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The apelinergic system: a perspective on challenges and opportunities in cardiovascular and metabolic disorders.

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Abstract

The apelinergic pathway has been generating increasing interest in the past few years for its potential as a therapeutic target in several conditions associated with the cardiovascular and metabolic systems. Indeed, preclinical and, more recently, clinical evidence both point to this G protein–coupled receptor as a target of interest in the treatment of not only cardiovascular disorders such as heart failure, pulmonary arterial hypertension, atherosclerosis, or septic shock, but also of additional conditions such as water retention/hyponatremic disorders, type 2 diabetes, and preeclampsia. While it is a peculiar system with its two classes of endogenous ligand, the apelins and Elabela, its intricacies are a matter of continuing investigation to finely pinpoint its potential and how it enables crosstalk between the vasculature and organ systems of interest. In this perspective article, we first review current knowledge on the role of the apelinergic pathway in the above systems, as well as the associated therapeutic indications and existing pharmacological tools. We also offer a perspective on the challenges and potential ahead to advance the apelinergic system as a target for therapeutic intervention in several key areas.

Graphical abstract

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Competing interests

The authors declare no competing interests.

This perspective article reviews current knowledge on the role of the apelinergic pathway in the cardiovascular and metabolic systems and discusses recent evidence pointing to this G protein–coupled receptor as a target of interest in the treatment of not only cardiovascular disorders, but also of conditions such as water retention/hyponatremic disorders, type 2 diabetes, and preeclampsia.

Keywords

apelin; Apela/Toddler/Elabela; apelin receptor/APLNR/APJ; cardiovascular protection; heart failure; pulmonary arterial hypertension; water retention/hyponatremic disorders; hypertension; septic shock; type 2 diabetes; preeclampsia

Introduction

Cardiovascular and metabolic diseases are prevalent in most societies and continue to rise, despite knowledge of important and preventable risk factors such as unhealthy diet, insufficient physical activity, and smoking.[1] These risk factors and their determinants are major contributors to vascular endothelial and multi-organ dysfunctions. The development of novel and more effective therapies is thus urgently needed to curb the incidence, morbidity, and mortality associated with cardiovascular and metabolic diseases.

Among systems contributing to the maintenance of vascular homeostasis, the apelinergic system stands out for its pleiotropic beneficial impacts on cardiovascular and metabolic insults. In this perspective article, we first review current knowledge of the apelinergic system as it pertains broadly to cardiovascular, body fluid, and metabolic homeostasis, then synthesize recent developments and identify questions to address in order to advance this target toward therapeutic applications. This manuscript is a joint effort resulting from the RegPep2018 symposium on the apelinergic system organized by the International Regulatory Peptide Society in October 2018.

Discovery of the apelinergic system

In 1993, O'Dowd *et al.* cloned from a human genomic library the cDNA of an orphan receptor that was then named APJ (putative receptor protein related to the type 1 angiotensin receptor).[2] Subsequently, the APJ receptor (also known as the apelin receptor (APLNR)) was isolated in amphibians[3] and rodents.[4, 5] The human receptor is 380 amino acids (aa) long and belongs to the seven transmembrane domain G protein–coupled receptors (GPCRs) superfamily. It shares 31% aa sequence identity with the human angiotensin type 1 (AT1) receptor,[2] yet does not bind radiolabeled angiotensin II (Ang II). Moreover, treatment of the rat apelin receptor with Ang II does not affect cyclic adenosine monophosphate (cAMP) production, confirming that it is not an angiotensin receptor subtype.[4]

As an example of reverse pharmacology, the apelin receptor was deorphanized in 1998 when apelin (APJ Endogenous LIgaNd) was isolated from bovine stomach tissue extracts as apelin-36, its longest endogenous ligand with sub-nanomolar affinity, by Tatemoto *et al.*[6] Apelin is generated from a 77-aa precursor, preproapelin. Alignment of preproapelin sequences from cattle, humans, rats, and mice shows strict conservation of the C-terminal

portion (aa 61–77), known as apelin-17 (also known as K17F).[6] *In vivo*, apelin circulates as several isoforms differing in length (corresponding to the 36-, 17-, or 13-aa of the C-terminal part of preproapelin), commonly called apelin-36, apelin-17, and apelin-13 (Fig.1).[7–9] Apelin-13 is naturally pyroglutamylated at its N-terminus (Fig.1). Apelin-36 predominates in rat lung, testis, uterus, and bovine colostrum, while it is detected together with Pyr[1]-apelin-13 in the rat mammary gland.[8] The most abundant forms of apelin in rat brain as well as rat and human plasma are apelin-17 and Pyr[1]-apelin-13, whereas the concentration of apelin-36 is much lower.[7, 10] In the heart, the most abundant form is Pyr[1]-apelin-13.[11] Due to the presence of pairs of basic residues in preproapelin, prohormone convertases are presumed to be responsible for the processing of its precursor to generate apelin-17 and apelin-13 (Fig.1). The ability of the proprotein convertase subtilisin/kexin 3 (also known as furin) to cleave proapelin directly into apelin-13 without generating longer isoforms was shown *in vitro*.[12]

A recent peculiar discovery came from the cloning by two independent groups of *Apela* (also known as Elabela or Toddler), a distinct gene encoding a second endogenous ligand of the apelin receptor with sub-nanomolar affinity.[13, 14] Mature Elabela is a 32-aa peptide relatively well conserved in vertebrates and secreted following the removal of its N-terminal 22 aa-long signal peptide. Although putative furin cleavage sites were predicted to generate shorter Elabela peptides,[13, 15] their *in vivo* detection still needs to be demonstrated. While Elabela is broadly expressed during development, its mRNA transcript remains highly expressed during adulthood in the prostate and kidney.[15] Yet other sources of Elabela secretion, such as the endothelial cells (ECs) of adult human arterial vessels,[16] may also significantly contribute to its presence in the circulation.

Proteolytic inactivation of apelins—Angiotensin-converting enzyme 2 (ACE2, EC 3.4.17.23), the first protease implicated in the physiological regulation of apelins, removes the C-terminal phenylalanine residue of apelin-36, apelin-17, apelin-13, and Pyr[1]-apelin-13 (Fig.1).[17, 18] Cleavage of apelin-17 by ACE2 generates apelin-16, which retains sub-nanomolar affinity for the apelin receptor and similar efficacy of $G_{\alpha i/o}$ protein coupling. However, apelin-16 exhibits an opposite profile of ligand-induced receptor internalization and blood pressure regulation,[19, 20] making apelin-16 a bona fide biased agonist of the receptor. Interestingly, the apelin–ACE2 axis forms a feedback loop as demonstrated by the necessity of apelin-13 to maintain and upregulate ACE2 expression *in vivo* (Fig.2).[21] Using a peptide-based fluorescent probe, the membrane-bound, zinc-dependent metalloprotease neprilysin (NEP, EC 3.4.24.11) was recently identified as another enzyme that cleaves apelin isoforms (Fig.1).[22] *In vitro,* proteolysis by NEP generated fragments lacking affinity for the apelin receptor, making NEP the first protease to fully inactivate apelin (Fig. 2).[23] Synthetic analogs of apelin modified within the NEP degradation site (“RPRL” motif, Fig.1) show improved *in vitro* proteolytic stability while maintaining receptor affinities. Many such analogs proved physiologically inactive even with relatively conservative modifications, highlighting the importance of this region for full agonist activity. The recognition that NEP is a major enzyme that completely inactivates apelin peptides provides a novel mode of action of NEP inhibitors as potential therapeutic agents in cardiovascular diseases.[22, 24, 25]

More recently, human plasma kallikrein (KLKB1) has been identified as a protease that cleaves the first three N-terminal amino acids (KFR) of apelin-17.[26]

Signaling—Evidence for the coupling of apelin receptor with $G_{\alpha i/o}$ protein was revealed by the team of Fujino and other laboratories (Fig.2).[4, 6, 8, 9, 27] Stable overexpression in CHO cells of the rat apelin receptor fused at its C-terminal portion with enhanced green fluorescent protein showed the sub-nanomolar efficacy of apelin isoforms (i.e., apelin-17 and Pyr^1-apelin-13) to inhibit forskolin-induced cAMP production.[4, 28] Pyr^1-apelin-13, apelin-13, and apelin-17 stimulation of ERK1/2 MAPK phosphorylation depends on a pertussis toxin–sensitive G protein in CHO cells overexpressing the receptor (Fig.2).[19, 28, 29] While the apelin receptor preferentially couples with protein $G_{\alpha i1}$ and $G_{\alpha i2,}$ but not $G_{\alpha i3}$,[30] the identity of the subtypes of the $G_{\beta\gamma}$ protein subunits released upon activation remains largely unknown. Additional coupling to the $G_{\alpha q}$ protein upon ligand binding was implied in studies of cultured rat basophil–derived cells, adipocytes, and neuronal cells.[31–33] However, the lack of Ca^{2+} mobilization and arachidonic acid release from CHO cells stably overexpressing the apelin receptor,[27] and the decreased amplitude of Ca^{2+} release upon apelin receptor–dependent signaling in healthy adult cardiomyocytes,[34] collectively suggest that significant $G_{\alpha q}$ protein signaling following apelin receptor activation, if any, may depend on cellular context.

As expected for a GPCR, agonist-induced apelin receptor activation also elicits recruitment of β-arrestins (Fig. 2) and subsequent internalization through a clathrin-dependent mechanism, while removal of apelin's C-terminal phenylalanine abrogates this process.[20, 35] Accordingly, apelin analogs biased toward $G_{\alpha i/o}$ protein signaling and inefficiently recruiting β-arrestins lose the ability to induce vasorelaxation and reduce blood pressure.[19, 20, 36] This functional dissociation between $G_{\alpha i/o}$ proteins and β-arrestin signaling has been shown to lead to the phosphorylation of MAPK ERK1/2 by two different pathways, one dependent on $G_{\alpha i/o}$ protein coupling and the other dependent on β-arrestins (Fig. 2).[19, 37] Interestingly, apelin-13 and apelin-36 determine different fates of internalized apelin receptor by modulating its interaction with β-arrestin1.[38] Based on accumulating evidence about β-arrestin–dependent signaling of internalized GPCRs,[39] it is likely that the endocytosed apelin receptor exerts important biological functions of the apelinergic system that have yet to be uncovered.

Distribution—Analysis of the apelin receptor transcript expression indicates its presence in rat and human brain (e.g., hypothalamus and medulla),[2, 5, 4, 40] anterior pituitary,[4, 5] and highly vascularized organs of the body (e.g., lungs, heart, spleen, and kidney).[8, 33, 40, 41] Important interspecies differences for the expression profile of transcripts of apelin and its receptor in tissues were noted.[33] However, expression of the receptor, as revealed by immunohistochemistry and binding assays performed on ECs of both murine[42] and human vessels,[43, 44] is in accordance with its potent cardiovascular and metabolic effects. Similarly, the endothelial production of apelin was demonstrated by *in situ* hybridization,[45] immunohistochemistry,[46] and reporter gene expression assays,[47] while the mRNA encoding Elabela was found to be highly expressed during adulthood mainly in the kidney.[15] Based on the distribution of the receptor and its endogenous ligands, apelin and Elabela, the

therapeutic potential of the apelinergic system has been predominantly explored based on its physiological roles in cardiovascular, respiratory, and metabolic functions (Fig. 3), as well as body fluid homeostasis (Fig. 4). As a result, the apelinergic system has emerged as a potential target for diseases associated with the vascular system, heart failure (HF), pulmonary arterial hypertension (PAH), water retention/hyponatremic disorders, and type 2 diabetes, but also more recently in conditions bearing a strong cardiac and/or vascular component, such as septic shock and preeclampsia. These are further elaborated below.

Vascular physiological effects of apelinergic signaling

Numerous studies have provided insight into the vascular effects of apelinergic signaling.[48, 49] For example, apelin-13 produced relaxation in normal human splanchnic arteries via NO release after activation of apelin receptors located in the endothelium (Fig.2).[50] While administration of apelin-36 and Pyr^{1}-apelin-13 to humans caused NO-dependent arterial vasodilation,[51] apelin-17 provoked NO-dependent vasorelaxation of rat renal glomerular arterioles or rat aorta that were precontracted with Ang II or norepinephrine, respectively.[41, 52] Establishing the expression profile of apelin and its receptor relied on several immunohistochemistry, *in situ* hybridization, and reporter gene expression studies investigating the exact cell types that express the ligand and/or its receptor (see above). The recent identification of Elabela as another endogenous ligand of the apelin receptor added another dimension of complexity to this signaling paradigm.[13, 14] In the developing vasculature of the mouse retina, apelin expression is highly restricted to tip cells that demarcate the leading edge of the developing vasculature, while apelin receptor expression is restricted to the endothelium of the stalk cells that form developing luminal structures.[53] Evaluation of ligand and receptor expression in adult mice demonstrated the predominantly endothelial expression of the ligand/receptor combination.[54, 55] An important caveat to these findings is that the expression profiling discussed above are limited to evaluation in normal homeostatic or developmental context. While other studies have described the expression of apelin or its receptor in disease models, the use of newly developed and highly specific knock-out validated antibodies[56, 57] and reporter gene expression models is expected to provide more rigorous, reproducible, and reliable findings in future studies.

Role of apelin and its receptor in vascular disease models

Multiple studies have focused on apelinergic signaling in vascular disease models.[49] Key vascular pathologies found to be impacted by the apelinergic system include atherosclerosis,[58] arterial hypertension,[45] and PAH (Fig. 3).[16, 59–61] In the context of atherosclerosis, loss of apelin resulted in exacerbation of atherosclerosis in $Apoe^{-/-}$ mice subjected to a high fat diet, and supplemental apelin administration led to significant improvement in atherosclerosis, which was due, at least in part, to its activation of the nitric oxide pathway and inhibition of Ang II signaling.[58] These findings contrast with the pro-atherosclerotic effect of targeting the apelin receptor in $Apoe^{-/-}$ mice fed with high fat diet,[62] but the experimental design of these two studies differ in several aspects. Indeed, the distinct mouse genetic backgrounds, the moderate vs. high cholesterol–containing diets, and the potential activation of compensatory loci alleviating the profound disruptive effect of targeting the apelin receptor gene on embryonic vascular development might explain these confounding results, as previously discussed.[58] Considering the increased expression of apelin in human

atherosclerotic plaques[63] and stenotic aortic valves,[64] the anti- or pro-atherosclerotic roles of the apelinergic system in this context still have to be clarified.

In diabetic (*Lepr*$^{db/db}$) mice, apelin-36 restores the altered aortic vascular responsiveness to acetylcholine and Ang II by potentiating phosphorylation of Akt and eNOS (Fig. 2).[65] In the context of blood pressure regulation, acute intravenous (i.v.) administration of various forms of apelin (i.e., apelin-36, apelin-17, Pyr^{1}-apelin-13, and apelin-12) in anesthetized rodents consistently leads to short-term lowering of blood pressure.[20, 28, 45, 66] Consistent with these data, *Apln*$r^{-/-}$ mice display an exaggerated pressor response to systemic Ang II, suggesting a counterregulatory effect of apelin on Ang II.[67] Interestingly, *Apln*$r^{-/y}$ mice do not have significant differences in systemic blood pressure, suggesting that compensatory mechanisms may be activated or downregulated to maintain blood pressure homeostasis.[68] This is discussed in more detail in the section on body fluid homeostasis.

With respect to PAH, a number of approved classes of drugs have led to improvements in clinical outcomes,[69] yet mortality remains exceedingly high.[70] PAH is a progressive pulmonary vasculature disease that results from an intricate combination of vasoconstriction, thrombosis, inflammation, and proliferative-obstructive remodeling of the pulmonary arterial vasculature, which subsequently leads to right ventricular failure.[71] In fact, impaired pulmonary arterial endothelial cell (PAEC) function is a key feature of PAH. Apelin has been identified among the principal genes affected by right HF and PAH.[72] Although acute hypoxia or hypoxia-inducible factor exposure induce apelin cellular expression/secretion,[73–75] low circulating levels and cardiopulmonary expression of apelin-13 and Elabela are ultimately observed in patients with established PAH.[16] Accordingly, PAECs from PAH patients display decreased expression of apelin, higher apoptotic index, and the promotion of pulmonary arterial smooth muscle cells proliferation that can be rescued by the administration of apelin or bone morphogenetic protein receptor 2 ligands.[76] However, contradictory data have been published on apelins and the proliferation of smooth muscle cells,[76, 77] which needs to be further investigated. Importantly, acute apelin infusion in patients with primary PAH produces beneficial hemodynamic effects without associated systemic hypotension and adverse effects,[78] suggesting a potential therapeutic role for apelinergic signaling in this devastating disease.

Apln$^{-/-}$ mice exhibit worsened hypoxia-induced PAH, and multiple downstream targets and upstream regulators of apelin signaling have been identified to have key roles in pulmonary vascular homeostasis.[16, 59, 61] For instance, significant disruption of an endothelial miRNA-dependent apelin–FGF2 link was demonstrated to trigger endothelial dysfunction and PAH.[61] Additional evidence of the deleterious impact of apelin disruption and/or antagonism of its receptor is exemplified by the serious adverse reactions sometimes observed using protamine (a sub-micromolar affinity competitive antagonist of the apelin receptor that reverses heparin anticoagulation), and by the occurrence of sudden rises in pulmonary arterial pressure, sustained hypotension, and impaired cardiac fractional shortening.[59, 79–82] Apelins can also potentially impact long-term vascular remodeling by reducing inflammation[83–87] and fibrosis.[88–92]

Cardiac effects of the apelinergic system and its potential for heart failure treatment

While HF is common and characterized by high morbidity and mortality,[24] better understanding and treatment of HF has significantly improved outcomes over the past two decades.[93] Despite better medical and surgical therapies, ischemic, hypertensive, and obesity-related heart diseases remain leading causes of HF.[94, 95] Acute and chronic HF are characterized by activation of several signaling pathways associated with pathological hypertrophy and maladaptive ventricular remodeling. HF is caused by damage to or loss of cardiomyocytes, and contributes to diminished systolic performance and diastolic dysfunction in the failing heart.[94, 96] HF involves changes in cardiac structure, myocardial composition, myocyte deformation, and multiple biochemical and molecular alterations, collectively referred to as adverse myocardial remodeling.[97, 98] The patient profile of HF has evolved significantly recently; while HF with reduced ejection fraction (HFrEF) is declining due to effective revascularization of patients with acute coronary syndromes, the prevalence and incidence of HF with preserved ejection fraction (HFpEF), mainly characterized by diastolic dysfunction, is still increasing.[99] Myocardial hypertrophy, systemic inflammation, and endothelial dysfunction are the major pathophysiological events in HFpEF.[100–102]

Lessons from loss-of-function experiments—Important observations on the potential impact of the apelinergic system in HF were obtained from loss-of-function approaches. Indeed, aged *Apln*$^{-/y}$ mice develop progressive impairment of myocardial contractility along with systolic dysfunction, and loss of apelin contributes to HF in response to pressure overload.[88] Deletion of *apelin* also leads to aggravated left ventricular injury following MI, while a synthetic apelin receptor agonist markedly protects from myocardial ischemia/reperfusion (I/R) injury.[103] This is accompanied by greater activation of survival pathways and promotion of angiogenesis. Stretch-dependent apelin receptor signaling, rather than ligand-induced apelin receptor signaling, contributes to increased cardiomyocyte cell size and myocardial hypertrophy (Fig. 2).[104] Since the apelin receptor responds to both mechanical stretch and endogenous apelin in cardiac hypertrophy (Fig. 2), interventions aiming to balance these stimuli may determine the resulting adaptive physiology of the apelinergic system.[104] In the two-kidney, one-clip hypertensive rats, reduction in apelin and its receptor in the heart and aorta aggravates pathophysiological responses to hypertension and HF.[25] Reduced plasma and myocardial apelin levels in patients with advanced HF, with concomitant reduction in expression of its receptor, clearly suggest that the apelinergic system is compromised in HF.[105, 106] However, discrepant findings have been reported, with no difference in plasma apelin levels noted in patients with dilated cardiomyopathy. Discrepancies among these studies may be related to the quality of the assays, often immunoassays with different qualities of antibodies. In this context, the development of antibody-free quantification methods based, for example, on tandem mass spectrometry, would be a great boost to the quality of future studies.[107–109]

Several mechanistic studies provide insight into the role of apelinergic signaling in cardiac function and disease. Inactivation of Forkhead box protein O1 (FOXO1) and inhibition of endothelial expression of fatty acid (FA) binding protein 4 (FABP4) were identified as key downstream signaling targets of apelin/receptor signaling (Fig. 2). Both *Apln*$^{-/-}$ and apelin receptor constitutive and EC-specific knock-out (KO) mice demonstrate increased

endothelial FABP4 expression and excess tissue FA accumulation.[55] In obese patients, higher left ventricular ejection fraction is associated with higher levels of plasma apelin, and in an obese murine model of I/R injury, apelin prevents nuclear translocation of FOXO3 in response to oxygen deprivation, providing insights into its potential clinical relevance in obese patients with HF.[110] Ang II infusion potentiated oxidative stress, pathological hypertrophy, and myocardial fibrosis in young *Aplnr*$^{-/y}$ mice, resulting in exacerbation of cardiac dysfunction in association with reduced ACE2 levels.[111] Downregulation of the apelin pathway action lowers ACE2 levels, leading to activation of the renin-angiotensin system (Fig. 2).[21, 88, 112] Indeed, deletion of *apelin* in mice leads to cardiac dysfunction, which is abrogated by blockade of the AT1 receptor or using AT1 receptor KO mice.[21] The intricate interplay between the apelin and angiotensin pathways is also demonstrated by the heterodimerization of their receptors induced by apelin-13, which negatively regulates the allosteric binding site and function of the AT1 receptor (Fig. 2).[113] These findings provide mechanistic insights that could greatly expand the therapeutic applications for the apelinergic system in cardiomyopathic, hypertensive, and metabolic disorders.[55, 88]

Gain-of-function approaches—Systolic and diastolic function, as well as adverse cardiovascular remodeling, were drastically improved in response to infusion of Pyr^{1}-apelin-13 in hypertensive rats, and this was accompanied by decreased expression of inflammatory cytokines.[114, 115] Apelin-mediated activation of PKCε and ERK1/2 was responsible for enhancement of cardiac contractility, myosin light chain kinase being the downstream target (Fig.2).[25, 116] Circulating apelin levels are reduced in patients with essential hypertension, and lower plasma apelin levels are independently associated with greater left ventricular systolic and diastolic dysfunction, indicating that apelin may serve as a biomarker for increased risk of chronic HF.[108, 117] However, the measurement of blood apelins, unlike N-terminal pro B-type natriuretic peptide and galectin-3, is reported not to be useful to fine tune the diagnosis of acute HF,[118] whereas the blood levels of cardiac troponin are more likely prognostic.[119] A bolus of exogenous apelin lowers blood pressure, an effect abrogated in *Aplnr*$^{-/-}$ mice.[67] These effects are paralleled in humans where apelin peptides induce peripheral and coronary vasodilatation while increasing cardiac output and contractility.[120] Importantly, both local vascular and systemic hemodynamic responses to apelin are preserved in patients with stable symptomatic chronic HF treated by current medical therapy.[120, 121] Expression of apelin and its receptor in human ECs is associated with shear stress, in which expression of the receptor is induced independently of its ligand.[122]

Plasma apelin levels predict the major cardiovascular event after percutaneous coronary intervention in patients with ST-segment elevation myocardial infarction, and adverse events are higher in patients with lower plasma apelin levels.[123] Apelin and its receptor are markedly upregulated in the heart and skeletal muscle following myocardial injury or systemic hypoxic exposure through a hypoxia-inducible factor-mediated pathway.[54] In mice fed with a high-fat diet subjected to myocardial I/R injury, apelin-13 alleviated infarct size, myocardial apoptosis, and mitochondrial damage.[25, 124] Apelin gene therapy increases vascular density and alleviates diabetic cardiomyopathy via a mechanism involving activation of Sirt3 and upregulation of VEGF/VEGFR2 expression (Fig. 2).[25, 125]

Intracellular Ca^{2+} abnormality and endoplasmic reticulum stress mediates cardiac dysfunction induced by myocardial I/R injury, and apelin-13 suppresses these pathogenic pathways.[25, 126] In cultured primary cardiomyocytes under hypoxia/re-oxygenation, apelin administration suppresses apoptosis and generation of reactive oxygen species. In addition, apelin improves cardiac dysfunction after myocardial I/R injury by inhibiting myocardial apoptosis and oxidative stress, along with upregulation of eNOS levels and activation of PI3K/Akt and ERK1/2 phosphorylation signaling.[25, 127] Apelin-mediated protection against cardiac fibrosis results primarily from direct modulation of plasminogen activator inhibitor-1 gene expression, associated with synergistic inhibition of Ang II signaling and increased production of NO.[25] Since apelin-13 upregulates ACE2 levels (Fig. 2),[21] it is also likely to decrease superoxide production and expression of hypertrophy- and fibrosis-related genes, as seen with daily recombinant human ACE2 treatment that significantly attenuated myocardial hypertrophy, fibrosis, and dysfunction in the Ang II-infused *Ace2*$^{-/y}$ mice.[128] Based on the above, the apelin receptor represents a promising target in the treatment of HF. As a result, a first clinical trial with the orally administered small-molecule apelin receptor agonist AMG986 represents an important milestone toward the validation of the target in this indication.[129]

Central and renal effects of apelin on body fluid homeostasis

The colocalization of apelin and arginine-vasopressin (AVP; also know as antidiuretic hormone), together with the apelin and vasopressin type-1 receptors in hypothalamic magnocellular neurons, suggested an interaction between these two hormones (Fig. 4).[10, 130] Early experiments were performed in lactating rats, which exhibit increased activity in magnocellular AVP neurons, leading to increased AVP synthesis and release in order to preserve water for maximal milk production. In this model, intracerebroventricular (i.c.v.) administration of apelin-17 triggered inhibition of the phasic electrical activity of AVP neurons, reduced AVP release in the bloodstream, and increased diuresis, with no change in sodium and potassium excretion.[10] Together, these suggest that apelin is likely released from AVP cell bodies to inhibit both AVP neuron activity and release by autocrine action on the apelin receptor expressed by AVP/apelin-containing neurons.

Moreover, following water deprivation in rodents, increased plasma AVP levels are associated with depletion of AVP neuronal contents in the paraventricular nucleus and supraoptic nucleus magnocellular AVP/apelin neurons (Fig. 4). Conversely, water deprivation decreases plasma apelin levels and increases apelin neuronal contents in the same neurons (Fig. 4).[10, 131] Interestingly, i.c.v. administration of a selective V1 receptor antagonist markedly reduces the increase in neuronal apelin contents induced by water deprivation, whereas i.c.v. infusion of AVP has similar effects to dehydration on neuronal apelin concentration.[131] Together, these imply that AVP and apelin are released separately by AVP/apelin magnocellular neurons in which they are produced, and that they reciprocally control their release into the bloodstream upon osmotic stimuli. Physiologically, this cross-regulation of apelin and AVP, which follows osmotic stimuli, maintains body fluid homeostasis by controlling water excretion at the kidney level, depending on the hydration state (Fig. 4). This makes apelin a natural physiological inhibitor of the antidiuretic effect of AVP. Such cross-regulation of apelin and AVP following osmotic stimuli has also been

observed in humans.[7] Indeed, increased plasma osmolality simultaneously raised plasma AVP levels and decreased plasma apelin levels. Conversely, decreased plasma osmolality simultaneously reduced plasma AVP levels and rapidly increased plasma apelin levels (Fig. 4).[7] These observations are consistent with the fact that plasma osmolality is a major physiologic regulator of plasma apelin in humans and rodents, strongly suggesting that apelin and AVP contribute to maintain body fluid homeostasis in both species.

In addition to its central action, the aquaretic effect of apelin probably involves a renal component because of the renal expression of the apelin receptor, Elabela, and preproapelin, as well as apelin immunoreactivity.[5, 13, 15, 33, 44, 132, 133] A high level of apelin receptor mRNA expression was also detected in glomeruli, and a moderate expression was observed in all nephron segments, especially in collecting ducts (CDs) that express AVP type-2 receptors (V2R).[41] Application of apelin-17 on glomerular arterioles precontracted by Ang II induced NO-dependent vasorelaxation by inhibiting Ang II-induced intracellular calcium mobilization.[41] This vasorelaxation induced by apelin in glomerular efferent arterioles, which gives rise to the vasa recta, is in agreement with a key role of apelin in the control of renal medullary circulation.[41] In addition, the expression of the apelin receptor mRNA in the CDs strongly suggests that apelin could act as an aquaretic peptide through direct action on this nephron segment. As confirmation, treatment of microdissected medullary CDs with apelin-17 inhibits cAMP production induced by dDAVP, an AVP analog agonist of the V2R expressed in CD cells.[134] Intravenous injection of increasing doses of apelin-17 in lactating rats dose-dependently increases diuresis, concomitantly with a decrease in aquaporin-2 insertion at the apical membrane of CD cells and a significant decrease in urine osmolality (Fig. 4).[134] Thus, the diuresis induced by i.v. injection of apelin-17 seems linked to a direct action of apelin on CDs. Similarly, in other studies, acute or chronic i.v. treatment with apelin-13 potently increased diuresis in male Sprague-Dawley rats (Fig.4).[133, 135]

In contrast with these studies demonstrating the aquaretic role of apelin in rodents,[136] water-deprived *Aplnr*$^{-/-}$ mice were unable to concentrate their urine to the same extent as wild-type mice.[137, 138] A possible explanation for this discrepancy is that in whole-body KO mouse model, the total absence of apelin receptor during fetal and adult life elicits compensatory mechanisms, leading to opposite effects on urine output observed following exogenous apelin administration. Hopefully, the study of an apelin receptor conditional KO mouse model[55, 68] will circumvent the limitations of those prior studies and provide insight. Altogether, these studies demonstrate that the aquaretic effect of apelin is elicited by its central effect that inhibits AVP release in the blood circulation, as well as its direct renal action that increases renal blood flow and counteracts the V2R-dependent antidiuretic effect of AVP at the CD (Fig. 4). In this context, the development of apelin receptor agonists or apelin analogs will represent potential therapeutic tools for the treatment of water retention/hyponatremic disorders.

Integrating cardiac and renal aspects and the impact on cardio-renal syndrome

Disturbance of the cardio-renal axis is common in acute and chronic HF (see above), septic shock, and severe forms of preeclampsia (see below).[139] Indeed, the bidirectional crosstalk between the heart and kidneys can induce acute or chronic dysfunction in other organs with

parallel and/or consecutive occurrences, leading to complex overlapping syndromic conditions difficult to treat and with poor outcomes.[140] Apelin circulating levels are strongly associated with and inversely proportional to cardiovascular risk and the progression of renal dysfunction in patients with type 2 diabetes mellitus.[141] Elabela and apelin-13 differentially affect the cardio-renal axis, mainly through opposing effects on counter-regulation in the vasopressinergic system, and possibly additional unknown mechanisms. Elabela is abundantly expressed in the kidney[142] and is more effective than apelin-13 in reducing renal dysfunction/injury.[15, 85, 143] Elabela and apelin-13 also significantly improve fluid homeostasis in experimental endotoxemia and septic shock, thus limiting hypovolemia.[85, 144] An anti-inflammatory activity of apelin-13 and Elabela may contribute to reduce systemic vascular permeability, thus preserving plasma volume and hemodynamics.[85, 144] The latter effects are closely related to a specific ligand-dependent interplay between the apelinergic and vasopressinergic systems (see above). In fact, i.v. injection of Elabela does not impact the increase of sepsis-induced AVP circulating levels, as opposed to apelin-13 which lowers AVP release in the blood circulation and rather tilts the balance toward undesired aquaresis and subsequent plasma volume loss.[85] Indeed, a dynamic reduction of AVP bloodstream release is observed in severe sepsis in humans, and AVP supply offers a non-catecholaminergic pathway that helps to limit both the extent and duration of low blood pressure in patients with septic shock.[145, 146]

Potential of the apelinergic system for the treatment of septic shock

Septic shock is essentially initiated by microbial infections growing systemically, and sepsis-induced myocardial dysfunction is a special condition related to acute HF, with its own specific molecular mechanisms, treatment, and outcome (Fig.3).[147, 148] The heart, along with the brain and lungs, is one of the three organs most strongly affected and associated with septic shock mortality,[149] often with a combination of systolic and diastolic dysfunctions.[147, 148, 150] Systemic vascular resistance (afterload) time-dependently and dynamically declines in septic shock, whereas it remains high to very high in non-septic acute HF.[151] However, as a member of the vasoplegia syndrome family, this shock is complex, and absolute-to-relative preload deficiency (fluid deficit and leakage) is almost always associated with this condition.[152–154] Vascular permeability during septic shock is an early concern, along with related organ dysfunctions and differential leaking intensity.[155, 156]

During experimental sepsis, circulating and myocardial apelin-13 and Elabela contents are low[85, 157] and apelin receptor expression is rather downregulated.[85] Receptor blockade further exacerbates myocardial dysfunction and mortality,[144] and apelin gene expression is down-regulated in the hearts of non-survivors.[158] On the other hand, circulating apelin-13 is marginally increased in early human sepsis, suggesting an inappropriately low reactivity of the system.[159]

Exogenous delivery of apelin-13 is beneficial in several experimental models of sepsis (e.g., endotoxemia, peritonitis), reducing multiple organ inflammation/injury and improving outcome.[144, 157, 160–162] More specifically and remarkably, Elabela is superior to apelin-13 in enhancing cardiac performance and hemodynamics in rats with peritonitis,[85] optimizing

both inotropy and pressure-volume relationships on the Frank-Starling curve, and reducing myocardial inflammation, oxidation, and stress.[144] Interestingly, the potency of cardiac response with apelinergic agonists is enhanced under systemic inflammatory conditions or polymicrobial infection, in spite of myocardial AR downregulation.[85, 144] In comparison, while adrenoceptor β1 agonists are actually recommended for supporting septic shock–related hemodynamic failure, sepsis dampens myocardial responsiveness,[163–165] with possible decreased expression of adrenoceptor β1,[166] and impairs GPCR signaling (e.g., enhanced $G_{\alpha i/o}$ and reduced $G_{\alpha s}$ activities).[167, 168]

Metabolic implications of apelin signaling and potential links with vascular effects.

Although most studies have addressed the vascular[49] and metabolic[48] effects of the apelinergic system independently, recent results suggest the two may be highly integrated, whereby the vasculature may be a critical driving force behind several beneficial metabolic effects of apelin. Indeed, preceded by a number of studies suggesting differential regulation of apelin expression in metabolically challenged states, including diabetes[169] and obesity,[170] Dray and colleagues reported that acute administration of apelin-13 led to robust reduction of blood glucose, both at baseline as well as in the setting of an oral glucose tolerance test, [171] without affecting circulating insulin levels. Subsequent studies corroborated these findings, including the demonstration of impaired insulin sensitivity in *Apln*$^{-/y}$ mice.[172] Recent human studies demonstrated that Pyr^{1}-apelin-13 injection in a group of obese but otherwise healthy humans led to improved insulin sensitivity.[173] These findings provide clinical evidence for yet another potential indication for the apelinergic system.

In addition to regulating glucose utilization and insulin sensitivity, the apelinergic pathway was recently found to have a key role in skeletal muscle function as related to aging. Indeed, apelin expression is decreased in aged skeletal muscles, and *Apln*$^{-/y}$ and *Aplnr*$^{-/-}$ mice were found to have impaired muscle function with age.[174] Apelin administration led to increased mitochondriogenesis, as well as increased proliferation and myogenic differentiation of muscle stem cells. Lastly, a strong correlation was found between activity/exercise levels and circulating apelin levels in humans, characterizing apelin as an exerkine, a recently coined term for peptides and nucleic acids released from skeletal muscle and other organs upon exercise and mediating systemic adaptations to exercise.[175]

While the profound metabolic effects of apelinergic signaling, including human disease implications, provide a potential therapeutic role for targeting this pathway, the significantly greater expression of apelin and its receptor in ECs over non-endothelial cell types provides a mechanistic enigma. A number of key questions emerge regarding how this signaling mechanism truly impacts the metabolic phenotypes observed in different KO strains: (1) Is the primary source of apelin the ECs, and is endothelially-derived apelin responsible for the bulk of metabolic effects in a paracrine manner? (2) Is the lower-level expression of the apelin receptor in the non-endothelial cell types, such as adipocytes and skeletal myocytes, sufficient to drive the paracrine effects of circulating apelin? And (3) can autocrine or paracrine endothelial apelin/receptor signaling be responsible for the metabolic effects attributed to this signaling pathway? These important questions are beginning to be addressed by the generation of conditional, cell type–specific deletions of *Aplnr.* Mice with

an endothelial-specific deletion of *Aplnr* were found to exhibit insulin resistance and significantly impaired glucose utilization, and failed to improve glucose utilization in glucose tolerance tests after exogenous apelin administration.[55] Moreover, these mice were found to have augmented trans-endothelial fatty acid transport and accumulation of fatty acids in the skeletal muscle, which at least partly explains their impaired insulin resistance. While these studies suggest that endothelial-specific apelin/receptor signaling may be a critical component of how apelin regulates metabolic balance, further studies involving selective deletion of *Aplnr* in other non-endothelial cell types, including skeletal myocytes, adipocytes, hepatocytes, and others, will provide further insights into both the metabolic and the myogenic effects of apelinergic signaling as we continue to develop this pathway into a therapeutic modality.

Emerging potential of the apelinergic system for treating preeclampsia

Preeclampsia, a clinical condition observed during pregnancy, is characterized by high blood pressure and damage to vital organs, most often liver and kidneys. Placental impaired trophoblast invasion, spiral artery transformation, and endothelial dysfunction are commonly observed in preeclampsia, with mostly unknown mechanisms.[176, 177] Several miRNAs, peptides, and proteins derived from the placenta were shown as potential biomarkers of preeclampsia,[178, 179] but valuable and safe therapeutic targets are still urgently needed. Interestingly, the apelin/Elabela/APLNR axis is a major player in the development, growth, and function of the vascular system, including the extensive angiogenesis necessary for placental growth as well as normal embryonic and fetal development.[180, 181] More specifically, Elabela is known to be a hormonal guide for angioblasts during angiogenesis.[182] Both apelin receptor and its ligands are strongly present in placental (including cytotrophoblast, syncytiotrophoblast, and stromal cells) and uterine tissues,[183] with possible overexpression in preeclampsia.[184] On the other hand, knockdown of LIN28B (an RNA-binding protein that influences stem cell maintenance and metabolism) suppresses Elabela expression[185] and Elabela deficiency is a promoter of preeclampsia and cardiovascular defects (including in angiogenesis) in mice.[186] This experimental link has to be further investigated, since two recent clinical studies did not confirm the lack of Elabela in the blood and placenta of women with early and late preeclampsia.[187, 188] This topic is a still emerging and relevant one with regards to both pathophysiological events and the potential function of the apelinergic system. There is no intervention along this axis currently in progress to our knowledge.

Toward pharmacological probes and novel drugs

In parallel to the above investigations on the role of the apelinergic axis in the physiology and pathology associated with multiple systems and diseases, efforts have been dedicated to understanding the structure–function of the endogenous ligands of the apelin receptor. Indeed, the apelinergic system possesses the rare characteristic of being associated with two classes of ligands detected *in vivo*: apelins (apelin-36, apelin-17, apelin-13, and Pyr^{1}-apelin-13) and Elabela. Not surprisingly, based on the nature of its endogenous ligands, peptide derivatives of apelin and, more recently, Elabela have dominated chemistry efforts along several lines aimed at (1) better characterizing the structure–signaling and structure–activity relationship (SAR) of apelin and Elabela; (2) providing biologically more stable

ligands; and (3) generating pharmacological probes to validate the potential of the apelin receptor as a target in the above indications. More recently, nonpeptide small molecules have also been reported, some of which have been clinically tested.[189, 190]

Peptides and mimetics—Initial efforts to understand the SAR of peptide molecules classically involve truncation, deletion, alanine scanning, as well as aspartate and *N*-methylation scanning. Together, these efforts usually lead to the identification of critical pharmacophoric elements, as well as preliminary information on the structure of the ligand. When applied to apelin fragments, N-terminal truncation was found to decrease forskolin-induced cAMP production, receptor internalization, AVP release, and the effect on blood pressure.[20, 28] Subsequently, single replacements with alanine (alanine scanning) and stereoinversion collectively pointed to Arg2, Arg4, Leu5, and Phe13 as important pharmacophoric elements.[22, 33, 191, 192] This is congruent with NMR structures of apelin-17, which identified these regions as structurally less flexible.[193, 194]

Important insights into the role of the C-terminal Phe residue in signaling came early on with the demonstration that its deletion in apelin-17 (i.e., generating apelin-16)[20] abrogated the hypotensive effect of the parent peptide, and its substitution by Ala in apelin-13 inhibited the apelin-13-induced transient blood pressure decrease.[195] Surprisingly, apelin-16 possesses similar affinity for the apelin receptor and efficacy to activate $G_{\alpha i/o}$ signaling as apelin-17.[196] The profound decrease in the hypotensive effect of apelin-16 was due to its reduced ability to promote β-arrestin recruitment and apelin receptor internalization. This demonstrated that β-arrestin signaling may account for the hypotensive activity of apelin-17.[19, 20] Recently, a broader analysis of signaling bias in a collection of compounds confirmed the correlation between the hypotensive effect of apelin and the activation of the β-arrestin pathway subsequent to binding to the receptor.[36] Knowing that the C-terminal Phe residue of apelin-17 or apelin-13 is removed by ACE2 (Fig.1), this suggests that ACE2 might be an important regulator of the activity of apelins in the control of blood pressure.[18]

Substitution of the C-terminal residue with unnatural aromatic amino acids led to compounds possessing mid-pM affinity.[191, 197, 198] This class of modifications also increased plasma stability. Furthermore, aromatic amino acid substitutions of the C-terminal residue also identified a determinant of signaling, likely associated with the presence of multiple aromatic residues on several transmembrane domains in that area of the binding pocket (*i.e.* residues Y35, W85, Y88, Y93 and Y299), as observed in the recently reported X-ray structure of the stabilized apelin receptor (modifications include N- and C-terminus truncations as well as the mutations V117A and W126K) complexed with a macrocyclic apelin-17 derivative.[17, 23, 33, 189, 196, 199] Additional work in this area relied on the introduction of Pro12Aib and Phe13(4-Br)Phe, which were beneficial for plasma stability of apelin-13 and apelin-17.[17, 23, 33, 192] Further stabilizing modifications involved N-Me, α-Me, *aza*-Arg, and *aza*-Leu in replacement of Arg4 and Leu5, combined with the above Pro12 and Phe13 replacements, which led to improved plasma stability while keeping low nanomolar binding affinity. N-PEGylation and N-palmitoylation of the native peptides also increased plasma stability by several orders of magnitude while maintaining good Ca^{2+} mobilization.[200, 201] Interestingly, preserved agonist activity with prolonged *in vivo* half-life was also achieved by fusing apelin-13 to IgG Fc fragment or by encapsulating Pyr^1-

apelin-13 in PEGylated liposomal nanocarriers.[202, 203] More recently, the addition of a fluoroalkyl chain to the N-terminal part of apelin-17 was shown to strongly increase plasma stability while maintaining its *in vitro* pharmacological properties and significantly improving the *in vivo* activity of the peptide on diuresis and arterial blood pressure.[192]

Finally, macrocyclization was applied to apelin-13 in different ways.[204] Among several attempts, double cyclization of apelin-13 turned produced a competitive antagonist with a K_i of 82 nM that demonstrated efficacy in reducing tumor growth in a mouse model of glioblastoma.[205, 206] MM07 was also reported as a G protein–biased agonist with potency comparable to Pyr^1-apelin-13 in the saphenous vein contraction assay, yet with lower potency on β-arrestin recruitment and receptor internalization.[207] MM07 significantly increased cardiac output in anesthesized rats, and dose-dependently increased forearm blood flow in human volunteers with higher efficacy than apelin. A further systematic study of macrocyclization in Pyr^1-apelin-13 identified the N- and C-termini as preferable sites for cyclization to retain good binding and signaling properties, and to significantly increase plasma stability. In this series, low nM, $G_{\alpha i}$-biased agonists were identified, and they demonstrated, as suggested above, very low hypotensive properties in rats *in vivo*, as well as strong inotropic properties in the Langendorff isolated perfused heart model.[135, 208]

Beyond apelin-13, a few studies have dissected the SARs of apelin-17, apelin-36, and Elabela. Essentially, conclusions drawn from SAR on apelin-13 have held true for the other apelins.[23, 33, 192] Importantly, the combined SARs of the three apelins point to the presence of two distant pharmacophoric elements, one corresponding to the N-terminus of Pyr^1-apelin-13, with cationic elements found in Arg2 and Arg4 interacting with anionic residues in the extracellular domain of the receptor, and the second involving a C-terminal aromatic moiety found in Phe13, buried deeper in the transmembrane domain.[196] This view is consistent with the X-ray structure of the human apelin receptor complexed with a macrocyclic derivative of apelin-17, [198] and site-directed mutagenesis studies on the rat apelin receptor.[196, 209] This is also consistent with a semi-peptide derivative reported by Iturrioz *et al.*, which links a cationic peptide portion with a polyaromatic fluorophore via a flexible linker.[52, 210] Interestingly, preliminary SAR of Elabela allowed the determination that the shortest significantly active fragment corresponds to its 14 C-terminal amino acids. [211] Despite large differences in primary sequence between apelins and Elabela, distant epitopes seem necessary, although their chemical nature varies somewhat.

Receptor structure—The X-ray structure of the apelin receptor complexed with a macrocyclic apelin-17 analog (AMG3054) was reported recently.[199] This follows several studies of either the ligand in solution, individual transmembrane domains by solution NMR, or site-directed mutagenesis, as summarized recently.[189] Altogether, these studies provide invaluable structural information on the binding and dynamics of the receptor. The C-terminal binding pocket described in the X-ray structure and 3D model of the apelin receptor, which is very rich in aromatic residues located on several transmembrane domains, helps to explain why and how modifications to the C-terminal Phe residue of apelins is such an important determinant of β-arrestin signaling.[19, 196, 199] Accordingly, this region also emerges as a region of interest for virtual screening for the identification of small molecules. Future efforts combining 3D solid structure with solution dynamics and computational

modeling will be critical for fully comprehending the ligand-receptor complex structure and dynamics, and how these relate to signaling. This knowledge will serve the dual purpose of identifying novel small molecule ligands and how to bias their signaling toward beneficial pathways at the expense of undesired ones. Indeed, as described in the previous sections, different signaling pathways may be necessary to optimize efficacious and safe drugs for different therapeutic indications.

Small molecules—Few small molecules have been reported to date as apelin receptor ligands. The presence of two distant pharmacophoric elements (see above) may contribute to the difficulty of identifying small enough molecules to bind and activate the receptor. As far as antagonists are concerned, two small molecule antagonists with low/sub-micromolar efficacy, ML221 and ML223, were identified from a high-throughput screen. Yet, although functional efficacy data were reported, these compounds have not been shown to actually bind the orthosteric apelin receptor site.[212, 213] Protamine was also identified as an apelin receptor antagonist with high nanomolar efficacy.[79] More recently, CMF-019 was identified as an agonist with EC_{50} on 47 nM on calcium mobilization. CMF-019 binds to the human left ventricle (pKi = 8.6), and potently inhibits cAMP production, with a low impact on β-arrestin recruitment and receptor internalization. As such, CMF-019 emerges as a $G_{\alpha i}$-biased apelin receptor agonist, and it showed increased cardiac contractility upon i.v. administration to rats. Several series of low/sub-nanomolar apelin receptor agonists were reported in the patent literature based on various heterocyclic scaffolds.[189, 190] Finally, the oral administration of the small-molecule apelin agonist AMG986 is currently in phase I clinical trials for the treatment of HF.[129]

Challenges and perspectives

Several key points remain to further establish the druggability of the apelinergic pathway. First, a clear understanding of an optimal candidate signaling profile for the therapeutic indications under scrutiny is required. At the moment, it is not clear, for each indication, whether a compound acting on cAMP signaling via the $G_{\alpha i/o}$ pathway, or a compound that mediates receptor internalization, or even a G protein–independent but β-arrestin–dependent signaling, is preferred. In order to validate this, compounds possessing strongly biased signaling profiles on these diverse pathways are necessary, as well as the validation of these compounds in relevant animal models. This will be invaluable to orient drug discovery on the right pathways. Second, and in the same vein, like many peptidergic GPCRs it has been difficult to identify small molecule agonists of the apelin receptor. Although peptide and semi-peptide compounds provide high levels of affinity and represent good probes to decipher the above structure-signaling relationship or potential drug candidates for indications compatible with parenteral administration, there is only a handful of orally bioavailable small molecules that would be compatible with indications such as heart failure, PAH, or diabetes. One of the difficulties in identifying such small molecules may be the necessity of two topologically distant epitopes for binding, as revealed by studies based on peptide molecules. Third, translational models, particularly those relying on genetic interference, all possess their drawbacks such as, for example, heavy compensation. This makes translation more difficult. Their predictive value will be enhanced by the validation of the observed effects with emerging molecules, and the availability of tissue-specific genetic

interference approaches. Finally, although several investigator trials have provided encouraging results on the role of apelins in several conditions, much remains to be done to demonstrate the clinical efficacy of apelin or Elabela in pathological conditions. Some differences are observed between the two peptide classes in animal models; however, these need to be clinically validated.

Conclusion

The apelinergic system is an emerging target with broad potential therapeutic impact in cardiovascular, renal, and metabolic disorders, as well as other indications with a strong vascular component. Animal models of diseases provide convincing evidence that it should be pursued in several indications. Among the most advanced indications in terms of preclinical and clinical validation are HF and PAH. However, type 2 diabetes, water retention/hyponatremic disorders, kidney diseases, septic shock, and preeclampsia are also among therapeutic indications that would benefit from intervention via the apelinergic system. Moreover, the interplay between the involved systems and the particular role of the vasculature emphasizes the need to better understand the impact of vascular dysfunctions in these diseases, as well as how intervention via the apelinergic system contributes to the positive effects observed.

Several classes of molecules are now available, some with biased signaling, to decipher which signaling pathway to favor for therapeutic intervention in the above areas. With these tools in hand, the coming years will certainly be very stimulating for answering some of the questions associated with the apelinergic system.

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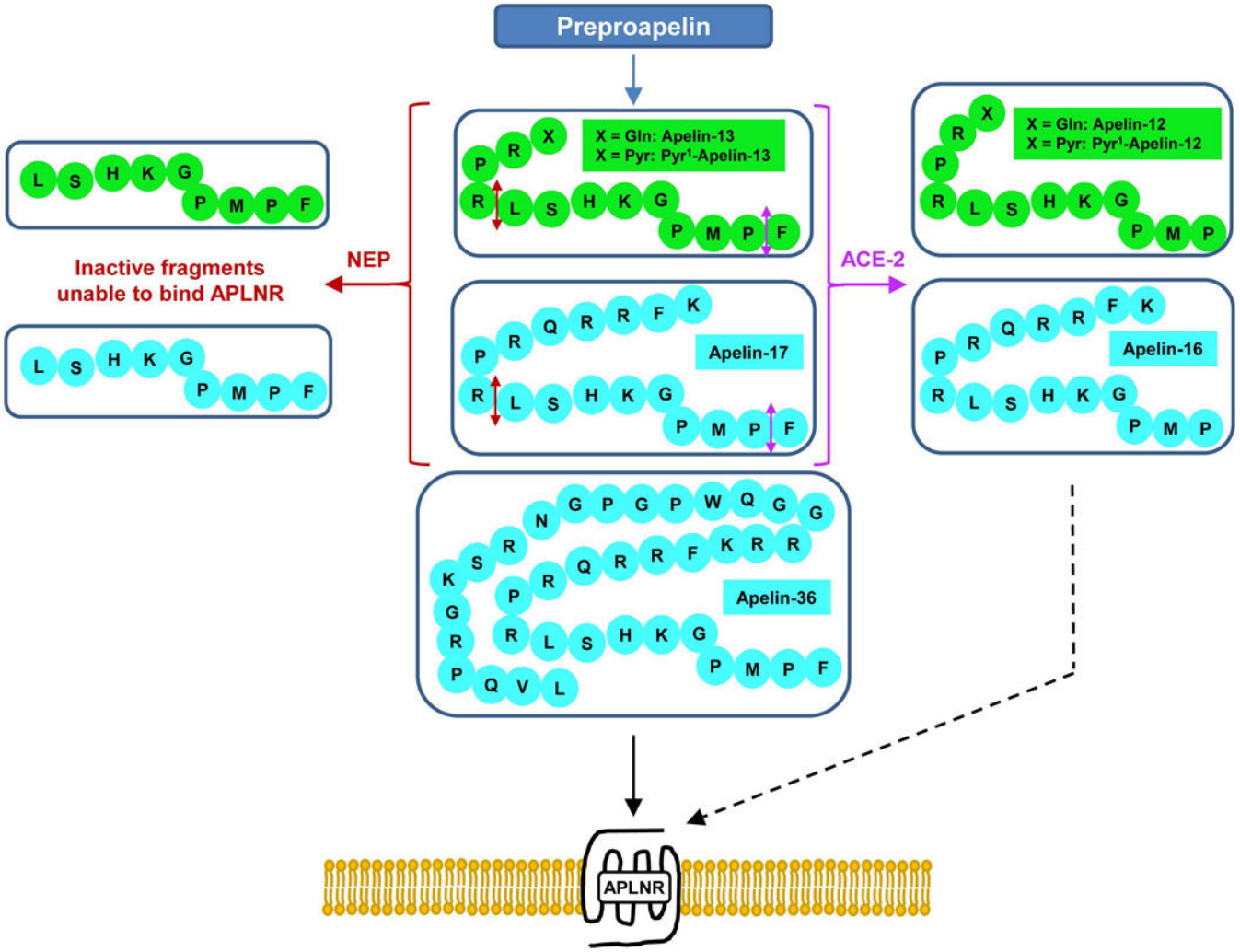

Figure 1.

The different forms of apelin. Proteolysis of preproapelin at putative cleavage sites leads to three fragments of 36, 17, and 13 amino acids named apelin-36, apelin-17, and apelin-13, respectively. Pyroglutamylated apelin-13 is formed by spontaneous cyclization of apelin-13. The cleavage sites for neprilysin and angiotensin-converting enzyme 2 on apelin-13 and apelin-17 are indicated by red and purple double-headed arrows, respectively. The straight and dashed black arrows represent full and biased agonism of the different apelin forms, respectively.

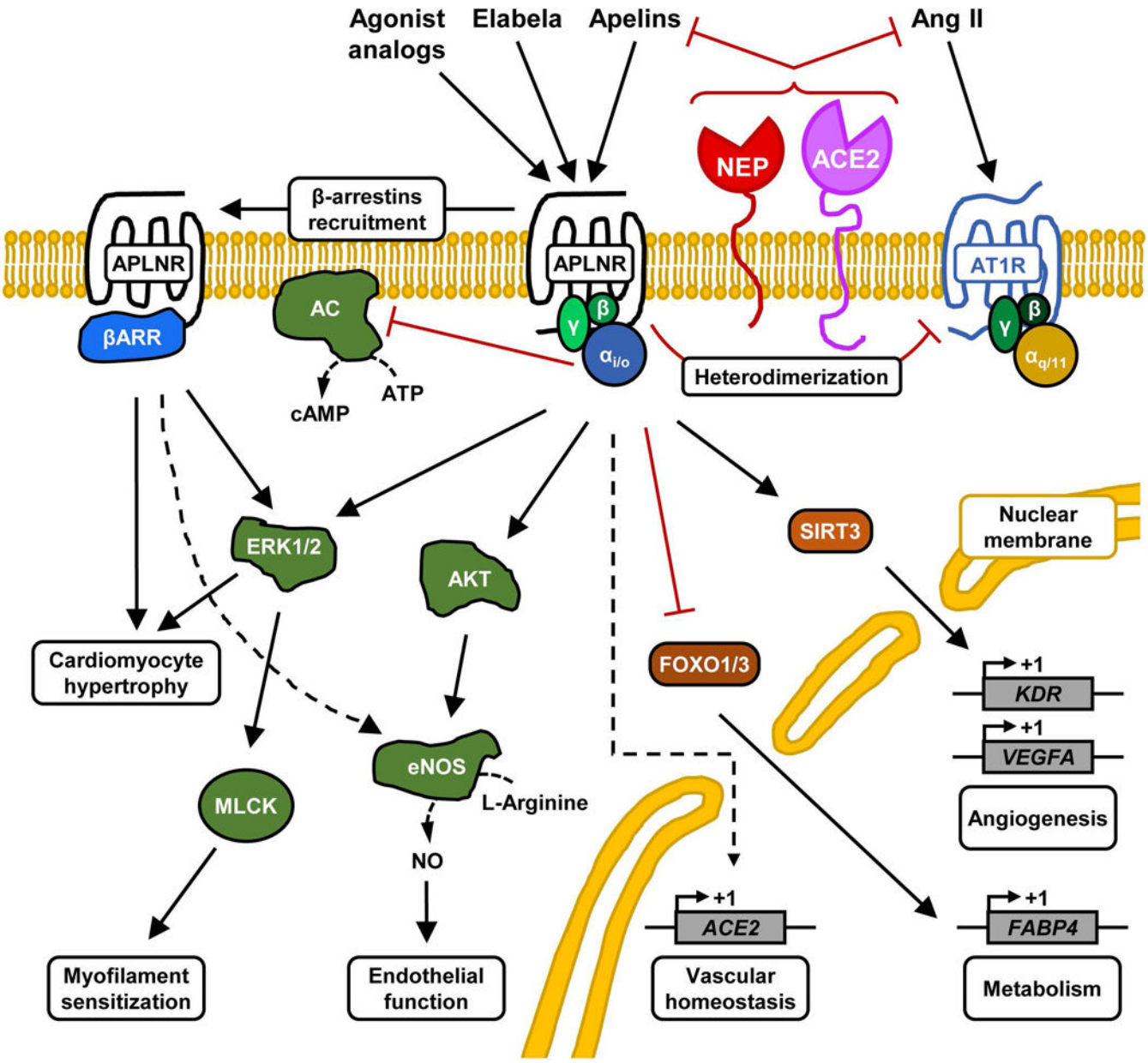

Figure 2.

Schematic of the different apelinergic signaling pathways. The activation of apelin receptor by its endogenous ligands, apelin or Elabela, triggers various signaling pathways that exert protective cardiovascular and metabolic effects in the organism. Some of the transcriptional gene regulation effects following the activation of apelinergic pathway are depicted in the nucleus. Abbreviations: AC, adenylate cyclase; ACE2, angiotensin-converting enzyme 2; Ang II, angiotensin II; APLNR, apelin receptor; AT1R, angiotensin type 1 receptor; βARR, β-arrestins; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular-regulated kinases 1/2; FABP4, fatty acid binding protein 4; FOXO1/3, forkhead box protein O1/3; KDR, kinase insert domain receptor (also known as VEGFR2); MLCK, myosin light chain kinase; NEP, neprilysin; SIRT3, sirtuin 3; VEGFA, vascular endothelial growth factor A.

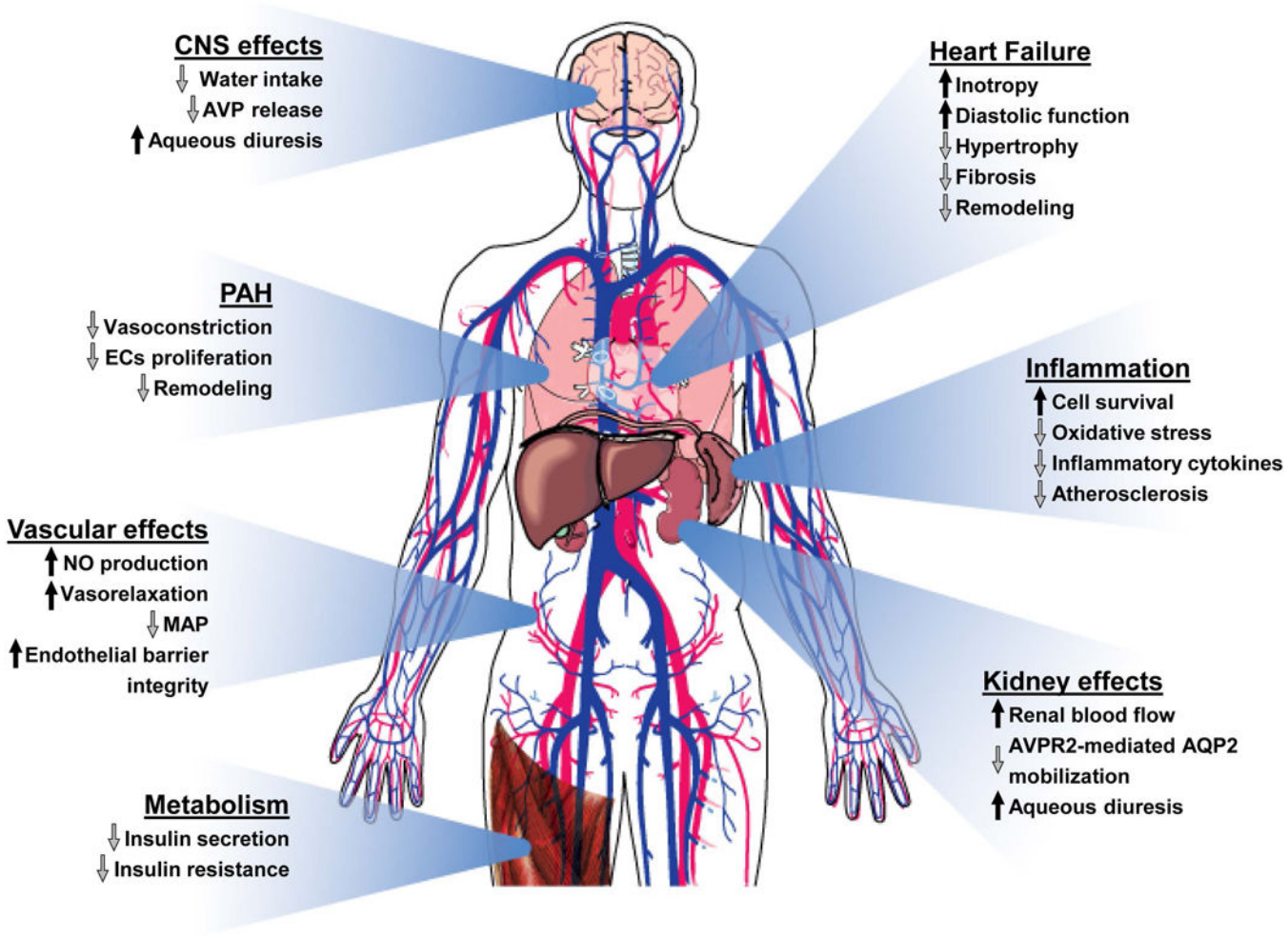

Figure 3.

The apelinergic system counteracts cardiovascular and metabolic failures. The activation of the apelinergic pathway results in multiple physiological effects that can alleviate an array of cardiovascular and metabolic disorders. Abbreviations: AVP, vasopressin; ECs, endothelial cells; MAP, mean arterial pressure; NO, nitric oxide; PAH, pulmonary arterial hypertension.

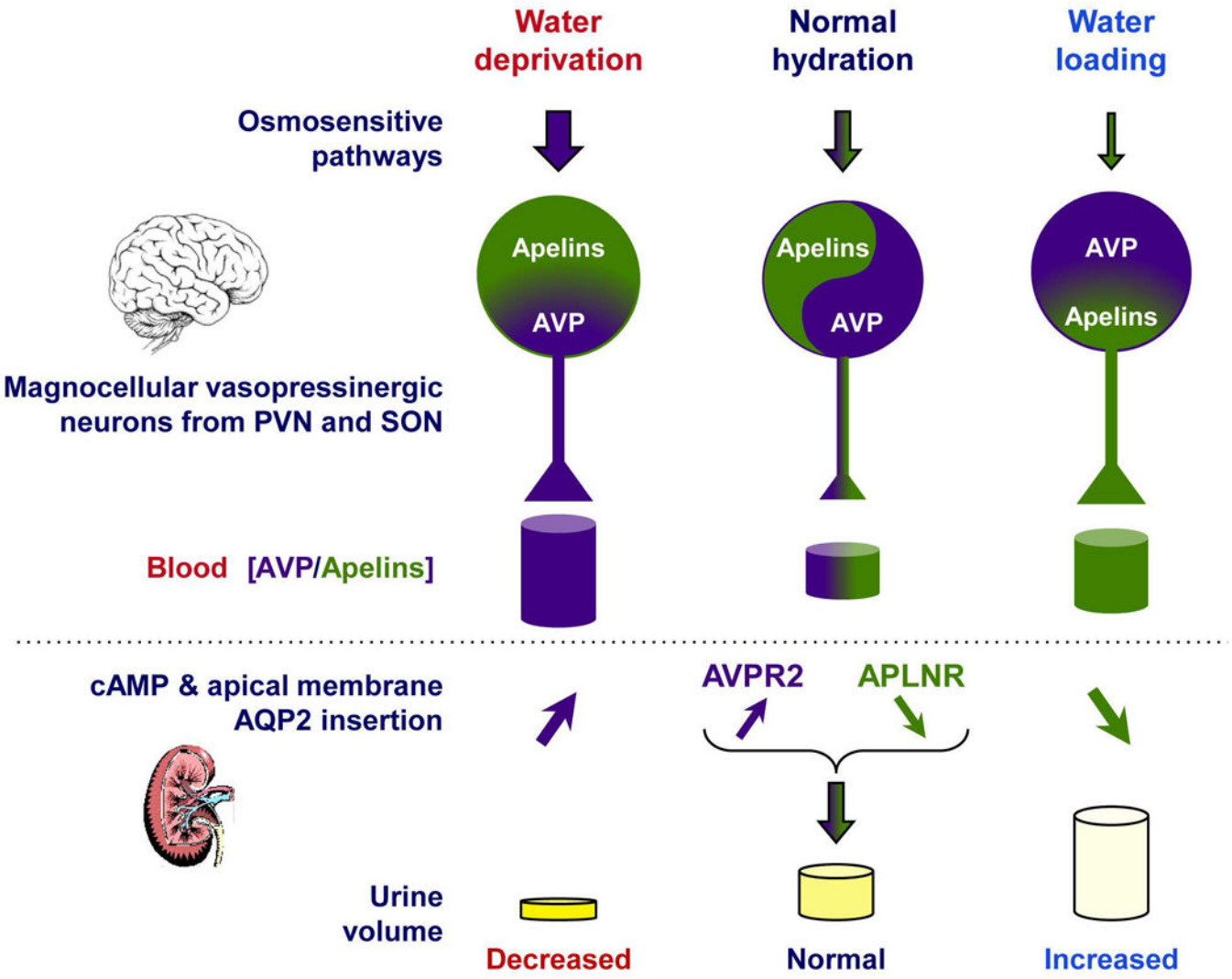

Figure 4.

Vasopressin and apelins—the yin and yang of water balance. The interrelated actions of apelins and vasopressin (AVP) are required to ensure and finely tune water balance in the entire organism. The hydration state determines the overall release of apelins and AVP from the hypothalamic magnocellular neurons into the bloodstream. Their opposing receptor-mediated functions in the brain and kidney results in the regulation of urine osmolality and volume.