP. [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. *Hypertension.* 2009; 54(3):598-604.
- Masri B, Knibiehler B, Audigier Y. Apelin signalling: a promising pathway from cloning to pharmacology. *Cell Signal.* 2005; 17(4):415-26.
- Najafipour H, Soltani Hekmat A, Nekooian AA, Esmaeili-Mahani S. Apelin receptor expression in ischemic and non- ischemic kidneys and cardiovascular responses to apelin in chronic two-kidney-one-clip hypertension in rats. *Regul Pept.* 2012; 178(1-3):43-50.
- O'Dowd BF, Heiber M, Chan A, et al. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene.* 1993; 136 (1-2):355-60.
- Pitkin SL, Maguire JJ, Kuc RE, Davenport AP. Modulation of the apelin/APJ system in heart failure and atherosclerosis in man. *Br J Pharmacol.* 2010; 160(7):1785-95.
- Simpkin JC, Yellon DM, Davidson SM, Lim SY, Wynne AM, Smith CC. Apelin-13 and apelin-36 exhibit direct cardioprotective activity against ischemia-reperfusion injury. *Basic Res Cardiol.* 2007; 102(6):518-28.
- Szokodi I, Tavi P, Földes G, et al. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ Res.* 2002; 91(5):434-40.
- Tatemoto K, Hosoya M, Habata Y, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Bioch Biophys Res Commun.* 1998; 251(2): 471-6.
- Tatemoto K, Takayama K, Zou MX, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul Pept.* 2001; 99(2-3):87-92.
- Wettschureck N, Offermanns S. Mammalian G proteins and their cell type specific functions. *Physiol Rev.* 2005; 85(4):1159-204.
- Zeng XJ, Zhang LK, Wang HX, Lu LQ, Ma LQ, Tang CS. Apelin protects heart against ischemia/reperfusion injury in rat. *Peptides.* 2009; 30(6):1144-52.