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Targeting the apelin pathway as a novel therapeutic approach for cardiovascular diseases

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ABSTRACT

The apelin/apelin receptor system is widely distributed and has a dominant role in cardiovascular homeostasis and disease. The apelin gene is X-linked and is synthesized as a 77 amino acid pre-pro-peptide that is subsequently cleaved to generate a family of apelin peptides that possess similar functions but display different tissue distribution, potency and receptor binding affinity. Loss-of-function experiments using the apelin and the apelin receptor knockout mice and gain-of-function experiments using apelin peptides have delineated a well-defined role of the apelin axis in cardiovascular physiology and diseases. Activation of the apelin receptor by its cognate peptide ligand, apelin, induces a wide range of physiological effects, including vasodilation, increased myocardial contractility, angiogenesis, and balanced energy metabolism and fluid homeostasis. The apelin/apelin receptor pathway is also implicated in atherosclerosis, hypertension, coronary artery disease, heart failure, diabetes and obesity, making it a promising therapeutic target. Hence, research is expanding to develop novel therapies that inhibit degradation of endogenous apelin peptides or their analogues. Chemical synthesis of stable apelin receptor agonists aims to more efficiently enhance the activation of the apelin system. Targeting the apelin/apelin receptor axis has emerged as a novel therapeutic approach against cardiovascular diseases and an increased understanding of cardiovascular actions of the apelin system will help to develop effective interventions.

1. Introduction

Apelin is an endogenous peptide capable of binding the apelin receptor, which was originally described as an orphan G-protein-coupled receptor [1]. Upon proteolysis through a not fully elucidated mechanism, pre-pro-apelin is converted into specific fragments ranging from 36 to 13 amino acids, with the 12 C-terminal amino acids conserved among all apelin isoforms [2]. Both apelin and apelin receptor are expressed across a wide range of eukaryotes including humans, in a variety of tissues including the central nervous and cardiovascular systems [3]. Apelin and its cognate receptor are essential for diverse biological processes and play important roles in the regulation of cardiovascular function [4,5]. The beneficial effects of the apelin/apelin receptor system are well established by treating with apelin in conditions as diverse as hypertension, atherosclerosis, myocardial infarction, heart failure (HF) and pulmonary arterial hypertension (PAH) [6–8]. Recently, the apelin receptor has been shown to be activated by a novel endogenous peptide ligand known as apela/Elabela/Toddler, with an important role in cardiovascular development and function [9,10]. This further enriched the understanding of the multiple physiological mechanisms of the apelin/apelin receptor system. The abilities of exogenous apelin to rescue heart and vascular diseases support the therapeutic potential of apelin in preventing and treating cardiovascular diseases. Nonetheless, therapeutic application of apelin is limited by its short half-life and parenteral administration. Thus great effort has been directed to the development of novel agonists or analogues, efficient delivery methods and improved efficacy of agonists at the apelin receptor [11]. This review summarizes the development and the latest advances concerning the

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apelin/apelin receptor signaling pathway and its role in cardiovascular physiology.

2. Physiology of the apelin pathway

2.1. Biochemistry

The apelin gene is located on the X chromosome and is synthesized as a 77 amino acids pre-pro-peptide that contains a high number of basic amino acid residues [1]. Pyr-apelin 13 and apelin 17 are the predominant isoforms in plasma whereas apelin 36 is predominant in lung, testis, uterus and colostrum [12–15]. Furthermore, pyr-apelin 13 has been detected as the primary isoform in human heart, bearing the greatest affinity for the apelin receptor [1,13,15]. Binding with apelin 13 leads to internalization of the apelin receptor followed by recycling to the cell surface whereas apelin 36 promotes intracellular sequestration of the receptor [16]. Although the apelin receptor is present in both vascular endothelium and vascular smooth muscle cell (VSMC), the predominant source of apelin appears to be located in the endothelium as well as in adipocytes [17,18].

Apelin 13 and 36 have short plasma half-lives in the range of 5–8 min. This is attributed to the degradation by endogenous circulating proteases [15,19]. Interestingly, apelin 13 and apelin 36 are substrates of angiotensin converting enzyme 2 (ACE2) [20,21], which is a pleiotropic monocarboxypeptidase capable of catalyzing a diverse range of peptide substrates [22,23]. There is a functional interplay between apelin and ACE2 characterized by increased ACE2 promoter activity *in vitro* and elevated expression of ACE2 in failing hearts *in vivo* in response to apelin treatment [24]. Our latest work revealed that ACE2 represents a major negative regulator of apelin in the vasculature and heart. ACE2 potentiated the degradation of pyr-apelin 13 and apelin 17 through cleavage of their C-terminal phenylalanine (Fig. 1), thereby producing pyr-apelin 12 and apelin 16 whose cardioprotective effects were negligible [20]. This observation was previously confirmed using synthetic C-terminally truncated apelin peptides in stably transfected CHO cell lines expressing the rat apelin receptor [25]. Sequential removal of the last three amino acids of apelin 17 showed equivalent receptor binding and comparable cAMP inhibition, exhibited a dramatic reduction in receptor internalization [25]. Computational modeling predicted a key pi-stacking interaction between the C-terminal phenylalanine with aromatic residues within the apelin receptor [26]. Disruption of this hydrophobic interaction via synthetic truncation [26–28], alanine substitution [26,29], or site-directed mutagenesis of receptor aromatic residues [26] significantly decreased receptor internalization and downstream cardioprotective effects. Therefore the C-terminus of apelin isoforms is essential for the full agonistic activity of the apelinergic system [27]. N-terminal truncation of apelin isoforms has minimal impact on receptor binding and corresponding physiological activity until the conserved C-terminal 12 amino acid core is disturbed [30]. Removal of any of the amino acids within the 'RPRL' motif completely abolishes receptor binding via disruption of the key electrostatic interactions with the apelin receptor [28,31]. Most recently, the metalloprotease neprilysin (NEP), a target for Entresto™ used in treatment of HF, was identified as an enzyme that degrades and inactivates apelin peptides via proteolysis within this region (Fig. 1). This new discovery contributes to our understanding of the mechanisms of NEP inhibition and the apelin/apelin receptor system in cardiovascular physiology and disease [32].

2.2. Mechanism of action

Apelin underlies a dual action in both animal and human tissues depending on the presence of an intact endothelium [33]. The net effects of the apelin/apelin receptor signaling in the intact endothelium are vasodilation. In contrast, vasoconstriction prevailed after damage of the vascular endothelium [13]. The hypotensive action of apelin has been demonstrated in healthy rats, to which intravenous injection of apelin leads to immediate lowering of both systolic and diastolic blood pressure [34]. In hypertensive rats, treatment with apelin has been shown to lower systolic blood pressure levels via a nitric oxide (NO)-dependent signaling [35,36]. Given the elevated expression during the formation of the retinal vessels, endogenous apelin is required for normal vascular development that is fueled by induced endothelial NO synthase (eNOS) phosphorylation (Table 1 & Fig. 2) [37,38]. Consistent with this, apelin 13 stimulates angiogenesis by potentiating the interaction between adenosine monophosphate activated protein kinase (AMPK) and Akt signaling in myocardial microvascular endothelial cells (ECs) (Table 2 & Fig. 2) [39]. The apelin receptor and neurotensin receptor 1 (NTSR1) can form a functional heterodimer which enhances activation of extracellular signal-regulated kinase 1/2 (ERK1/2) signaling and proliferation in human umbilical vein endothelial cell (HUVEC), providing a new potential pharmaceutical target for cardiovascular disease [40].

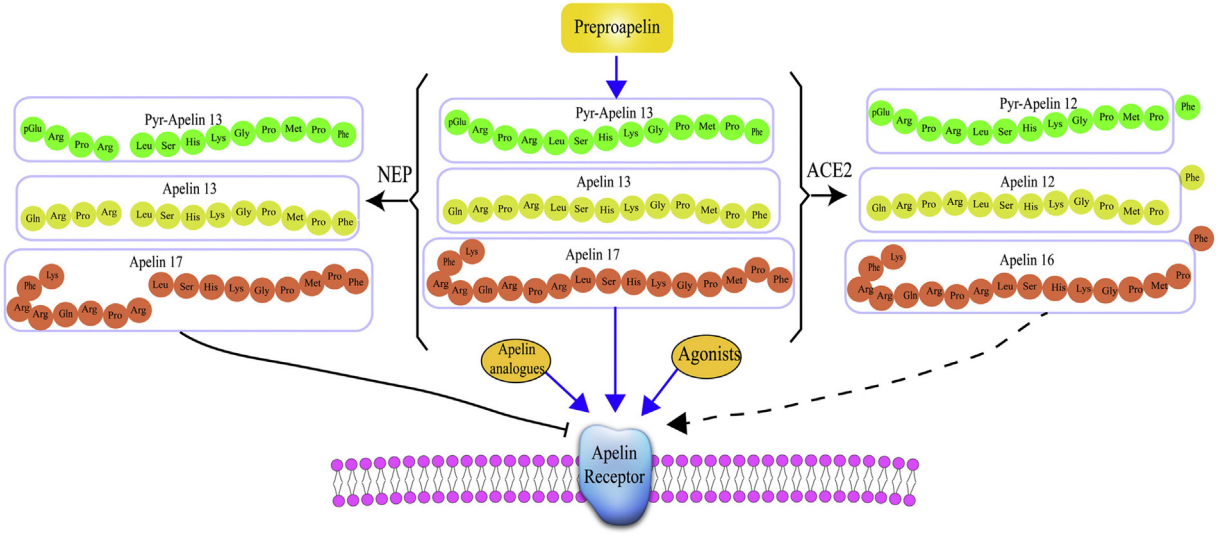

Fig. 1. The biochemistry of the apelin/apelin receptor system. The 77 amino acids pre-pro-apelin is cleaved to generate fragments of various size. ACE2 potentiates the degradation of pyr-apelin 13, apelin-13, -17 and -36 through cleavage of their C-terminal phenylalanine, subsequently producing pyr-apelin 12, apelin-12, -16 and -35, respectively. Neprilysin degrades apelin at the conserved C-terminal 12 amino acids, abrogating the binding of apelin to its receptor. ACE2, angiotensin-converting enzyme 2; NEP: neprilysin.

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