The emerging role of the apelinergic system in kidney physiology and disease

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ABSTRACT

The apelinergic system (AS) is a novel pleiotropic system with an essential role in renal and cardiovascular physiology and disease, including water homeostasis and blood pressure regulation. It consists of two highly conserved peptide ligands, apelin and apela, and a G-protein-coupled apelin receptor. The two ligands have many isoforms and a short half-life, and exert both similar and divergent effects. Vasopressin, apelin

and their receptors colocalize in hypothalamic regions essential for body fluid homeostasis and interact at the central and renal levels to regulate water homeostasis and diuresis in inverse directions. In addition, the AS and renin angiotensin system interact both systemically and in the kidney, with implications for the cardiovascular system. A role for the AS in diverse pathological states, including disorders of sodium and water balance, hypertension, heart failure, pre-eclampsia, acute kidney injury, sepsis and diabetic nephropathy has recently been reported. Furthermore, several metabolically stable apelin analogs have been developed, with potential applications in diverse diseases. We review here what is currently known about the physiological functions of the AS, focusing on renal, cardiovascular and metabolic homeostasis, and the role of the AS in associated diseases. We also describe several hurdles and research opportunities worthy of the attention of the nephrology community.

Keywords: apela, apelin, apelin receptor, cardiovascular, kidney, water homeostasis

INTRODUCTION

1. The apelinergic system

The apelinergic system (AS) consists of two peptide ligands, apelin and apela, and a G-protein-coupled apelin receptor (AR - originally called APJ for apelin peptide jejunum). The gene encoding the human AR (*APLNR*, chromosome 11) was cloned in 1993 [1]. Five years later, apelin (*APLN*, X chromosome- APJ endogenous ligand), a peptide isolated from bovine stomach extracts, was reported to induce signaling in cells expressing AR [2]. A second peptide ligand, apela (apelin receptor early endogenous ligand, also known as elabela or toddler, (*APELA*, chromosome 4), was discovered in 2013 [3, 4]. Apelin and apela display little sequence identity, but both are produced as precursors that are processed to generate several isoforms, as described in previous reviews [5, 6]. The distribution, function and processing of these specific isoforms have yet to be fully elucidated. *APLN* is expressed as a 77-amino acid precursor, preproapelin, which is further processed by tissue-specific proteases [7]. The longest secretable form is apelin-55 [8, 9]. Several enzymes that process apelin to generate

both active fragments and inactive fragments lacking various N-terminal and C-terminal residues have been identified [8, 10], and the list of “orthodox” isoforms is toolimited.

The apela gene encodes a 54-amino acid precursor [4]. After removal of the signal peptide, apela-32 is secreted [3] and bioactive [11, 12]. This protein is predicted to undergo further cleavage to produce several fragments that have been pharmacologically characterized [12, 13]. The shortest C-terminal apela-11 fragment is fully conserved between species. The apelinergic peptides can undergo further posttranslational modifications. The best characterized of these modifications is the spontaneous cyclization of the N-terminal glutamine of apelin-13 to form pyroglutamate, resulting in the more stable pyr-apelin-13 [14], but other modifications have also been predicted. All isoforms have a short half-life, implying rapid and transient responses to homeostatic changes, and they may differ in potency and signaling bias. The human AR is a conserved class A rhodopsin-like G-protein-coupled

receptor [15]. The AR preferentially binds to multiple G-proteins, including Gai, Gaq and β -arrestin, mediating different effects in different cell types (figure 1). The AS is widely distributed and plays a pleiotropic but essential role in various physiological and pathological processes, as exemplified by recent reviews [6, 16-20]. Here, we highlight the complexity of the AS, and its role in pathophysiology, focusing on the kidney and the cardiovascular system. We also describe several hurdles and research opportunities worthy of the attention of the nephrology community.

2. The expression of AR, apelin and apela in the kidney

Data on renal AS expression are provided in table 1. All the elements of the AS have been detected in human and murine kidneys. The most detailed data available were obtained in murine studies [11, 21, 22]. In several of these studies, the AR was detected mostly in vascular structures. The AR mRNA is highly abundant in rat glomeruli and *in situ* hybridization showed the AR to be present in the endothelial and vascular smooth muscle cells of glomerular arterioles [23]. Lower levels of expression

have been detected in the renal tubules, including the proximal convoluted tubule, the proximal straight tubule of the cortex, the thick ascending limb from the outer medulla, the thick ascending limb from the cortex, and the collecting duct (CD) from the cortex, outer medulla, and inner medulla [23]. Other studies reported low levels of AR expression in the distal convoluted tubule and cortical thick ascending loop of Henle but not in the CD [22, 24]. In addition, high levels of AR mRNA have been detected in the non-tubular interstitial cells of the medulla [22]. This study found that most AR-positive cells also expressed apelin.

Apelin expression has been detected in the glomeruli, vascular epithelial cells and tubular epithelial cells [25], but other studies have reported its restriction largely to isolated cells in the vicinity of AR-positive cells in the medulla [22].

Triple labeling revealed that more cells express apela than AR or apelin. In addition, apela-expressing cells do not appear to overlap with AR- and apelin-expressing cells

[22]. Apela is expressed principally in the murine medullary renal tubular epithelial cells, with expression strongest in the loops of Henle and CD and very weak or absent in the glomeruli [22, 24, 26]. In a single-cell RNA-seq study of renal CD cell types, apela was found to be selectively expressed in the principal cells [27]. Xu *et al.* found both apela and apelin in the apical membrane of the principal cells of the medullary CD [24].

Data concerning AS expression in the human kidneys are scarce. Double-immunostaining of the human kidney revealed that apela does not colocalize with endothelial cells and hematopoietic cells, with labeling instead specifically overlapping with tubular epithelial cells, whereas AR is expressed by all these cells [11]. Another study demonstrated strong AR immunoreactivity in the tubular epithelium [28]. In human embryonic kidney (HEK) 293 cells cotransfected with both receptors, the AR was found to form heterodimers with the bradykinin B2 receptor [29].

The ligands can be secreted in both blood and urine [30], suggesting a possible paracrine function. Moreover, AR-independent effects have been described [26, 31]. It is not clear how gene expression translates into protein levels, which isoforms predominate in the kidneys and the relative influences of circulating and local renal apela and apelin. The relative contribution of the kidney to circulating apelin and apela levels also remains unknown.

THE PHYSIOLOGICAL ROLE OF THE AS

1. Interaction of the AS with the renin-angiotensin system

The kidney has a fundamental role in the renin-angiotensin system (RAS), which regulates blood pressure, salt retention, and fluid balance [32-34]. In the classical system, renin cleaves angiotensinogen to form angiotensin I (AngI), which is subsequently converted to AngII by angiotensin-I converting enzyme (ACE). The processing of AngI and II by ACE-2 to generate angiotensin 1-7 and angiotensin 1-9 is part of a counterregulatory system

[32]. The prorenin receptor (PRR) increases the conversion of angiotensinogen to AngI in the bloodstream and kidney [35].

Both apelin and apela influence the RAS (figure 2). Apelin can downregulate AngII-AngII receptor type 1 (AT1R) signaling through the heterodimeric interaction of AR and AT1R [36, 37]. Apelin also upregulates ACE-2 in models of heart failure, where ACE-2 expression and activity are downregulated in apelin-deficient mice, and apelin administration increases ACE-2 expression [38-40]. Apelin isoforms are probably the main non-RAS substrate of ACE-2, which metabolizes bioactive apelin peptides in a negative feedback loop [41], but the interaction is complex. For example, apelin-16 (i.e. ACE-2-converted apelin-17), has an affinity similar to that of apelin-17 for the AR, but has the opposite effect on β -arrestin signaling, with less potent hypotensive effects. By contrast, apela downregulates ACE expression (converting AngI to AngII) with little effect on ACE-2 expression in the stressed heart [42]. AngII levels are chronically high in heart disease, and they increase plasma aldosterone and vasopressin levels via AT1R activation, causing vasoconstriction, hypertension, cardiac hypertrophy, renal

fluid retention, inflammation and insulin resistance [32]. By contrast, angiotensin 1-7 and 1-9 induce vasodilation and hypotension and attenuate inflammation and cardiac hypertrophy [32]. Enhancing the beneficial actions of apelin peptides and ACE2 axes while complementing existing pharmacological blockades of detrimental pathways constitutes a novel pathway for the development of treatments.

Apelin opposes the vasoconstrictive effects of AngII in the efferent and afferent glomerular arterioles in rat kidney [23]. There is also evidence to suggest that apelin influences blood pressure regulation by inhibiting RAS expression in the distal nephron [24]. Intravenous (IV) apelin-32 administration to salt-loaded salt-sensitive Dahl rats decreases medullary PRR and renin levels, urinary soluble PRR, renin, angiotensinogen and AngII excretion, blood pressure and kidney injury. It has been suggested that the apelinergic ligands antagonize renal PRR expression and RAS activation through cAMP-dependent but AR-independent signaling in the CD [34]. Soluble PRR decreases renal vasopressin V2 receptor (V2R) expression, adding another layer of potential complexity to the role of the AS in water homeostasis [43].

Conversely, components of the RAS influence the AS. Mice with whole-kidney and CD-specific knockouts of PRR have higher levels of medullary apela and apelin expression and urinary excretion than wild-type mice [24]. By contrast, the whole-kidney knockout of PRR decreases medullary AR levels, whereas no such decrease is observed with the CD-specific knockout. The pathways regulating the AS downstream from PRR remain unknown. The soluble PRR and the apelinergic peptides may be considered to act in opposition, each antagonizing the expression of the other, with opposite effects on intrarenal RAS, with major implications for blood pressure regulation, kidney injury, and urine concentrating capability. Improvements in our understanding of the molecular mechanisms underlying these interactions will open up new possibilities for the treatment of hypertension and renal diseases.

2. The role of the AS in water homeostasis

Vasopressin, apelin and their receptors colocalize to hypothalamic regions essential for body fluid homeostasis, and neurons with apelin immunoreactivity project into the

posterior pituitary gland [44]. These two hormones interact at the central and renal levels, with inverse effects on water homeostasis.

In rats, water deprivation or the central administration of vasopressin increases the number and density of apelin-immunoreactive hypothalamic cells, induces apelin-positive axons along the hypothalamo-hypophyseal tract, and decreases the release of apelin into the bloodstream, with the opposite effect on vasopressin-immunoreactive hypothalamic cells and axons, and vasopressin release [45]. These effects are attenuated by antagonists of vasopressin V1 receptor (V1R). IV or central apelin treatment decreases circulating vasopressin levels and increases diuresis [46, 47]. In the rat CD, apelin inhibits V2R agonist-induced cAMP production and Ca^{2+} influx, and decreases AQ2 insertion into the apical membrane [46]. In a mouse CD cell line, AR was found to be present, and apelin both decreased cAMP production and antagonized vasopressin-induced AQ2 [48]. Apelin also amplified the Ca^{2+} signal resulting from V1R stimulation, suggesting an enhancement of the physiological antagonistic effects

of V1R. Further evidence for an effect on renal hemodynamics is provided by the observation that apelin induces the vasorelaxation of AngII-precontracted efferent and afferent arterioles in the rat kidney by inhibiting AngII-induced Ca^{2+} signals [23]. Data for human cell models are lacking and *in vivo* data are scarce. In healthy volunteers, increasing plasma osmolality has been shown to increase plasma vasopressin and decrease plasma apelin levels, whereas a decrease in plasma osmolality after water loading inhibits vasopressin and increases circulating apelin levels [49].

Apela administration increases diuresis and water intake in rats in a dose-dependent manner, whereas AR antagonism decreases diuresis and water intake, also in a dose-dependent manner [50]. One study reported a diuretic response five times stronger than that observed with apelin, whereas another showed the diuretic responses to apela and apelin to be similar [47]. As the relative concentrations and half-lives of the isoforms in the kidneys remain unknown, it is unclear what this means *in vivo*. Synthetic analogs have more potent effects on diuresis and glomerular hemodynamics [51].

The evidence is not unequivocal. Other studies have shown no effect or even a decrease in water intake after central apelin administration [52, 53]. In addition, AR KO mice have a lower water uptake under euvoletic conditions and an impaired urine concentration capacity in response to hyperosmotic conditions [54], whereas apelin KO mice display no alteration to drinking behavior [55]. Apelin KO mice have a dehydration-induced drinking response similar to that of wild-type mice [56], suggesting a compensatory role of apela. Important progress has also recently been made towards understanding the cellular logic underlying different types of thirst, including the use of optogenetic gain-of-function in thirst type-specific cell types [57-59]. The study of the AS with these techniques could shed additional light on the potential role of the AS in central thirst regulation.

THE ROLE OF THE AS IN RENAL PATHOPHYSIOLOGY

1. Disorders of sodium and water balance

As mentioned above, apelin interacts with vasopressin and is influenced by water status. Apelin-immunoreactive cells are found in the hypothalamic region involved in the control of drinking behavior [60, 61], and a role for the AS in thirst sensation and drinking behavior is suggested by the observation that the central injection of apelin in water-deprived rats decreased water intake [53]. Copeptin (a surrogate for vasopressin) is the best validated parameter, but the apelin-to-copeptin balance is altered in several disorders. The precise contribution of apelin to pathogenesis requires further clarification. We summarize here the most important water homeostasis-related disorders and current knowledge of the role of apelin (table 2).

1.1 Syndrome of inappropriate antidiuretic hormone secretion (SIADH)

Patients with SIADH have concentrations of apelin and copeptin 26 and 75% higher, respectively, than those in healthy subjects [62]. During acute osmotic challenge, plasma copeptin concentration was found to be higher and plasma apelin

concentration lower than expected from plasma sodium concentration and the plasma apelin-to-copeptin ratio decreased exponentially with natremia, demonstrating that apelin and apelin-to-copeptin ratios are also “inappropriate” in SIADH patients. These findings led to suggestions that the increase in plasma apelin secretion is insufficient to compensate for the higher levels of vasopressin release in these patients. However, only apelin was explored, and no data are available for apela or the local renal AS in this context.

1.2 Congestive heart failure

In patients with congestive heart failure and hyponatremia, plasma copeptin concentrations are 190% higher than those in healthy subjects, plasma apelin concentrations are 25% higher, and the plasma apelin-to-copeptin ratio is lower than in controls. It has been suggested that the slight increase in apelin secretion is insufficient to counteract the increase in vasopressin secretion, leading to abnormal water metabolism and hyponatremia [62].

1.3 Nephrogenic/central diabetes insipidus:

Patients with nephrogenic diabetes insipidus have higher plasma copeptin concentrations than healthy volunteers, and their plasma apelin levels are also 61% higher than those in controls. As expected, patients with central diabetes insipidus were found to have plasma copeptin concentrations 52% lower than those in healthy volunteers, whereas plasma apelin concentrations were 18% lower and the plasma apelin-to-copeptin ratio was higher in patients than in controls [61].

1.4 Primary polydipsia

Patients with primary polydipsia have normal plasma copeptin concentrations, with a significantly lower plasma apelin concentration than healthy volunteers, but with an apelin-to-copeptin ratio that remains within the normal range. This suggests either balanced water homeostasis or a role for the AS in the pathogenesis of primary polydipsia [61].

2. The role of the AS in cardiovascular diseases

The AS is expressed in the myocardium and in all three layers of the blood vessel wall, but its anatomic distribution and the distribution of isoforms vary between vascular beds. Both ligands are potent inotropes, and AR agonism typically causes vasodilation, but conflicting data have been published concerning this aspect, as illustrated in table 3 and recently reviewed [63]. In humans, the systemic administration of apelin lowers mean arterial blood pressure and reduces peripheral vascular resistance by arterial vasodilation [64-66]. AR expression levels are low in the heart, kidney and aorta of hypertensive rats [67]. Plasma apelin levels are low in patients with essential hypertension [68]. Apelin-deficient mice have low ACE-2 levels and high levels of myocardial hypertrophy and myocardial fibrosis following AngII infusion, whereas apelin infusion is protective [38-40]. Conversely, ACE-2 upregulation can attenuate the detrimental effects of a loss of apelin action [38, 69]. In addition, apelin- and AR-deficient mice develop progressive myocardial dysfunction with aging and are

susceptible to heart failure in response to pressure overload [40, 70, 71], whereas a loss or blockade of the AT1R is protective. Interestingly, stretch-dependent, rather than ligand-induced AR signaling contributes to the induction of hypertrophy [72]. Apelin and apela are positive inotropes in rodents and humans [13, 65, 73]. Acute apelin administration causes peripheral and coronary vasodilatation and increases cardiac output in healthy volunteers and patients with heart failure [74].

3. The role of the AS in pre-eclampsia and pregnancy-related hypertension

The AS is highly expressed in the placenta and participates in angiogenesis. It has been suggested that apela is a circulating hormone secreted both maternally and by the embryo and that its levels increase during pregnancy in mice [75]. Apela deficiency in mice induces placental insufficiency due to poor vascularization, inducing a hypoxic response, leading to hypertension, proteinuria and glomerular endotheliosis, as in human preeclampsia. No such effect is observed for apelin deficiency. Treatment with apela rescues this phenotype, whereas the overexpression of apelin does not,

suggesting the involvement of different signaling pathways. The interplay between the two ligands is fascinating. Apela represses the expression of apelin in allantoic explants, and the embryos with the most severe apela deficiency have the highest levels of apelin expression, suggesting that the restoration of apelin levels in the absence of apela is pathogenic. The two ligands have opposite effects on the expression of genes associated with hypoxia. These data open up new possibilities for treatment, but the clinical relevance of this work in mice remains to be demonstrated, given the high degree of species specificity and the evolutionary differences in the placenta between species. The consistency of human data is poor, due partly to the use of unreliable measurement methods [76]. Some studies have reported human placental AR expression to remain unchanged during pre-eclampsia, whereas others have reported a decrease, whereas apelin mRNA levels seem to be unaffected. Plasma apela concentrations in pregnant women with pre-eclampsia have been reported to be lower than, higher than or similar to those in healthy pregnant controls [77].

4. The role of the AS in acute kidney injury

Both apelin and apela have been shown to protect against acute kidney injury (AKI) [26]. Apela levels have been shown to be markedly low after AKI, and apela administration inhibits the DNA damage response, apoptosis, inflammation, fibrosis and renal functional parameters after renal ischemia/reperfusion in mice [26]. The protective effects of apela observed in a cell line with AR knockdown (KD) suggested that these effects were independent of AR. Treatment with a combination of apela and apelin results in synergistic protection against ischemia/reperfusion-induced loss of cell viability. Apelin exerts its protective effects by inhibiting TGF-beta and restoring histone methylation, whereas apela inhibits the increases in TGF-beta and inflammation levels without altering methylation. These findings suggest that these peptides have both overlapping and specific functions. Furthermore, apela was shown to have renoprotective effects in a murine model of myocardial infarction [78]. During experimental sepsis, circulating apelin levels decrease and myocardial apelin and AR

are downregulated, whereas circulating and myocardial apela concentrations remain unaffected [47, 79]. Renal AS expression is unaffected by sepsis [47], whereas circulating apelin-13 levels increase early in human sepsis [80]. The administration of apelin and apela reduces inflammation, vascular permeability, and multiple organ failure and improves hemodynamics, fluid homeostasis and outcome in experimental models of sepsis [47]. Interestingly, the potency of the cardiac response to apelinergic agonists seems to be unaffected or enhanced during sepsis, contrasting with the lower responsiveness of the myocardium to commonly used adrenoceptor β 1 agonists [17].

5. The role of the AS in diabetic nephropathy and renal fibrosis

The deletion of the AR gene in pancreatic beta cells lowers glucose tolerance [81], an effect that can be reversed by apelin administration [82]. Apelin administration increases glucose uptake in muscle, and reduces blood insulin and glucose concentrations [83]. Circulating apelin concentration increases in type II diabetes and

obesity [84]. Apela concentration has been shown to be inversely associated with proteinuria and kidney function [85] and apelin concentration has been correlated with cardiovascular mortality and kidney function decline in patients with type II diabetes [86]. AR signaling can inhibit renal fibrosis by decreasing the expression of transforming growth factor- β (TGF β) [87] in various cell types, including podocytes [88], and attenuating epithelial mesenchymal transition (EMT), in various cell types, including renal tubular cells [89], suggesting a potential role in diabetic nephropathy. Apelin levels are high in the glomeruli of diabetic mice and have a protective effect, inhibiting TGF β and EMT in podocytes [88]. However, the potential role of the AS in diabetic nephropathy remains unclear. The prolonged subcutaneous administration of apelin decreases glomerular hypertrophy, renal inflammation and albuminuria in mice with type I diabetes [90], and could have a beneficial effect through the regulation of histone acetylation [91]. In another study, the prolonged administration of apela decreased renal inflammation, fibrosis and podocyte apoptosis in diabetic mice [92]. By contrast, apelin can also induce podocyte dysfunction and disrupt glomerular

angiogenesis [93]. The AS has also been associated with inflammatory renal conditions. Patients with advanced Alport syndrome display a marked upregulation of apelin and AR, associated with inflammatory markers [94]. The stimulation of primary human glomerular endothelial cells with apelin-13 or pyr-apelin-13 separately increased ICAM1 and VCAM1 expression, whereas costimulation with both isoforms did not, indicating that different apelin isoforms may be required for the pro-inflammatory activation of glomerular endothelial cells [94].

6. The AS in autosomal dominant polycystic kidney disease and renal cancer

Two small studies have reported lower plasma apelin concentrations in patients with autosomal dominant polycystic kidney disease (ADPKD) than in healthy subjects [95, 96], associated, like increases in copeptin concentration, with disease progression [96]. As vasopressin antagonism is currently the only disease-specific treatment option for ADPKD [97], these findings are interesting and require validation in larger cohorts.

The AR is mutated in several cancers [98]. It was recently identified as essential for the effector function of CD8⁺T cells, with loss-of-function mutations of the AR gene associated with a failure of immunotherapy [99]. Given the importance of immune evasion mechanisms in renal cell carcinoma (RCC), Tolkach et al. explored the role of the AS [28]. They reported lower levels of AR expression in higher grade, higher stage and metastatic chromophobic RCC tumors, and an association of AR levels with survival (table 4). Apela downregulation has been observed in all types of renal cancer, and apela has been shown to upregulate mTORC1 [11]. Apelin and AR can induce the morphological and functional maturation of blood vessels in tumors, thereby enhancing the efficacy of immunotherapy and anti-angiogenic therapy [100, 101]. Apelin is a marker of disease progression and has prognostic implications in several cancer types, via diverse putative mechanisms reviewed briefly in table 4. Cells have recently been engineered for the recognition of the AR as a marker for angiogenesis in proliferating endothelial cells, resulting in promising new tools for the detection and treatment of

solid tumors [102]. The AS thus constitutes an active area of research in oncology that may lead to promising new treatments.

THE AS AS A TREATMENT TARGET

The administration of apelin agonists in SIADH and heart failure could increase water excretion and reverse hyponatremia. However, to date there are no published preclinical or clinical studies targeting the AS in these conditions. A first clinical trial (NCT03276728) with an oral small-molecule AR agonist in patients with heart failure was recently discontinued, probably due to a lack of efficacy.

FUTURE PERSPECTIVES

1. Development of novel drugs

The therapeutic targeting of the AS is an active area of research. More stable peptide and non-peptide derivatives of the two ligands would increase the potential for pharmacological use. Ligands biased towards signaling beneficial in specific contexts could also be developed. Small-molecule and peptide-based agonists, antagonists and

allosteric modulators of AR described between 2014 and 2019 have been reviewed elsewhere [103]. The N-PEGylation and N-palmitoylation of the native peptides, the fusion of apelin-13 to an IgG Fc fragment, and the encapsulation of Pyr1-apelin-13 in PEGylated liposomal nanocarriers have all been used to increase pharmacological stability [17, 104-106]. The addition of a fluoroalkyl chain to apelin-17 improves its activity, in terms of diuresis and blood pressure, *in vivo* [51]. An apelin analog with two cyclized β -turn RPRL motifs has been shown to act as a competitive antagonist of the AR [107]. The cyclic analog MM07 is a biased agonist that has been shown to increase forearm blood flow by vasodilation in human volunteers and to increase cardiac output more effectively than apelin in rodents [66]. Several small-molecule AR ligands have been developed. IV administration of the G α_i -biased agonist CMF-019 increases cardiac contractility in rats [108]. The characteristics of the oral small-molecule apelin agonist AMG986 [109] and of the first antibody agonist against the AR to be developed [110] have recently been published.

2. Challenges for future research

Apelin and apela protein levels have mostly been measured with commercial immunoassays based on polyclonal antibodies raised against various synthetic peptide fragments. Only 0.5-5% of these antibodies bind to the putative target [111]. Furthermore, they probably recognize a plethora of apelin fragments [112], resulting in a large range of reported plasma concentrations. Alternative methods combining high-performance liquid chromatography with radioimmunoassay detection have also been used. For one such method, based on rabbit polyclonal antibodies directed against apelin 17 [53], a relative specificity of the antibodies for apelin 13, 17 and 36 fragments was confirmed, with iodinated apelin-13 and 17 used as tracers [44, 49, 53]. The detection of naturally circulating apelin peptides by mass spectrometry has been attempted, but only one group has successfully detected apelin *in vivo*. [8, 14, 73]. The short half-life of apelinergic peptides, probably only a few minutes [13] has also hampered attempts to determine their levels, and validated protocols for sample conservation before analysis are lacking. There is still no reliable method for apela

detection [76]. Further insight will depend on an ability to detect individual apelin fragments, but the most reliable way for accurately determining the levels of endogenous apelinergic peptide fragments *in vivo* has yet to be established; published results should therefore be interpreted with caution [14, 112].

Furthermore, given the omnipresence of the AS and the various physiological processes involved, there is a considerable potential for both target and undesired effects. More insight into the physiology and pathophysiology of the AS is required, for definition of the desired signaling profile for any given therapeutic indication. Signaling bias is currently preferred in treatment strategies for the potential indications discussed above. The clinical efficacy of AS targeting in any of the aforementioned pathological conditions has yet to be unequivocally demonstrated.

CONCLUSION

The AS is a novel pleiotropic system with an essential role in renal and cardiovascular (patho) physiology, including water homeostasis and blood pressure regulation. Both the physiological role of the AS and its potential as a treatment target have been less studied than those of its counterparts, vasopressin and the RAS. Many research questions remain unanswered, including the renal distributions and physiological roles of apelinergic isoforms, the main signaling pathways of the AS in the kidney, the interplay between the AS, RAS and vasopressin, and the establishment of reliable detection methods. The development of novel molecules targeting the AS is rapidly expanding the potential therapeutic arsenal.

Figure 1 Signaling pathways of the apelin receptor. Different ligand isoforms can elicit variable signaling pathways (biased agonism,) with distinct intracellular and physiological consequences. The primary G α coupling of the AR is with Gai/o, leading to an inhibition of cAMP production by apelin and apela isoforms in subnanomolar quantities. Evidence shows that the AR preferentially binds Gai1 and Gai2, but not Gai3, while the subtypes of the G $\beta\gamma$ subunits associated with the AR remain unknown. Coupling of the AR to the Gq protein and subsequent Ca²⁺ signaling has been described. Coupling of the AR to the Gq protein and subsequent Ca²⁺ signaling has been described [70], but others have described a lack of Ca²⁺ mobilization by apelin isoforms [39] or a decreased amplitude of Ca²⁺ release upon AR activation in mouse cardiomyocytes [113]. Thus, the nature of Gq protein interaction and Ca²⁺ signaling after AR activation, remains to be elucidated and could be cell- or tissue specific. Apela inhibits store-operated Ca²⁺ entry in AR-expressing HEK cells [11]. Both apelin and apela isoforms can stimulate ERK1/2 MAPK phosphorylation in a Gai-dependent fashion [50, 114]. Other transduction cascades

include PLC/ PKC ϵ , AMPK/ eNOS, and PI3K/Akt/p70S6 kinase signaling. AR binding can lead to β -arrestin recruitment and subsequent internalization through a clathrin-dependent mechanism. Depending on the apelin isoform and mediated by different Rab proteins, β -arrestin binding to the AR can both be followed by rapid dissociation and recycling of the AR to the cell surface or by more stable binding and subsequent intracellular sequestration [115]. β -arrestin can also activate further cascades, including the phosphorylation of MAPK ERK1/2. The contexts in which rapid or slow resensitization or degradation and what other pathways -if any- of the arrestin interactome are subsequently influenced remain to be elucidated. Generally, the longer apelin isoforms lead to β -arrestin binding to the AR, causing internalization and degradation of the AR. In contrast, apelin-13 allows for disassociation of the receptor from β -arrestin and the subsequent recycling of the receptor following internalization. In addition, a ligand independent mechano-response of AR can trigger myocardial hypertrophy in a β -arrestin-dependent manner, while apelin can oppose this effect by lowering Ca²⁺ transients [72]. Although a 31% homology with the angiotensin 1 receptor, the AR does not bind angiotensin II. However, homo-and heterodimerization of AR with the AT1R (induced by apelin-13 or independent of a ligand), opioid receptor, neurotensin receptor-1 and bradykinin 1 receptor has been reported [114], either inhibiting or potentiating signaling. The interplay between the AR and AT1R negatively regulates the allosteric binding site and function of the AT1 receptor. Abbreviations: AC: adenylate cyclase; AKT: Protein kinase B; AMPK: Adenosine monophosphate-activated protein kinase; AR: Apelin Receptor;; AT1R: angiotensin 1 receptor; ATII: angiotensin II.; ATP: adenosine triphosphate; BARR: β -arrestin; cAMP: Cyclic adenosine monophosphate; eNOS: endothelial nitric oxide synthase; ERK: Extracellular signal-regulated kinase; FOXO: forkhead box protein; PKC: Protein kinase C; SIRT3: Sirtuin-3

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

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TABLES

Organ system	Anatomic region	AR	Apelin	Apela	Ref
<i>Kidney</i>	Glomeruli	M ^R P ^R R ^R	R ^P	M ^R R ^R	[22, 23, 25]
	Tubules	R ^R P ^P	R ^P	M ^R P ^P R ^R H ^P	[11, 22-26]
	CD	R ^R	M ^P R ^P	M ^R P ^P R ^R P ^P	[22-24, 26, 116]
	Medullary interstitium	M ^R R ^R	M ^R R ^R		[22]
	Vasa recta	M ^R R ^R			[22, 23]
	Podocytes	M ^P R ^R	M ^R		[117]
<i>Heart</i>	Left ventricle	M ^R R ^R H ^R P ^P	M ^R H ^R	M ^R H ^R	[12, 21, 22, 118-122]
	Cardiomyocytes	R ^P R ^R H ^P R ^R	R ^R H ^R	R ^R	[21, 22, 118, 119, 122-124]
	Endothelium	M ^R H ^R P ^P	M ^R H ^P	M ^R R ^R	[118, 119, 122, 125, 126]
<i>Vasculature</i>	Arteries (media & intima)	H ^P	H ^P	H ^R	[12, 119, 127]

	Veins (media & intima)	H ^P	H ^P	H ^R	[12, 119, 127]
	Coronary arteries	R ^{RP} H ^P	H ^P		[118, 127, 128]
	Vascular smooth muscle cells	R ^P H ^P			[118]
	Endothelium (venous and arterial)	M ^R R ^P H ^P	H ^P		[118, 129]
<i>Lung</i>		M ^R R ^{RP} H ^R P	M ^R R ^R H ^R	H ^R	[12, 21, 127, 130, 131]
<i>Central</i>	Hypothalamus (PVN, SON)	M ^R R ^{RP} H ^R	R ^P R ^R H ^R P		[53, 60, 131-134]
	Pituitary gland	M ^R R ^{RP} H ^R	R ^{RP} H ^R		[21, 131, 133, 135-137]
<i>Pancreas</i>		M ^P R ^P H ^P R	M ^R H ^R		[138]
<i>Placenta</i>		R ^R H ^R P	M ^R H ^R P	M ^{RP} H ^R	[21, 75, 130, 135, 139, 140]

Table 1. Overview of AS expression. M=mouse, R=rat, H=human. ^R=gene expression. ^P=protein detection. During

adulthood, AR and apelin are widely expressed, whereas apela expression is more restricted. Interspecies differences in (sub)anatomic localization are observed. Abbreviations: PVN: periventricular nucleus; SON: supraoptic nucleus. Protein detection was based on immunohistochemistry or radioligand binding.

Condition, No. patients	Apelin*	Copeptin*	Apelin:Copeptin Ratio*	Pathophysiological Hypothesis	Remarks	Ref
SIADH, 16	26% Increase	75% Increase	-27% (86% outside the physiological range)	Insufficient increase in plasma apelin secretion to compensate for high vasopressin levels.	Neither apela nor the local renal AS studied.	[62]
Congestive heart failure, 32	26% Increase	173 % -291 Increase°	Lower than predicted in 59%	Small increase in apelin secretion, insufficient to counteract the increase in vasopressin levels, resulting in hyponatremia. Advanced cardiac dysfunction blunts the apelin secretion induced by hyponatremia. Opposite patterns of plasma apelin regulation by hypovolemia and hyponatremia	Neither apela nor the local renal AS studied.	[62]

Central DI, 15	17% Decrease	53% Decrease	56% higher than in healthy controls	Decrease in plasma apelin concentration insufficient to counteract the decrease in vasopressin concentration. Imbalance between the antidiuretic and diuretic effects resulting in polyuria.	Neither apela nor the local renal AS studied.	[61]
Nephrogenic ic DI, 7	63% Increase	1280% Increase	92% lower than healthy controls	Parallel increase in apelin and copeptin concentrations. Renal resistance to vasopressin and an increase in apelin levels resulting in polyuria.	Neither apela nor the local renal AS studied. . Small number of patients, mostly with lithium- induced disease	[61]

Primary polydipsia, 19	25% Decrease	4.9% Decrease	Similar to healthy controls	Normal apelin-to-copeptin ratio consistent with balanced water homeostasis. Apelin involved in the pathogenesis of primary polydipsia	Neither apela nor the local renal AS studied.	[61]

Table 1. Apelin, copeptin and apelin:copeptin ratio in disorders of sodium and water balance *relative to healthy controls Abbreviations: DI diabetes insipidus ° normonatremic and hyponatremic patients, respectively

Ligand	Effect	Specifications	Vascular bed, species	Ref
<i>Apelin-13</i>	Vasodilation	NO-dependent, endothelium- dependent	Coronary arteries, rat	[128]
<i>Pyr- apelin 13</i>	Vasodilation	Endothelium dependent, NO- independent	Internal mammary arteries, human	[141]
<i>Apelin-13</i>	Attenuation of angiotensin II-induced vasoconstriction	Endothelium- independent	Portal vein, rat	[142]
<i>Apela-32</i>	Vasodilation	NO-independent	Aorta, mouse	[143]
<i>Pyr- apelin 13</i>	Vasoconstriction	NA	Atherosclerotic coronary artery, human	[144]
<i>Pyr- apelin 13</i>	Vasoconstriction	Intact vasculature	Saphenous veins of humans receiving coronary bypass grafts	[127]

<i>Apelin-17</i>	Vasodilation	Precontracted by ATII	Juxtamedullary afferent arteriole	[23]
<i>Apelin-13</i>	Vasodilation	Inhibition of NO-induced and endothelium-dependent vasodilation	Cerebral artery, rat	[145]
<i>Apelin-13, Apela-32</i>	Hypotensive, inotropic	IV or IP administration	Mouse, rat	[13, 17]
<i>Apela-32</i>	Hypotensive, inotropic	Attenuation of angiotensin-II-induced pressor effect	Mouse	[42]
<i>Pyr-apelin-13</i>	Hypertensive	IV systemic	Conscious mice	[146]

<i>Apelin-36, Pyr-apelin 13</i>	Vasodilation	Arterial but not venous vasodilatation after local IV or IA infusion	Healthy human volunteers, dorsal hand vein, brachial arterial flow	[64]
<i>Pyr-apelin 13</i>	Vasodilation, hypotension, inotropic	Local and systemic administration	Healthy human volunteers and patients with heart failure	[65]

Table 3. Overview of the potential vascular and hemodynamic effects of apelinergic agents. Discrepancies have been described in terms of vascular and hypotensive effects, depending on the vascular bed, species and experimental conditions. In denuded vessels, apelin induces vasoconstriction. Abbreviations: ATII angiotensin II; CI cardiac index; IA intra-arterial; IP intraperitoneal; IV intravenous; LVEF left ventricular ejection fraction.

Type of Cancer	Main Findings	Interpretation	Ref
<i>Renal cell carcinoma</i>	AR expression is correlated with chromophobic RCC aggressiveness. AR is expressed in the tumor vasculature and tumor cells at different levels, with opposite effects on tumor aggressiveness. Cytoplasmic staining of tumor cells was associated with poor patient survival, whereas vascular staining was associated with good overall patient survival. Apelin expression was only weakly associated with the histological grade of the tumor.	Upregulation of AR and apelin by hypoxia-inducible factor-1 alpha. The inverse relationship between AR expression and tumor aggressiveness is explained by the fact that AR activation induces maturation of the tumor vasculature, rendering immunotherapy more effective.	[28]
<i>Renal cell carcinoma</i>	Apela gene expression was repressed, with no apela protein detected in clear cell, papillary, and chromophobe renal cell carcinomas. Apela induced apoptosis and repressed cell proliferation and migration in an mTORC1-dependent manner.	Apela has tumor suppressor features. Potential new role of mTORC1 activation by apela, suggesting the potential use of apela and/or derivatives as a therapeutic approach in kidney cancers.	[11]

	<p>Apela acted in synergy to repress tumor growth.</p> <p>Apela expression in renal carcinoma cells either induced or repressed AR expression.</p>		
<i>Renal cell carcinoma</i>	<p>Microarray analysis showed an upregulation of apelin in the RCC tissues relative to the adjacent normal tissues, whereas RT-qPCR showed no significant difference in apelin gene expression between the RCC and adjacent normal tissues.</p>		[147]
<i>Colon carcinoma</i>	<p>AR and apelin were upregulated in colon adenomas and adenocarcinomas. AR antagonist decreased the proliferation of colorectal cancer cells.</p>	<p>Presence of an autocrine loop, stimulating tumor growth.</p>	[148]
<i>Melanoma</i>	<p>Apelin promoted lymphangiogenesis and the lymph node metastasis of melanoma cells.</p>	<p>Apelin plays an important role in lymphatic tumor progression and has potential for use as an anticancer therapy targeting lymphangiogenesis.</p>	[149]

<i>Glioblastoma</i>	Apelin is a central regulator of the endothelium-mediated maintenance of glioblastoma patient-derived cells with stem-like properties. The competitive AR antagonist MM54 impaired glioblastoma growth.	Apelin release by endothelial cells is a potential treatment target for glioblastoma and other cancers.	[150]
<i>Glioblastoma</i>	Apelin, released by glioblastoma and/or endothelial cells, is required for tumor angiogenesis. The apelin/AR signaling pathway inhibits glioblastoma cell invasion.	Clinical perspective for combination treatments based on apelin-F13A and angiogenesis inhibitors.	[151]
<i>Mammary and lung cancers</i>	Apelin inhibition remodels the tumor microenvironment, reducing angiogenesis, normalizing tumor vessels and blunting tumor growth. Apelin ablation prevents resistance to anti-angiogenic therapy, reducing growth and angiogenesis in lung and breast cancer models, without increasing hypoxia in the tumor microenvironment. Apelin levels were correlated with a poor prognosis in patients receiving sunitinib.	Apelin ablation strikes an Achilles heel of antiangiogenic therapy, reducing tumor angiogenesis and primary tumor growth while maintaining vascular structure, with a higher degree of pericyte coverage, preventing progression, hypoxia and distant metastasis.	[100]

<i>Non-small cell lung cancer</i>	Apelin mRNA levels in human NSCLC samples were higher than those in normal lung tissue. High tumor apelin protein levels were associated with high microvessel densities and poor overall patient survival.	Apelin is a novel angiogenic factor in human non-small cell lung cancer.	[152]
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Table 4. The apelinergic system in oncology

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180x253mm (300 x 300 DPI)

NDT-01745-2020.R2-fig2

204x241mm (300 x 300 DPI)