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Apelin receptors: From signaling to antidiabetic strategy

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ABSTRACT

The G protein-coupled receptor APJ and its cognate ligand, apelin, are widely expressed throughout human body. They are implicated in different key physiological processes such as angiogenesis, cardiovascular functions, fluid homeostasis and energy metabolism regulation. On the other hand, this couple ligand–receptor is also involved in the development and progression of different pathologies including diabetes, obesity, cardiovascular disease and cancer. Recently, a new endogenous peptidic ligand of APJ, named Elabela/Toddler, has been identified and shown to play a crucial role in embryonic development. Whereas nothing is yet known regarding Elabela/Toddler functions in adulthood, apelin has been extensively described as a beneficial adipokine regarding to glucose and lipid metabolism and is endowed with anti-diabetic and anti-obesity properties. Indeed, there is a growing body of evidence supporting apelin signaling as a novel promising therapeutic target for metabolic disorders (obesity, type 2 diabetes). In this review, we provide an overview of the pharmacological properties of APJ and its endogenous ligands. We also report the activity of peptidic and non-peptidic agonists and antagonists targeting APJ described in the literature. Finally, we highlight the important role of this signaling pathway in the control of energy metabolism at the peripheral level and in the central nervous system in both physiological conditions and during obesity or diabetes.

1. Introduction

The apelin receptor, also called APJ or angiotensin receptor-like 1 was first cloned in 1993 due to its strong sequence homology with the angiotensin II receptor (AT_1) (54% in transmembrane domains and 31% for the entire sequence). Nevertheless, APJ does not bind angiotensin II (O'Dowd et al., 1993).

Human apelin receptor gene encodes for a protein of 380 amino acids which belongs to the class A (rhodopsin-like receptor) G protein-coupled receptor (GPCR) family. Examining APJ protein sequence, Glu^{20} and Asp^{23} in the extracellular N-terminal tail were first identified as crucial residues for binding of its endogenous ligand called apelin (Langelaan et al., 2013; Zhou et al., 2003b). In addition, combining three-dimensional molecular modeling with site-directed mutagenesis, Gerbier et al. (2015) recently established that Asp^{94}, Glu^{174} and Asp^{284} are also involved in apelin binding.

Apelin and APJ are both widely expressed in human organism and can be detected in the central nervous system and in the periphery (heart, lung, kidney, adipose tissue, muscle…). Indeed, apelinergic system is notably expressed in hypothalamus where it participates to the regulation of fluid homeostasis, food intake and glucose metabolism (De Mota et al., 2004; Drougard et al., 2014; Duparc et al., 2011; Lv et al., 2013). APJ stimulation or inhibition in the brain may have consequences on behavior (memory, food and water intake) and physiology (neuroprotection, pain, metabolism). Moreover, it is well established that apelin signaling participates to the peripheral regulation of cardiovascular function playing an essential role in physiological (Cox et al., 2006; Kalin et al., 2007; Kang et al., 2013; Kasai et al., 2010, 2008; Saint-Geniez et al., 2002) and pathological (Berta et al., 2014, 2010; Kalin et al., 2007; Liu et al., 2015; Sorli et al., 2007, 2006) angiogenesis (for reviews, see Audigier et al., 2014).

Furthermore, the apelin–APJ system has been extensively described as a major factor involved in energy metabolism. Consequently, dysregulation of apelin signaling is associated with pathological states such as cardiac hypertrophy, type 2 diabetes (T2D) and obesity (Castan-Laurell et al., 2011, 2012). Given the broad range of pathophysiological actions of apelin, APJ represents a promising target for pharmacological agent design.

2. APJ signaling

2.1. The endogenous agonists of APJ

2.1.1. Apelin

APJ was an orphan receptor until 1998 when its first

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endogenous ligand named apelin for *APJ* endogenous *lig*and was identified from bovine stomach extracts (Tatemoto et al., 1998). Apelin gene encodes for a 77 preproprotein containing a signal peptide in its amino-terminal (N-terminal) sequence thus directing apelin in the secretory pathway. Interestingly, apelin propeptide contains several basic amino acids doublets implicating potential proteolytic cleavage sites for endopeptidases which would give rise to several bioactive carboxy-terminal (C-terminal) fragments including apelin-36, apelin-17 and apelin-13 (Fig. 1A) (Masri et al., 2005). Moreover, the N-terminal glutamate of apelin-13 can be post-translationally modified thus creating the pyroglutamate apelin-13 ([pyr-1]-apelin-13) which is more protected from exopeptidase degradation (Habata et al., 1999).

Actually, [pyr-1]-apelin-13 represents the most common fragment in heart and brain whereas apelin-36 predominates in lung, testis and uterus while both fragments are prevalent in mammary gland as demonstrated by chromatography (Kawamata et al., 2001; Maguire et al., 2009). Moreover, apelin fragments have also been found in plasma where apelin-17 and [pyr-1]-apelin-13 may represent the predominant forms (Azizi et al., 2008; De Mota et al., 2004). Even if apelin maturation process is yet poorly understood, Shin et al. recently described direct cleavage of proapelin in bioactive apelin-13. This process is specific of proprotein convertase subtilisin/kexin type 3 but does not require proprotein convertase subtilisin/kexin type 7 (Shin et al., 2013). Thus, it will be of great interest to search for the existence and expression sites of proprotein convertase subtilisin/kexin type 3 and other unknown endoproteases potentially involved in apelin cleavage. Supporting tissues-specific cleavage hypothesis, proprotein convertase subtilisin/kexin type 3 and apelin are both expressed in the heart where apelin-13 represents the predominant form of apelin (Beaubien et al., 1995). Moreover, they are co-expressed in adipose tissue and their expression level is upregulated with obesity (Shin et al., 2013). Accordingly, tissue specific expression of endopeptidases involved in apelin maturation process could explain why the various apelin forms are differentially represented in organism.

Alignment of apelin sequences from different species revealed a full conservation of the C-terminal amino acids. Moreover, the 13 carboxy-terminal residues represent the smallest physiological entity with maximal activity (Habata et al., 1999; Tatemoto et al., 1998). Deletion of the different amino acids from the N-terminal side of apelin-13 revealed that apelin-12 reduced blood pressure whereas apelin-11 was inactive (Lee et al., 2005; Tatemoto et al., 2001). Accordingly, these data suggest that the 12 carboxy-terminal residues correspond to the minimal active structure. Crucial residues for apelin biological function were characterized using an alanine scanning approach. In this way, different studies have highlighted that residues within the Arginine–Proline–Arginine–Leucine motif and the Lysine–Glycine–Proline–Methionine motif of apelin-13 are important for binding activity, inhibition of adenylyl cyclase or internalization of APJ receptor (Fan et al., 2003; Medhurst et al., 2003).

Another important residue for apelin biological activity *in vitro* and *in vivo*, is the C-terminal phenylalanine. As well as angiotensin II, dynorphin A and des-Arg^9-bradykinin, apelin fragments are also targeted by Angiotensin-Converting Enzyme 2 which catalyses the cleavage of the C-terminus phenylalanine (Vickers et al., 2002). Apelin-17 lacking this phenylalanine (apelin-K16P) can no longer induce APJ internalization or lower blood pressure while it retains binding and signaling properties (inhibition of forskolin-induced cAMP production) (El Messari et al., 2004). Attesting these results, Ceraudo et al. recently demonstrated that apelin-K16P was still a bioactive peptide (Ceraudo et al., 2014). This point will be discussed later in the review.

2.1.2. Toddler/Elabela

Different observations in the literature suggested the possible existence of another ligand for APJ or another receptor for apelin. Firstly, apelin knock-out (apelin KO) mice are viable with a Mendelian inheritance pattern, which is not found for APJ knock-out (APJ KO) mice (Kang et al., 2013). Secondly, the animal phenotype after functional deletion of apelin and APJ genes should be similar, if not identical. In fact, APJ KO mice present developmental defects mainly at the cardiovascular level and this prominent phenotype is not found in apelin KO animals. The same observations were also made in developmental studies in zebrafish where knockdown of apelin protein expression using antisense morpholinos oligonucleotides does not phenocopy loss of APJ (Scott et al., 2007; Zeng et al., 2007). Third, APJ is expressed early during gastrulation and throughout the subsequent development stages (Devic et al., 1996, 1999) whereas apelin expression starts only at the end of gastrulation (Zeng et al., 2007) suggesting the possible existence of another unknown ligand for APJ or ligand-independent functions of APJ.

Recently, two different studies aiming at discovering novel signals involved in embryonic development regulation identified a new endogenous peptide ligand for APJ, named Elabela or Toddler (Chng et al., 2013; Pauli et al., 2014). Elabela/Toddler is highly expressed during gastrulation and its knockdown in zebrafish mirrors the phenotype of loss of APJ expression. Like apelin, Elabela/Toddler encodes a conserved vertebrate protein of 54 amino acids consisting of an N-terminal signal-peptide addressing the protein to the secretory pathway and a mature 32 amino acids peptide. Elabela/Toddler may generate several fragments due to the presence of doublets of basic residues predicting cleavage sites in its peptide sequence (Fig. 1B). This new APJ ligand plays a crucial role in heart morphogenesis inducing mesendodermal cells movement and endoderm differentiation.

As far as Elabela/Toddler is concerned, nothing is yet known regarding its functions in adults. Since Elabela/Toddler is a critical ligand for APJ regarding development, it will be important in the future to define if this new endogenous ligand is also expressed in adulthood but also to characterize its expression profile and its cellular and tissular functions.

2.2. Signal transduction pathways activated by APJ

The canonical signaling pathway stimulated by apelin involves activation of the *pertussis toxin* (PTX) sensitive G proteins, $G_{i/o}$. The first report of this G_i protein coupling has been established in assays measuring extracellular acidification rates with apelin-13 and apelin-36 (Hosoya et al., 2000). These results were then confirmed by numerous studies demonstrating a PTX sensitive activation of intracellular effectors such as Extracellular signal-Regulated Kinases (ERKs), protein kinase B (PKB or Akt) and p70S6 kinase induced by apelin but also the inhibitory action of apelin on forskolin-induced cAMP production (D'Aniello et al., 2009; Masri et al., 2002, 2004). The murine apelin receptor is preferentially coupled to $G_{\alpha i1}$, $G_{\alpha i2}$ but not $G_{\alpha i3}$ in Chinese Hamster Ovary cells through which apelin induces ERKs and Akt activation and inhibits adenylyl cyclase (Masri et al., 2006). Likewise, the same results were obtained in HEK293 cells expressing the human APJ receptor which activates $G_{\alpha i2}$ (Bai et al., 2008; Chen et al., 2014). However, depending on the cell type studied, apelin receptor can be associated with other G proteins. Indeed, in Human Umbilical Vein Endothelial Cells (HUVECs), APJ has been shown to activate $G_{\alpha 13}$ resulting in histone deacetylases type 4 and type 5 phosphorylation and cytoplasmic translocation (Kang et al., 2013) whereas in adipocytes APJ is coupled to G_i and $G_{q/11}$ proteins (Yue et al., 2011). This latter double coupling of APJ was confirmed *in vivo* where PTX, protein kinase C or phospholipase C inhibitors pretreatment

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