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Vascular Effects of Apelin: Mechanisms and Therapeutic Potential

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Abstract

Apelin is a vasoactive peptide and is an endogenous ligand for APJ receptors, which are widely expressed in blood vessels, heart, and cardiovascular regulatory regions of the brain. A growing body of evidence now demonstrates a regulatory role for the apelin/APJ receptor system in cardiovascular physiology and pathophysiology, thus making it a potential target for cardiovascular drug discovery and development. Indeed, ongoing studies are investigating the potential benefits of apelin and apelin-mimetics for disorders such as heart failure and pulmonary arterial hypertension. Apelin causes relaxation of isolated arteries, and systemic administration of apelin typically results in a reduction in systolic and diastolic blood pressure and an increase in blood flow. Nonetheless, vasopressor responses and contraction of vascular smooth muscle in response to apelin have also been observed under certain conditions. The goal of the current review is to summarize major findings regarding the apelin/APJ receptor system in blood vessels, with an emphasis on regulation of vascular tone, and to identify areas of investigation that may provide guidance for the development of novel therapeutic agents that target this system.

Keywords

Apelin; APJ receptors; Elabela; vasodilation; vasoconstriction

1. Introduction

Apelin and its receptor, APJ, are widely expressed throughout the cardiovascular system (Kleinz & Davenport, 2004; Kleinz, et al., 2005). This expression pattern has prompted considerable interest in the roles of apelin and APJ receptors in cardiovascular health and disease (Kalea & Batlle, 2010). Indeed, several clinical trials are evaluating the potential benefits of apelin and novel APJ receptor agonists in treating various cardiovascular disorders. A growing body of knowledge continues to shed new light on the apelinergic system, including the discovery of novel endogenous APJ receptor ligands (e.g. Elabela/

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Conflict of Interest Statement

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Toddler) (Chng, et al., 2013; Pauli, et al., 2014), synthetic analogs (e.g. E339-3D6, ML-233, MM07) (Brame, et al., 2015; Iturrioz, et al., 2010; Khan, et al., 2010) and receptor antagonists (e.g. F13A, ML221) (Lee, et al., 2005; Maloney, et al., 2012). With regard to the apelinergic system in blood vessels, there are several features involved in apelin-APJ signaling that make it a potential, but challenging target for drug discovery: (1) apelin has multiple effects on vasomotor tone (Salcedo, et al., 2007; Maguire, et al., 2009; Mughal, et al., 2018), (2) APJ receptors are expressed in the intimal and medial layers of the blood vessel wall, and possibly on cells in the adventitia as well (Kleinz, et al., 2005; Pope, et al., 2012; Mughal, et al., 2018), (3) APJ receptors are associated with multiple G-protein subunits (Hashimoto, et al., 2006; Kang, et al., 2013; Szokodi, et al., 2002), and (4) apelin may act via central nervous system mechanisms to regulate peripheral vascular function (Kagiyama, et al., 2005; Zhang, et al., 2009). The present review summarizes the current state of knowledge regarding the vascular effects of apelin, with an emphasis on the regulation of vasomotor tone, as well as novel pharmacologic agents that interact with APJ receptors in blood vessels.

2. Apelin

Discovered in 1998, apelin was initially identified as the sole endogenous ligand for the APJ receptor (Tatemoto, et al., 1998). Apelin-77 (pre-pro-apelin) is the precursor for various pharmacologically active apelin isoforms (e.g. apelin-12, -13, -17 and -36), and it shares 75–95% sequence homology among various species including rat, mice and human (Lee, et al., 2000). N-terminal residues of apelin-77 are post-translationally modified by endopeptidase to form pro-apelin-55, which is further cleaved to apelin-36, -17, -13 and -12. Each of these fragments has a conserved C-terminal region, which is essential for APJ receptor binding and functional activity (Pitkin, et al., 2010a). Post-translational modification of the N-terminal residue of apelin-13 with cyclized glutamine forms [Pyr]-apelin-13, which evades enzymatic degradation and results in a longer biological duration of action (Zhen, et al., 2013). The different apelin isoforms (i.e. apelin-12, -13, -17 and -36) have variable potency, but apelin-13 and [Pyr]-apelin-13 are identified as the more predominant and potent isoforms in the cardiovascular system (Tatemoto, et al., 1998; Maguire, et al., 2009). More recently, another endogenous APJ receptor-activating peptide, known as Elabela/Toddler (referred to as Elabela) and encoded by the APELA gene, has been discovered (Chng, et al., 2013; Pauli, et al., 2014).

Apelin has potent vascular effects but it is highly prone to proteolytic degradation, thereby limiting its duration of action (Japp, et al., 2008). Studies have shown that angiotensin converting enzyme type-2 (ACE-2), a zinc-containing carboxy monopeptidase, is partially responsible for apelin metabolism (Vickers, et al., 2002; Wang, et al., 2016; Yang, et al., 2017a). [Pyr]-apelin-13 is more susceptible to ACE-2 degradation than is apelin-17, and results in metabolites, i.e. [Pyr]-apelin-12 and apelin-16, respectively, that have reduced functional activity (Wang, et al., 2016). Another recent report showed that ACE-2 metabolizes [Pyr^1]-apelin-13 to biologically active [Pyr^1]-apelin-13 (1–12), suggesting a beneficial effect of increased ACE-2 expression during cardiovascular diseases (Yang, et al., 2017a). The metalloprotease, neprilysin, has also been shown to be involved in the degradation of apelin. Neprilysin, by truncating the RPRL (Arg2- Leu5) region of apelin,

forms a peptide that is devoid of the ability to activate APJ receptors (McKinnie, et al., 2016).

The apelin gene is located on band q25–26.1 of chromosome X and is expressed in vascular endothelial cells of both conduit and resistant arteries (Kawamata, et al., 2001; Medhurst, et al., 2003; Pitkin, et al., 2010b; Pope, et al., 2012). Kleinz et al. reported that the apelin gene is localized to endothelial cells, but not to vascular smooth muscle cells or adipocytes in human saphenous vein, and coronary, pulmonary, and mammary arteries; however, intrarenal arterial endothelial cells lack apelin-like immunoreactivity (Kleinz & Davenport, 2004). It is worth noting that although the apelin peptide is usually absent in vascular smooth muscle cells, it is expressed in smooth muscle cell positive atherosclerotic plaques (Pitkin, et al., 2010b). Moreover, atherosclerotic plaque apelin is co-localized with APJ receptors, suggesting the possibility of apelin-APJ signaling in coronary atherosclerosis.

Regulation of apelin gene expression and secretion of apelin from intracellular sources in the blood vessel wall (e.g. adipocytes, endothelial cells) is complex. Numerous factors and conditions increase apelin gene expression, including cytokines (tumor necrosis factor-alpha) (Daviaud, et al., 2006), lipopolysaccharides (Han, et al., 2008), hypoxia (Eyries, et al., 2008; Glassford, et al., 2007), dehydration (Reaux-Le Goazigo, et al., 2004), and insulin (Boucher, et al., 2005). In humans, hyperinsulinemia up-regulates apelin gene expression in adipose tissue and increases apelin release via phosphoinositide 3-kinase (PI3K) and protein kinase C (PKC)-dependent pathways (Castan-Laurell, et al., 2008). Hypoxia increases apelin gene expression in cultured human pulmonary arterial endothelial and smooth muscle cells via hypoxia inducible factor–1α (HIF-1α) dependent mechanism (Eyries, et al., 2008). Activation of α-retinoic acid receptors by all-trans retinoic acid increases apelin gene expression in HUVEC cells and in carotid arteries from balloon-injured rats (Shi, et al., 2017). Although these studies provide insight into several key pathways involved in apelin regulation, the association between apelin gene expression and release of apelin into the circulation and/or surrounding tissues remains poorly understood. Moreover, although administration of exogenous apelin has beneficial effects on cardiovascular diseases (Japp, et al., 2008; Tatemoto, et al., 2001), our knowledge of the role of endogenous apelin, as well as the stimuli for apelin release, in cardiovascular health and disease is limited.

Patients with cardiovascular disorders typically have lower plasma apelin levels than matched healthy controls. For example, plasma apelin levels are reduced in patients with coronary artery disease (CAD), and among CAD patients with unstable angina or acute myocardial infraction there is a further reduction in apelin levels as compared with asymptomatic CAD patients (Kadoglou, et al., 2010). Similarly, plasma apelin levels are reduced in patients with stable angina, essential hypertension and acute coronary syndrome (Li, et al., 2008b; Gupta, et al., 2016). In patients with heart failure, apelin levels vary depending on the stage and severity of the disease, as plasma apelin levels are elevated in early stage heart failure, but significantly reduced in patients with severe disease as compared to healthy controls (Chen, et al., 2003). Moreover, the concentration of apelin in myocardial tissue is reduced in patients with heart failure (Chandrasekaran, et al., 2010). In contrast to cardiovascular diseases, apelin levels are elevated during metabolic disorders. During obesity, plasma apelin levels are significantly higher than those observed in subjects

with normal weight (Boucher, et al., 2005), and are further increased in patients with morbid obesity (Heinonen, et al., 2005). Similarly, plasma apelin levels are increased in patients with diabetes (Castan-Laurell, et al., 2011; Habchi, et al., 2014). It is not yet known whether these changes in apelin levels during cardiovascular and metabolic disorders are associated with protective or compensatory roles of the apelinergic system. For example, in some human and animal studies administration of apelin is shown to restore decreased cardiac function, consistent with a beneficial therapeutic effect of apelin (Koguchi, et al., 2012; Nagano, et al., 2013).

In 2013, a second endogenous ligand for APJ receptors was discovered. Elabela has a 32 amino acid sequence but does not share sequence homology with apelin (Chng, et al., 2013; Pauli, et al., 2014); however, the affinity of the peptide for APJ receptors is similar to that of apelin (Perjes, et al., 2016; O'Carroll, et al., 2017). Nonetheless, evidence suggests that the peptides may not bind in the same way to the orthostatic binding site. Structure-activity relationship studies reveal that the C-terminal moiety (Arg28, Val29, Pro30, Phe31, and Pro32) and His26 residues of Elabela are most important for receptor binding and signaling (Murza, et al., 2016). This contrasts markedly with apelin-13, in which the key pharmacophores (Arg2, Pro3, Arg4, Leu5) are primarily located at the N-terminal. Elabela was initially identified as an embryonic APJ receptor regulator, but recent studies suggest its role in adult tissues as well (Perjes, et al., 2016; Yang, et al., 2017b). Similar to apelin, Elabela is also expressed in vascular endothelial cells (Perjes, et al., 2016; O'Carroll, et al., 2017). An understanding of the functional role of Elabela in the vascular system is still emerging but recent evidence suggests that Elabela can compensate for down-regulated functions of apelin during various cardiovascular diseases. Administration of exogenous Elabela improves right ventricular systolic pressure and hypertrophy, as well as pulmonary vascular remodeling, in a rat model of pulmonary hypertension in which apelin signaling is downregulated (Yang, et al., 2017b). Infusion of Elabela, which was associated with an increase in cardiac output and reduced systemic vascular resistance, improves cardiovascular function and survival in a cecal ligation puncture model of sepsis (Coquerel, et al., 2017). On the other hand, reduced levels of Elabela may lead to cardiovascular abnormalities, inasmuch as pregnant Elabela knockout mice display preeclampsia-like symptoms and defective placental angiogenesis (Ho, et al., 2017).

3. APJ Receptor

The APJ receptor is a G-protein coupled receptor located on chromosome 11 and composed of an intron-less gene (*APLNR),* which is conserved in many species including human, monkey, chimpanzee, mouse and rat (Pitkin, et al., 2010a). APJ receptors were first discovered in 1993 during a search for vasopressin receptors (O'Dowd, et al., 1993). Immunocytochemical and autoradiographic studies have detected APJ receptors in endothelial and smooth muscle cells in numerous blood vessels, including human coronary, internal mammary, radial, and pulmonary arteries, human saphenous vein, and rat aorta, coronary, cerebral and pulmonary arteries (Katugampola, et al., 2001; Kleinz, et al., 2005; Pitkin, et al., 2010b; Mughal, et al., 2018).

APJ receptors have a 380 amino acid sequence with a characteristic G-protein structure, including seven transmembrane domains and post-translation modification sites for phosphorylation, palmitoylation and glycosylation along with association sites for β-arrestin (O'Dowd, et al., 1993). APJ receptor signaling involves multiple G-protein subunits. Initial work suggested the involvement of pertussis toxin sensitive $G\alpha_{i/o}$ subunits that inhibit forskolin-induced cyclic adenosine monophosphate (cAMP) formation in CHO cells (Tatemoto, et al., 1998). This signaling pathway is also present in blood vessels, inasmuch as apelin inhibits large conductance, calcium-activated potassium (BK_{Ca}) channel currents in cerebral vascular smooth muscle cells in a pertussis toxin-sensitive manner (Modgil, et al., 2013). Apelin was also found to increase Ca^{2+} mobilization in neuronal cells (Choe, et al., 2000), suggesting the involvement of other G-protein subunits. Subsequent studies demonstrated that apelin-induced cardiac and smooth muscle contractile responses were attenuated by inhibitors of phospholipase C and PKC, suggesting a role for $G\alpha_{q/11}$ subunits in APJ receptor signaling (Hashimoto, et al., 2006; Szokodi, et al., 2002). Moreover, apelin was shown to increase phosphorylation of myosin light chain (MLC) in vascular smooth muscle cells via activation of $G\alpha_{q/11}$ subunits and a PKC-dependent mechanism (Hashimoto, et al., 2006). In addition, a functional role for $G\alpha_{13}$ subunits has been identified in apelin-induced cytoplasmic translocation of histone deacetylase during cardiac and vascular development (Kang, et al., 2013).

Various G-protein subunits, e.g. $G\alpha_{i/o}$, $G\alpha_q$ and βγ dimers, can activate PI3K/Akt signaling (Murga, et al., 1998), which is indeed involved in apelin-induced vascular responses. For example, apelin inhibits calcification of vascular smooth muscle cells via a PI3K/Akt signaling mechanism (Shan, et al., 2011). Similarly, apelin attenuates apoptosis in vascular smooth muscle cells by activating PI3K/Akt and extracellular signal-regulated kinase (ERK) signaling (Cui, et al., 2010; Tang, et al., 2007). Apelin also inhibits BK_{Ca} channel currents by activation of PI3K/Akt pathways (Modgil, et al., 2013). Apelin-induced PI3K/Akt signaling can increase phosphorylation of endothelial nitric oxide synthase (eNOS) to modulate aortic vascular tone (Zhong, et al., 2007b). Likewise, Elabela improves self-renewal and regenerative ability of human progenitor stem cells via PI3K/Akt activation (Ho, et al., 2015). These latter actions of Elabela might be mediated by a cell surface receptor different from the APJ receptor in human embryonic stem cells (Ho, et al., 2015), suggesting the possible existence of another subtype or novel type of receptor; however, the bulk of evidence to date continues to support the view that the peptide is acting solely on the currently identified APJ receptor. Elabela also inhibits forskolin-induced cAMP formation by activating $ERK_{1/2}$ signaling pathways in CHO cells (Wang, et al., 2015b).

In addition to these intracellular events, APJ receptors also have the ability to form homo- and heterodimers. APJ receptors are reported to hetero-dimerize with angiotensin (AT_1) receptors (Siddiquee, et al., 2013), bradykinin (B_1) receptors (Bai, et al., 2014b), neurotensin receptor-1 (NTSR1) (Bai, et al., 2014a) and κ-opioid receptors (KOR) (Li, et al., 2012). These receptors are involved in cardiovascular regulation and their ligands (apelin, des-Arg(9)-bradykinin, neurotensin and dynorphin) are sensitive to ACE-2 proteolysis (Vickers, et al., 2002), suggesting the importance of these heterodimers in cardiovascular pharmacology. For example, APJ receptors can allosterically modify the functional state of AT_1-receptors to a low affinity state towards angiotensin II (Siddiquee, et al., 2013; Sun, et

al., 2011) and these actions can be independent of its putative ligand, apelin (Siddiquee, et al., 2013). Similarly, constitutive heterodimers of APJ- and bradykinin (B_1)-receptors are shown to generate higher intracellular Ca^{2+} and upregulate eNOS phosphorylation, possibly by strengthening the association of the dimer with $G\alpha_q$ subunits (Bai, et al., 2014b). APJ-NTSR1 or APJ-KOR heterodimers also cause significant increases in phosphorylated $ERK_{1/2}$ levels by increasing intracellular Ca^{2+} (Li, et al., 2012; Bai, et al., 2014a). A recent report demonstrated the existence of homodimers-oligomers of the human APJ receptor, which could possibly mediate different signaling events in comparison to APJ monomers (Cai, et al., 2017).

APJ receptor expression is altered in various cardiovascular diseases, and both increases as well as decreases in APJ receptor expression have been reported. A decrease in mRNA and protein levels of APJ receptors was observed in patients with ischemic heart disease or idiopathic dilated cardiomyopathy (Földes, et al., 2003; Pitkin, et al., 2010b); however, APJ receptor protein levels were significantly increased in ischemic heart failure (Atluri, et al., 2007; Sheikh, et al., 2008). Hypoxia, one of the major consequences of ischemic injury, may lead to increased APJ receptor expression, as hypoxia-activated HIF-1α has been shown to cause aberrant increases in APJ receptor protein expression (Kerkela, et al., 2013; Kong, et al., 2015; Zhang, et al., 2015). APJ receptor mRNA levels are up-regulated in patients with aortic valve stenosis in comparison to controls (Peltonen, et al., 2009). Both mRNA and protein levels of APJ receptors are reduced in the heart, kidney and aorta of hypertensive rats (Najafipour, et al., 2012; Najafipour, et al., 2015), whereas in obese women, there is an increase in APJ receptor mRNA levels, which was attenuated by improving insulin resistance (Castan-Laurell, et al., 2008).

4. Vascular Effects of Apelin

Apelin has complex vasomotor effects as it can cause either vasodilation or vasoconstriction depending on the vascular bed and underlying conditions. These dual actions of apelin are attributed to the presence of APJ receptors in both the endothelial and smooth muscle cell layers of the blood vessel wall. Vasoactive agents may act directly on vascular smooth muscle cells to cause contraction or relaxation, or on endothelial cells, which can secrete substances that mediate vasodilation (e.g. NO, prostacyclin) as well as vasoconstriction (e.g. endothelin) (O'Rourke, et al., 2006).

4.1 Role of Apelinergic System in Vasodilation

A hypotensive effect of apelin was first reported in 2000, when it was shown that intraperitoneal injection of apelin reduces both systolic and diastolic pressure in anesthetized normotensive animals (Lee, et al., 2000). Shortly thereafter, intravenous administration of apelin was shown to produce hypotensive effects (Reaux, et al., 2001), as well as cause venodilation (Cheng, et al., 2003). The various apelin isoforms may have different potencies in different experimental models. For example, apelin-12 is more potent in reducing mean arterial pressure in comparison to apelin-13 and -36 in anesthetized normotensive rats (Tatemoto, et al., 2001), whereas apelin-13 has higher potency in comparison to apelin-12 in spontaneously hypertensive rats (Lee, et al., 2005). In addition, apelin-17 produces a greater

depressor response than [Pyr]-apelin-13 and apelin-15 in normotensive rats (El Messari, et al., 2004).

A role for NO in the hypotensive effect of apelin was demonstrated in studies where the NO synthase inhibitor, N(G)-nitro-l-arginine methyl ester (L-NAME), abolished the reduction in mean arterial pressure produced by intravenous administration of apelin (Tatemoto, et al., 2001). Similarly, intravenous infusion of apelin-36 and [Pyr]-apelin-13 produced coronary vasodilation that was inhibited by an "NO clamp" (L-N(G)-monomethylarginine co-infused with sodium nitroprusside), but not by inhibition of cyclooxygenase with aspirin (Japp, et al., 2008). Experiments performed with isolated human and rat peripheral arteries also suggested a role for NO in apelin-induced relaxations. Relaxation of isolated human mesenteric, but not hepatic arteries was attenuated in the presence of L-NAME (Salcedo, et al., 2007). In isolated rat aorta, apelin caused concentration- and time-dependent increases in eNOS activity and NO formation (Jia, et al., 2007). Apelin produced endothelium-dependent relaxation of isolated rat coronary arteries by stimulating the release of NO, which activated BK_{Ca} channels without altering cGMP levels in the underlying smooth muscle cells of these arteries (Mughal, et al., in press). Activation of mechanosensor pathways by flow-mediated shear stress increases APJ receptor expression and apelin and NO formation in human endothelial cells (Busch, et al., 2015). A role for prostanoids in apelin-induced relaxation was established in human mammary arteries, where several apelin isoforms (apelin-13, -36 and [Pyr]-apelin-13) caused concentration-dependent relaxations that were inhibited in the presence of indomethacin, but not L-NAME (Maguire, et al., 2009). Moreover, in the isolated portal vein of the rat, apelin attenuated angiotensin II-induced contractions, but the effect of apelin was only partially inhibited by endothelial denudation, suggesting that apelin may act directly on vascular smooth muscle to cause relaxation (Gurzu, et al., 2006). By contrast to apelin-induced relaxation, Elabela-induced relaxation in isolated mouse aortae was unchanged in the presence of L-NAME, suggesting the involvement of NO-independent mechanism(s) in the relaxation response to Elabela (Wang, et al., 2015b).

Apelin has been shown to have beneficial effects in several vascular or metabolic disorders. In animal models of pulmonary hypertension and diabetes, apelin treatment lowered mean arterial pressure and reduced the levels of vasoconstrictor mediators such as angiotensin-II and endothelin-1 (Akcilar, et al., 2013; Falcao-Pires, et al., 2009). Similarly, intravenous bolus administration of apelin caused a significant reduction in mean pulmonary arterial pressure during acute pulmonary embolism (Feng, et al., 2010), and apelin signaling via APJ – KOR heterodimers caused a depressor response during renovascular hypertension (Yeganeh-Hajahmadi, et al., 2017; Rostamzadeh, et al., 2018). Moreover, by increasing eNOS phosphorylation, apelin reversed the impaired relaxation response to acetylcholine and abrogated abnormal Ang II-induced contractile tone in intrarenal arteries from diabetic mice (Zhong, et al., 2007a). In human subjects, apelin-induced vasodilation was preserved in patients with chronic heart failure whereas the vasodilator response to acetylcholine was significantly impaired (Japp, et al., 2010). In middle-aged and older adults, an exercise-associated increase in plasma apelin levels was positively correlated with a decrease in carotid arterial stiffness, which commonly occurs with aging (Fujie, et al., 2014).

Like apelin, the novel peptide, Elabela, also has the ability to cause vasodilation in coronary arteries (Perjes, et al., 2016). Elabela has been shown to reverse vasopressor responses during pulmonary arterial hypertension and angiotensin-II-induced hypertension (Yang, et al., 2017b; Sato, et al., 2017). Moreover, sustained Elabela gene therapy delayed blood pressure elevation in hypertensive rats (Schreiber, et al., 2017).

The renin-angiotensin system is a key pathway in the development and progression of hypertension, heart failure and other cardiovascular disorders. APJ and AT_1-receptors share significant sequence homology and have a similar tissue distribution pattern (O'Dowd, et al., 1993); however, apelin does not bind AT_1-receptors nor does angiotensin II (Ang II) bind to APJ receptors (O'Dowd, et al., 1993; Tatemoto, et al., 1998). Nonetheless, activation of the apelinergic system has an antagonistic effect toward AT_1-receptor mediated responses, either by allosteric regulation of the receptor (Sun, et al., 2011; Siddiquee, et al., 2013) or by increasing NO-dependent signaling (Siddiquee, et al., 2011). Both acute and chronic apelin-induced vasodilation and depressor responses are preserved during renin-angiotensin system activation in healthy humans and in patients with heart failure (Barnes, et al., 2013). Moreover, apelin abrogated Ang II-induced atherosclerosis in ApoE-deficient mice by increasing NO formation to quench superoxide-induced changes in the vascular wall (Chun, et al., 2008). The antagonistic effects of apelin on Ang II signaling may be dependent on the underlying pathologic condition, since apelin attenuated Ang II-induced contractions in pulmonary arteries from normoxic animals, but not in arteries from animals exposed to chronic hypoxia (Andersen, et al., 2009). Apelin-APJ receptor signaling can also increase ACE-2 gene expression, which could increase conversion of Ang II to $Angiotensin_{1–7}$ to modulate cardiovascular functions (Sato, et al., 2013).

4.2 Role of the Apelinergic System in Vasoconstriction

APJ receptors are expressed on vascular smooth muscle cells and their activation results in vasoconstriction (Katugampola, et al., 2001; Maguire, et al., 2009; Han, et al., 2013; Mughal, et al., 2018). Indeed, some studies have reported a blood pressure-elevating effect of systemic administration of apelin, consistent with a vasoconstrictor response to the peptide. For example, a biphasic hemodynamic response to an intravenous bolus of apelin was observed in conscious sheep, where there was an initial transient fall in arterial pressure followed by a rise in arterial pressure and peripheral vascular resistance (Charles, et al., 2006). In conscious rats, intravenous administration of apelin failed to produce a depressor response, but caused a dose-dependent increase in mean arterial pressure (Kagiyama, et al., 2005).

Though few studies have been performed with isolated blood vessels, apelin generally causes relaxation of isolated arteries with intact endothelium (as described above), whereas vasoconstriction has been observed in isolated vessels denuded of endothelium and under conditions where the endothelial cells are damaged or dysfunctional. [Pyr]-Apelin-13 causes vasoconstriction in endothelium-denuded human saphenous veins and mammary arteries (Katugampola, et al., 2001; Maguire, et al., 2009), and in coronary arteries obtained from atherosclerotic patients (Pitkin, et al., 2010b). Apelin may also permeate through endothelial cells damaged by asymmetric dimethylarginine (Wang, et al., 2011), and cause

concentration-dependent vasoconstriction in isolated caudal arteries by increasing phosphorylation of MLC in vascular smooth muscle cells (Han, et al., 2013). That apelin passes through dysfunctional endothelial cells and activates APJ receptors on vascular smooth muscle is supported by in vivo studies in which apelin caused an increase in systolic blood pressure in mice treated with L-NAME, and in rats with vascular endothelium damaged by asymmetric dimethylarginine (Han, et al., 2013; Nagano, et al., 2013). In mouse thoracic aorta, apelin causes vasoconstriction by increasing myosin light chain phosphorylation via $G\alpha_{i/o}$-dependent activation of PKC and Na^+-Ca^{2+} exchanger-dependent pathways (Hashimoto, et al., 2006). In addition to MLC phosphorylation as a mechanism of vasoconstriction, apelin may also exert a net vasoconstrictor influence by inhibiting NO-induced relaxation, an effect of apelin that is mediated by inhibition of NO-induced activation of BK_{Ca} channels in cerebral arterial smooth muscle cells (Modgil, et al., 2013; Mughal, et al., 2018).

Vasopressor responses to apelin have also been observed following injection of the peptide into cardiovascular regulatory regions of the brain (Seyedabadi, et al., 2002; Kagiyama, et al., 2005). Moreover, overexpression of apelin in the rostral ventrolateral medulla of normotensive rats results in increased mean arterial blood pressure via NADPH oxidase-dependent formation of superoxide(s), and apelin gene expression in the rostral ventrolateral medulla is increased in spontaneously hypertensive rats (Yao, et al., 2011; Zhang, et al., 2009). The vasopressor response to centrally administered apelin is abolished in animals fed a high fat diet or subjected to chronic stress (Cudnoch-Jedrzejewska, et al., 2015; Gomolka, et al., 2015).

The role of apelin-APJ receptor signaling during cardiovascular disorders associated with endothelial dysfunction remains unclear. On one hand, increased apelin/APJ receptor expression and bioavailability produced beneficial vascular effects in conditions with endothelial dysfunction such as end-stage heart failure (Fukushima, et al., 2010), atherosclerosis (Chun, et al., 2008) and obesity (Schinzari, et al., 2017), while others have shown that apelinergic signaling may be a mediator for atherosclerosis (Hashimoto, et al., 2007). Indeed, up-regulated apelin genes are localized in atherosclerosis plaques (Pitkin, et al., 2010b) and stenotic aortic valves (Peltonen, et al., 2009). Thus, it will be important to decipher whether increased apelin-APJ receptor signaling has a role in disease progression or is involved in compensatory feedback mechanism(s).

4.3 Other Vascular Effects of Apelin

In addition to its direct regulatory role in controlling vascular tone, the apelin/APJ receptor signaling system also plays an important role in vascular stabilization, including angiogenesis, proliferation and permeability.

Cell Migration and Angiogenesis—Cell migration is an important feature of several physiological and pathological processes, including embryogenesis, would healing, inflammation, cardiovascular disease and cancer (Staff, 2001). High expression of APJ receptors during embryogenesis and postnatal retinal blood vessel formation provided initial support for the importance of apelinergic signaling during angiogenesis (Devic, et al., 1996;

Saint-Geniez, et al., 2002). In addition, Elabela increases migration of mesendodermal cells during gastrulation in a tightly regulated fashion (Pauli, et al., 2014).

Angiogenesis can be either beneficially adaptive (e.g. ischemia, myocardial infarction) or potentially deleterious (e.g. tumor growth, retinopathy, nephropathy). Hence, the role of the apelinergic system during angiogenesis can have either beneficial or detrimental effects based on the underlying physiology or pathological conditions. For example, intra-myocardial delivery of apelin-overexpressing bone marrow cells to ischemic mice increased the homing of bone marrow derived progenitor cells in ischemic heart progressing to neovascularization (Li, et al., 2013b). Similarly, increased coronary collateral development is correlated with increased plasma apelin levels in patients with coronary artery disease, establishing the beneficial angiogenic potential of apelin (Akboga, et al., 2014). Moreover, loss of apelin-APJ signaling has been shown to impair differentiation of endothelial, hematopoietic and cardiac progenitor cells (Inui, et al., 2006; Zeng, et al., 2007; Wang, et al., 2013). Treatment with apelin also alleviates diabetic cardiomyopathy in mice by increasing angiogenesis via SirT3-dependent pathways (Zeng, et al., 2014). By contrast, apelin-induced angiogenesis of retinal and glomerular endothelial cells is associated with increased risk of retinopathy and nephropathy during type 2 diabetes mellitus (Zhang, et al., 2013; Du, et al., 2014). Furthermore, apelin-induced migration of vascular smooth muscle cells suggests the possible involvement of smooth muscle APJ receptor signaling in the development of atherosclerosis (Liu, et al., 2013; Wang, et al., 2015a).

Cell Proliferation—Cellular proliferation is a complex process and apelin is shown to have both proliferative and anti-proliferative actions. Apelin stimulates vascular smooth muscle cell proliferation via PI3K/Akt/ERK (Liu, et al., 2010) and Jagged-1/Notch3 (Li, et al., 2013a) signaling pathways with a common downstream mediator, cyclin D1, to accelerate cell cycle progression from G0/G1 to S phase (Li, et al., 2008a). In addition, hypoxia-induced apelin gene expression also increases proliferation and differentiation of stem cells and progenitor cells, suggesting the possible therapeutic potential of apelin in ischemic reperfusion injury (Zhang, et al., 2015; Hou, et al., 2017). By contrast, apelin treatment reduces pulmonary vascular smooth muscle cell proliferation in hypoxic conditions via PI3K/Akt/mTOR signaling pathways (Zhang, et al., 2014b). Likewise, down-regulation of apelin signaling during pulmonary hypertension is correlated with hyper-proliferation of pulmonary endothelial cells and smooth muscle cells (Kim, et al., 2013).

Cell Permeability—Cell adhesion molecules regulate cell-to-cell adhesion and paracellular permeability. By regulating the permeability of pericytes, apelin modulates vascular development and remodeling (Chen, et al., 2015). The putative beneficial effects of apelin during obesity and cancer are associated with improved vascular integrity and decreased permeability (Kidoya, et al., 2012; Sawane, et al., 2013). Similarly, overexpression of apelin-APJ signaling during acute lung injury and respiratory distress syndrome can reduce capillary-alveolar leakage to alleviate symptoms (Fan, et al., 2015). These beneficial actions of apelin suggest a protective role of apelin during inflammation and immunotoxicity. In this regard, apelin is shown to have protective actions during post-burn sepsis via PI3K signaling pathways (Luo, et al., 2015).

5. Apelin Mimetics and Inhibitors

At present, only a limited number of apelin-like agonists and APJ receptor antagonists have been discovered. There is still a great need for the development of potent and selective drugs that target the apelin/APJ receptor-signaling pathway, not only as potential therapeutic agents but also as pharmacologic tools for dissecting the role of apelin in vascular biology. Those agents most commonly being used at the present time are described below.

5.1 Apelin Mimetics

There are three general types of apelin-like agonists, i.e. non-peptide agonists, biased agonists, and peptide agonists. In addition, novel drug delivery approaches, such as PEGylation and nano-encapsulation, have been employed in order to increase the biological half-life and activity of native apelin isoforms (Narayanan, et al., 2015).

E339-3D6 was the first non-peptide APJ receptor agonist to be developed (Iturrioz, et al., 2010). E339-3D6 has a binding affinity of 90 nM towards APJ receptors and it relaxes precontracted rat thoracic aorta at lower concentrations than acetylcholine (pD_2= 8.6 ± 0.4 vs. 6.8 ± 0.4 for E339-3D6 and acetylcholine; respectively). Moreover, E339-3D6 showed full agonist behavior with regard to β-arrestin-dependent APJ receptor internalization, but behaved as a partial agonist in inhibiting cAMP formation (Iturrioz, et al., 2010). E339-3D6 was later shown to be a mixture of polymethylated species, which were further purified to generate analogs with higher affinity and full agonist activity (Margathe, et al., 2014). Another synthetic non-peptide analog, ML-233, also reduced forskolin-induced cAMP formation, with maximum effects at 100 μM, and increased APJ receptor internalization by β-arrestin recruitment (Khan, et al., 2010). ML-233 has ~ 21-fold selectivity towards APJ receptors as compared to AT_1-receptors. Further in vitro and in vivo characterization of these molecules is required in order to better understand their vasoactive therapeutic potential.

Target receptor internalization and β-arrestin dependent receptor silencing are major limitations for G-protein coupled receptors, including APJ receptor mediated signaling (Evans, et al., 2001; Masri, et al., 2006). Novel strategies are being employed to develop biased agonists with higher affinity towards the APJ receptor and with minimal effects on β-arrestin dependent pathways. Brame et al. designed the first cyclic biased APJ receptor agonist, MM07 (Brame, et al., 2015). MM07 has nanomolar potency towards the human APJ receptor (pD2: 9.54 ± 0.42) and higher circulatory life (~17 min), but significantly lower potency towards β-arrestin internalization (pD2: 6.16 ± 0.07) in comparison with [Pyr]-apelin-13. Repeated administration of MM07 caused an increase in forearm blood flow without desensitization of APJ receptors. MM07 also caused a significant increase in cardiac output with no evidence of hemodynamic instability (Brame, et al., 2015). This same group later designed another small molecule APJ receptor biased agonist, CMF-019. This molecule has longer half-life (~38 minutes) with nanomolar affinity (pD_2=10.00 ± 0.13) towards APJ receptors, with lesser potency on receptor internalization (pD2=6.16 ± 0.21). Moreover, CMF-019 increased cardiac contractility with limited effects on the vasculature (Read, et al., 2016).

Two other synthetic analogs of apelin-17, P92 and LIT01-196, were designed by classic chemical substitution and fluorocarbon chain addition, respectively (Gerbier, et al., 2017). These analogs resulted in higher circulatory half-life (P92: 24 min and LIT01-196: >24 hr) with sub-nanomolar binding affinity (K_i= 0.09 ± 0.02 nM and 0.08 ± 0.01 nM for P92 and LIT01-196, respectively). Both P92 and LIT01-196 caused potent relaxation of rat thoracic aorta (EC_{50}= 7 ± 5 nM and 2 ± 1 nM for P92 and LIT01-196, respectively), which was abolished in the presence of L-NAME. Moreover, both P92 and LIT01-196 caused a marked increase in the diameter of rat glomerular arterioles precontracted with angiotensin-II. Intravenous injection of LIT01-96 and P92 in normotensive anesthetized rats caused a potent and prolonged depressor response in comparison to apelin-17. Likewise, both compounds showed a potent inotropic and diuretic response suggesting their ability to activate multiple signaling mechanisms to maintain physiological homeostasis (Gerbier, et al., 2017).

Considerable efforts have been made to structurally modify apelin in order to improve its biological half-life. One approach has been to design molecules that are resistant to enzymatic degradation by neprilysin. Recently, three metabolically stable analogs of apelin-17 have been synthesized by modifying neprilysin-sensitive Leu-Arg (RPRL) residues. These analogs include modified N-methyl Leu9 analogs and aza-analogs (azaArg and azaLeu), which demonstrated sub-nanomolar binding affinity (K_i= 0.14 – 0.15 nM) with beneficial cardiovascular effects, including vasodepressor and inotropic effects (McKinnie, et al., 2017). Another approach has been to structurally modify apelin by PEGylation of the N-terminal residue. Conjugation of a 40 kDa PEG moiety to apelin-36 resulted in a longer circulatory half-life with minimal effect on the binding constant (K_i= 0.3 nM vs. 0.05 nM with and without PEG-conjugation)(Jia, et al., 2012). PEG-apelin-36 demonstrated an extended effect on cardiac ejection fraction, but had no effect on aortic pressure, similar to that observed with apelin-36 (Jia, et al., 2012). It is not yet known if developing similar conjugates with the more vasoactive isoforms of apelin, i.e. apelin-13 and/or [Pyr]-apelin-13, will further prolong and enhance their vasoactive effects.

5.2 Apelin Inhibitors

The first antagonist of the cardiovascular actions of apelin is known as F13A, which was synthesized by chemical substitution of the C-terminal phenylalanine (Phe13) residue of the native apelin peptide with an alanine residue (Lee, et al., 2005). F13A demonstrated dose-dependent inhibition of apelin-induced hypotensive effects, with no effect on Ang II-induced hypertensive effects, indicating selectivity of F13A toward apelin-induced responses (Lee, et al., 2005). F13A also attenuated apelin-induced depressor responses during renovascular hypertension (Rostamzadeh, et al., 2018) and, in isolated rat cerebral arteries, F13A abolished the inhibitory effects of apelin on BK_{Ca} channel function and NO-induced relaxation (Mughal, et al., 2018). Moreover, pressor responses to centrally administered apelin in both normotensive and hypertensive rats are significantly attenuated by pre-treatment with F13A (Zhang, et al., 2014a; Griffiths, et al., 2017).

A second peptide APJ receptor antagonist was designed using a bivalent ligand approach (Macaluso, et al., 2011). The cyclic APJ receptor antagonist, [cyclo(1–6)CRPRLC-KH-cyclo(9–14)CRPRLC], demonstrated a K_i value of 82 nM in CHO cells transfected with

human APJ receptors and a K_D value of 3.2 μM in competition binding studies in human left ventricle (Macaluso, et al., 2011). This cyclic receptor antagonist has yet to be evaluated in in vivo or in vitro studies designed to determine its antagonist activity versus apelin-induced vasoactive effects.

ML-221 is the first non-peptide APJ receptor antagonist to be developed (Maloney, et al., 2012). ML-221 has micromolar potency in cell-based assays (IC_{50}: 0.70 μM in the cAMP assay, and 1.75 μM in the β-arrestin assay) and > 37-fold selectivity towards APJ receptors as compared to AT_1-receptors. However, ML-221 has low aqueous solubility, moderate stability, limited membrane permeability, and high protein binding, all of which can limit its utility as an in vitro or in vivo pharmacologic tool (Maloney, et al., 2012). Nonetheless, it was recently reported that ML-221 caused a reduction in apelin-induced microvascular endothelial cell proliferation and that intraperitoneal administration of ML-221 reduced the retinal angiogenesis commonly observed during ischemic retinopathy (Ishimaru, et al., 2017). ML-221 has also been shown to attenuate Elabela-induced APJ receptor activation and signaling (Yang, et al., 2017b). The effects of ML-221 on the vasomotor actions of apelin are not yet known.

6. Conclusions and Future Directions

Targeting the apelinergic system provides exciting opportunities for the treatment of cardiovascular disease. Apelin/APJ receptor signaling has beneficial effects in several cardiovascular and metabolic disorders, including hypertension, heart failure, pulmonary hypertension, and obesity. Promising apelin-mimetics are in development, but several challenges remain. A key limitation at present is that our knowledge of the vasomotor effects of apelin in specific vascular beds (e.g. coronary, cerebral, renal) is limited. As there is potential for apelin to cause both vasodilation and vasoconstriction, there is a need to improve our understanding of apelin signaling and how it relates to these opposing functional effects. Other challenges associated with the therapeutic use of apelin and its isoforms include their short biological half-lives, complex intracellular signaling pathways, and the possible existence of more than one APJ receptor subtype or another cell surface receptor that has affinity for both apelin and Elabela. Overcoming these challenges will provide guidance on how to optimize opportunities to target the beneficial effects of apelin and improve the development of novel therapeutic agents for treating cardiovascular diseases.

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Abbreviations

APJ	apelin receptor
ACE-2	angiotensin converting enzyme type-2

AT-1	angiotensin-1
BK_{Ca}	large conductance calcium-activated potassium
CAD	coronary artery disease
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
ERK	extracellular signal-regulated kinase
HIF-1α	hypoxia inducible factor - 1α
KOR	κ-opioid receptors
L-NAME	N(G)-nitro-l-arginine methyl ester
MLC	myosin light chain
NO	nitric oxide
PI3K	phosphoinositide 3-kinase
PKC	protein kinase C

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