



# The therapeutic potential of apelin in kidney disease

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**Abstract** | Chronic kidney disease (CKD) is a leading cause of global morbidity and mortality and is independently associated with cardiovascular disease. The mainstay of treatment for CKD is blockade of the renin–angiotensin–aldosterone system (RAAS), which reduces blood pressure and proteinuria and slows kidney function decline. Despite this treatment, many patients progress to kidney failure, which requires dialysis or kidney transplantation, and/or die as a result of cardiovascular disease. The apelin system is an endogenous physiological regulator that is emerging as a potential therapeutic target for many diseases. This system comprises the apelin receptor and its two families of endogenous ligands, apelin and elabela/toddler. Preclinical and clinical studies show that apelin receptor ligands are endothelium-dependent vasodilators and potent inotropes, and the apelin system has a reciprocal relationship with the RAAS. In preclinical studies, apelin regulates glomerular haemodynamics and acts on the tubule to promote aquaresis. In addition, apelin is protective in several kidney injury models. Although the apelin system has not yet been studied in patients with CKD, the available data suggest that apelin is a promising potential therapeutic target for kidney disease.

Chronic kidney disease (CKD) is an increasingly common public health concern with a global prevalence of ~10%<sup>1</sup>. This disease now ranks as the 12th leading cause of death worldwide<sup>1</sup>. CKD results from a heterogeneous group of conditions that lead to a progressive and irreversible impairment in kidney function. It is defined as a reduction in estimated glomerular filtration rate (eGFR) to <60 ml/min/1.73 m<sup>2</sup> and/or the presence of markers of kidney damage on at least two occasions at least 3 months apart<sup>2</sup>.

CKD is independently associated with cardiovascular disease<sup>3</sup>. As eGFR decreases, the risks of major cardiovascular events, cardiovascular mortality and all-cause mortality increase<sup>4</sup>. Importantly, patients with stage 1–3 CKD (eGFR >30 ml/min/1.73 m<sup>2</sup>) are more likely to die from cardiovascular disease than they are to reach kidney failure<sup>4,5</sup> and around 50% of patients with kidney failure die from cardiovascular causes<sup>6</sup>. Not only are cardiovascular events more common in patients with CKD, but outcomes following such events are worse than in the general population<sup>7</sup>. Almost 8% of global cardiovascular deaths in 2017 were attributable to CKD<sup>1</sup>.

Hypertension is both a cause and a consequence of CKD. As kidney function declines, blood pressure rises, and more than 85% of patients with CKD are hypertensive<sup>8</sup>. Thus, reducing blood pressure in CKD is a key

therapeutic strategy that not only slows the progression to kidney failure but also reduces cardiovascular risk<sup>9</sup>. However, more than 30% of patients with CKD require four or more antihypertensive agents to achieve adequate blood pressure control and up to 50% never reach their target blood pressure<sup>8</sup>. Uncontrolled hypertension promotes the development of left ventricular hypertrophy (LVH). The prevalence of LVH increases as kidney function declines and it is present in ~50% of patients with an eGFR of <25 ml/min/1.73 m<sup>2</sup> (REF.<sup>10</sup>). Alongside hypertension and LVH, arterial stiffness, endothelial dysfunction and proteinuria are characteristic features of CKD and important independent predictors of cardiovascular disease<sup>4,11–14</sup>.

Current evidence-based management of CKD is limited to blockers of the renin–angiotensin–aldosterone system (RAAS) that slow CKD progression<sup>4,8</sup>. Sodium–glucose co-transporter 2 (SGLT2) inhibitors, which were originally developed for the treatment of type 2 diabetes mellitus (T2DM), have also now been shown to improve kidney and cardiovascular end points in patients with and without T2DM<sup>15,16</sup>. However, an urgent unmet need remains for novel treatments<sup>17</sup>. The ideal therapy would provide direct renoprotection and reduce proteinuria, while also offering broad cardiovascular protection. The apelin system has exciting therapeutic potential in this regard. In this Review, we focus on current

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## Key points

- Chronic kidney disease (CKD) is common worldwide and is associated with high cardiovascular morbidity and mortality; however, treatment options are limited.
- The apelin system is a broad regulator of physiology that opposes the renin–angiotensin–aldosterone system (RAAS) and has beneficial cardiovascular effects in health and disease.
- Increasing evidence indicates that apelin has favourable effects on renal physiology, including roles in the regulation of fluid balance and glomerular haemodynamics.
- Preclinical models demonstrate direct anti-inflammatory and anti-fibrotic actions of the apelin system in models of kidney injury; targeting the apelin receptor in combination with RAAS blockers might offer synergistic benefits.
- The apelin system is an attractive potential therapeutic target for CKD, offering direct renal protection in addition to targeting the associated cardiovascular complications.

understanding of the apelin system, its cardiovascular and renal benefits, and the next steps required to explore its utility as a potential therapy for CKD.

### Biology of the apelin system

The apelin system comprises the apelin receptor and its two endogenous ligands, apelin and elabela/toddler (ELA; also known as apelin receptor early endogenous ligand).

#### Apelin receptor

The *APLNR* gene (also known as *APJ*) was identified and cloned<sup>18</sup> in 1993. This gene was found to encode the apelin receptor, a novel G protein-coupled receptor (GPCR) that has ~50% homology with the type 1 angiotensin II (AT<sub>1</sub>) receptor. The apelin receptor system opposes the actions of angiotensin II (Ang II) agonism at the AT<sub>1</sub> receptor in vitro and in vivo<sup>19–27</sup>. In the cardiovascular system, apelin and AT<sub>1</sub> receptors are co-expressed. The apelin receptor is highly conserved between species with ~90% sequence similarity between mouse, rat and human proteins<sup>28</sup>. Only one apelin receptor has been identified in mammals, although two are expressed in fish and amphibians<sup>29–31</sup>.

#### Apelin receptor ligands

The two endogenous ligands of the apelin receptor are products of distinct genes, which is unusual for a peptide–ligand GPCR. The first to be discovered, apelin (encoded by *APLN*), was identified in 1998 and the second, ELA (encoded by *APELA*), was identified in 2013 (REFS<sup>32–35</sup>).

**Apelin.** Apelin peptides are produced by C-terminal cleavage of a 77-amino-acid precursor, pre-pro-apelin. Peptide fragments of varying lengths circulate in vivo; the major isoforms are apelin-36, apelin-17 and apelin-13 (REF.<sup>28</sup>) (FIG. 1a). The pyroglutamated form of apelin-13, [Pyr<sup>1</sup>]apelin-13, is structurally more resistant to metabolism by aminopeptidases than apelin-13 and is the most abundant apelin isoform in the cardiovascular system and human plasma<sup>36,37</sup>.

The mechanisms of apelin peptide metabolism are not yet fully defined. Experimental evidence suggests that furin (also known as PCSK3), plasma kallikrein and neprilysin cleave apelin peptides, but only neprilysin has

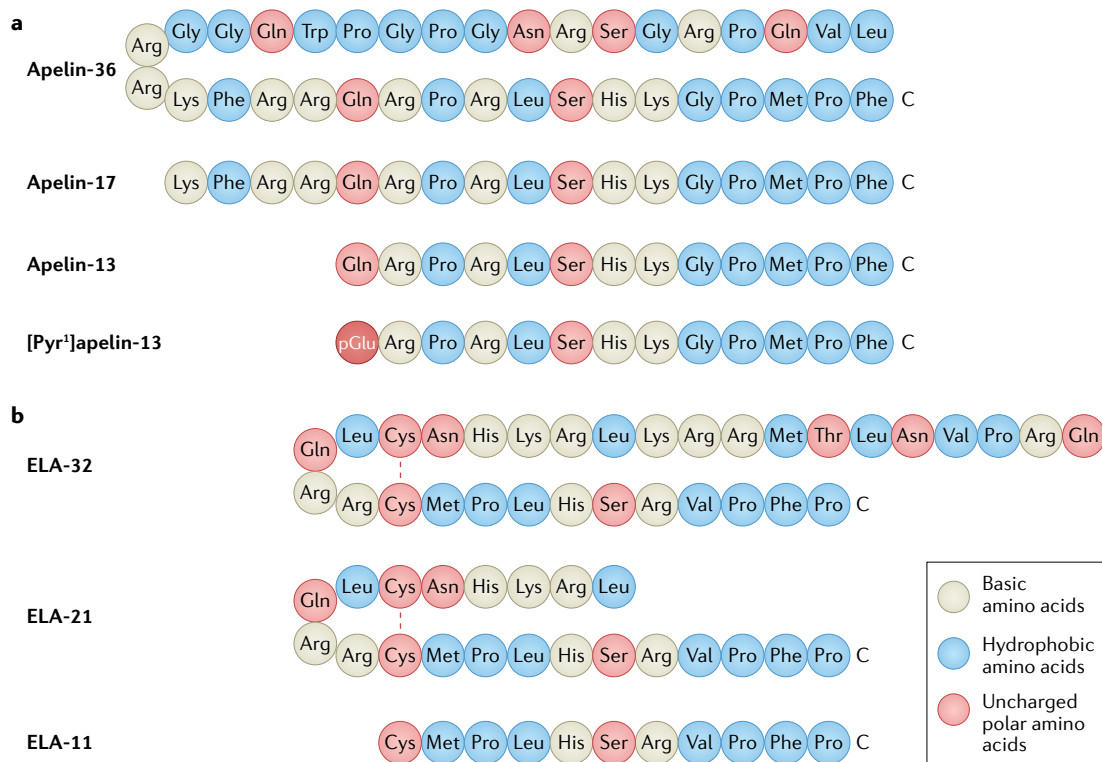
been shown to completely inactivate these peptides<sup>27,38–41</sup>. This finding is of interest when considering the cardio-protective effects of neprilysin inhibition in heart failure<sup>42</sup>. The mechanisms that underlie these effects remain undefined but prevention of the neprilysin-mediated degradation of apelin peptides could potentially contribute.

The carboxypeptidase angiotensin-converting enzyme 2 (ACE2) has been clearly demonstrated to cleave apelin isoforms both in vitro and in vivo, resulting in the removal of the common C-terminal phenylalanine<sup>43–45</sup>. This enzyme is highly expressed on the surface of lung alveolar epithelial cells and enterocytes and is expressed at lower levels in most organs including kidney and heart and on arterial and venous endothelial cells<sup>45–47</sup>. ACE2 promotes vasodilatation by converting Ang II into angiotensin-1-7 (Ang(1-7))<sup>43</sup>. In addition, ACE2 acts as a functional cellular entry receptor for severe acute respiratory syndrome coronaviruses, including SARS-CoV-2, which causes COVID-19 (REFS<sup>48–50</sup>). ACE2 was thought to inactivate [Pyr<sup>1</sup>]apelin-13 (REF.<sup>44</sup>); however the [Pyr<sup>1</sup>]apelin-13 metabolite, [Pyr<sup>1</sup>]apelin-13<sub>(1–12)</sub>, is detectable in human tissues and retains biological activity in vitro and in vivo although it is less active than [Pyr<sup>1</sup>]apelin-13 (REFS<sup>44,45</sup>). In healthy volunteers who received [Pyr<sup>1</sup>]apelin-13 infusions, the major metabolite generated in the plasma was [Pyr<sup>1</sup>]apelin-13<sub>(1–12)</sub>; [Pyr<sup>1</sup>]apelin-13<sub>(1–10)</sub> and [Pyr<sup>1</sup>]apelin-13<sub>(1–6)</sub> were also identified<sup>51</sup>.

**Elabela.** ELA is a 54-amino-acid peptide that is predicted to be processed to form mature peptides including ELA-32, ELA-21 and ELA-11 (FIG. 1b). The discovery of ELA as the second endogenous ligand for the apelin receptor provided an explanation for the unexpected and marked discrepancy between the phenotypes of apelin receptor-knockout mice, which were not born in expected Mendelian ratios and had substantial cardiovascular developmental defects, and apelin-knockout mice, which developed normally<sup>52</sup>.

In zebrafish, most ELA mutants die early in development as a result of cardiovascular abnormalities, including poorly developed or absent hearts. This phenotype could be rescued by injection of ELA, and the cardiovascular abnormalities could be recapitulated by knocking out the apelin receptor but not by knocking out apelin<sup>30,34,35,53</sup>.

Mice that lack ELA show low-penetrance embryonic lethality and cardiovascular abnormalities; however, their phenotype differs from that of apelin receptor knockouts<sup>54</sup>. Loss of ELA leads to yolk sac vascular remodelling defects with cardiac abnormalities and disrupted fetal circulation, whereas mice with loss of the apelin receptor have a lower incidence of such defects and some embryos show abnormal tail bending. The abnormalities in coronary vascularization in ELA knockouts and apelin receptor knockouts may be overcome by alternative progenitor cell pathways as development progresses, perhaps explaining the low-penetrance lethality<sup>55</sup>. ELA is detectable in human plasma but its specific tissue isoform expression has not yet been explored<sup>56</sup>.



**Fig. 1 | The peptide sequences of apelin and ELA isoforms. a** | The amino acid sequences of apelin-36, apelin-17, apelin-13 and [Pyr<sup>1</sup>]apelin-13 aligned by the C terminus, which is crucial for receptor binding and biological activity. The N-terminal Gln residue of apelin-13 undergoes translational modification to pyroglutamate, which increases resistance to metabolism by aminopeptidases. The resulting pyroglutamated form of apelin-13, [Pyr<sup>1</sup>]apelin-13, is the most abundant apelin isoform in humans<sup>34,35</sup>. **b** | The predicted amino acid sequences of ELA-32, ELA-21 and ELA-11 isoforms<sup>23,33</sup>. The N-terminal Gln of ELA-32 is predicted to undergo transformation to pyroglutamate. ELA-32 and ELA-21 form bridges between Cys residues. The occurrence and relative abundance of ELA isoforms have not yet been confirmed experimentally. Apelin and ELA isoforms have similar physicochemical properties, particularly within the C terminus.

### Expression of the apelin system

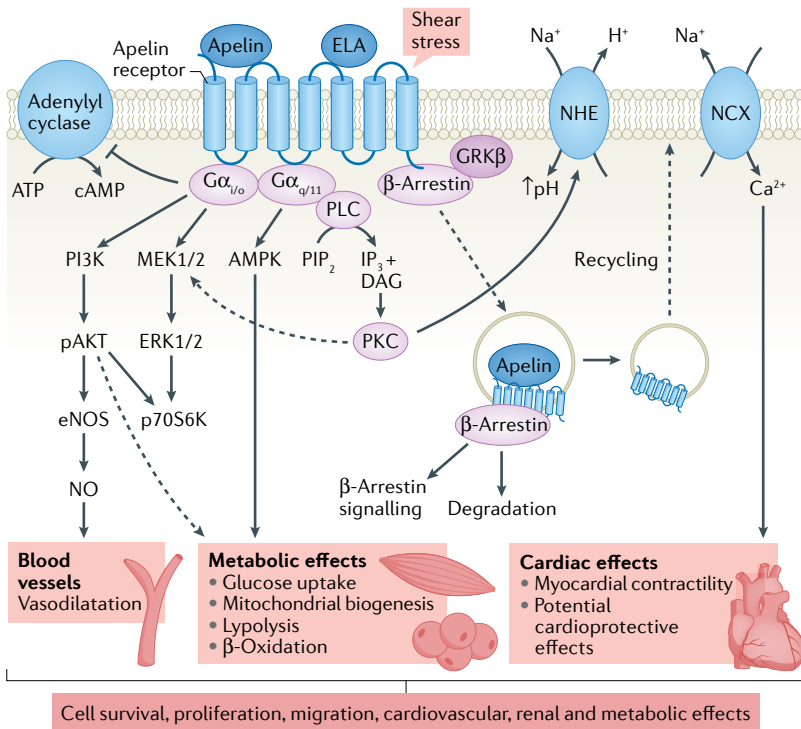
Apelin and the apelin receptor are widely expressed within the central nervous system and peripheral organs. In rodents and humans, apelin receptor mRNA is detected in many tissues, particularly in brain, spinal cord, placenta, lung, heart, kidney, adipose tissue and skeletal muscle<sup>57–60</sup>. Apelin receptor protein has been identified in the brain, spinal cord, heart, kidney and lung of rats and humans<sup>59,61,62</sup>. In human heart, apelin receptor is expressed by cardiomyocytes, endothelial cells of the endocardium and intramyocardial blood vessel, endothelial cells and smooth muscle of conduit artery and vein<sup>59,61,62</sup>. Within the kidney, apelin receptor protein localizes to the cortex and vasculature<sup>61,62</sup>.

In humans and rats, mRNA encoding apelin is widely expressed throughout the brain, spinal cord, lung, kidney, heart and stomach with lower expression in other tissues<sup>33,58,59,63</sup>. In the periphery, apelin protein localizes predominantly to vascular endothelial cells, for example, in human heart, kidney and adrenal gland and to the endocardial endothelial cells that line the atria and ventricle<sup>64</sup>. Apelin is also an adipokine<sup>65</sup> that localizes to adipose tissue and is produced by and secreted from human and rodent adipocytes<sup>60,66</sup>. However, apelin does not seem to be a major circulating hormone, and plasma concentrations are lower than tissue levels<sup>36,67</sup>.

Given this finding and the similar locations of apelin peptide and apelin receptor expression, it seems probable that the apelin system has a predominantly autocrine or paracrine mechanism of action. In contrast to apelin, ELA was initially reported to show kidney-specific expression in adult rat, largely confined to the renal tubules, but was subsequently identified in rodent heart<sup>68–70</sup>. To date, human studies have detected ELA expression only on the vascular endothelium and within the kidney<sup>36,71</sup>.

### Apelin receptor binding and signalling

The endogenous apelin isoforms bind with nanomolar affinity to their cognate receptor in cell expression systems and in rat and human cardiovascular tissues<sup>45,57,59,61,67,72,73</sup>. However, structure–activity studies suggest that the longer isoforms, such as apelin-36, and particularly apelin-17, have somewhat higher affinity than the shorter isoforms such as [Pyr<sup>1</sup>]apelin-13 (REFS<sup>45,74,75</sup>). The N-terminal RPRL sequence of [Pyr<sup>1</sup>]apelin-13, which is present in all longer apelin isoforms, is required for receptor binding with some contribution from other residues such as Pro12 (REFS<sup>76–78</sup>) (FIG. 1a). The smallest apelin fragment to retain biological activity, although with reduced affinity, is the 10-amino-acid apelin-13<sub>(2–11)</sub><sup>45,57,79</sup>.



**Fig. 2 | Apelin receptor activation leads to a broad range of physiological actions that are mediated by several signalling pathways.** In vascular endothelial cells, binding of apelin or elabela/toddler (ELA) to the apelin receptor results in  $G_{\alpha_i}$ -mediated inhibition of cAMP production and activation of phosphoinositide 3-kinase (PI3K)–AKT signalling cascades, leading to vasodilatation. In cardiomyocytes, activation of the apelin receptor leads to a  $G_{\alpha_q}$ -mediated increase in cardiac contractility and cardiac output through activation of the phospholipase C (PLC)–protein kinase C (PKC) pathway with subsequent enhanced activity of the  $\text{Na}^+/\text{H}^+$  exchanger (NHE) and increased intracellular pH. The increase in intracellular  $\text{Na}^+$  activates the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX), leading to a rise in intracellular calcium and increased myocardial contractility. Apelin-13 is thought to promote cardiomyocyte hypertrophy via PI3K–AKT–ERK1/ERK2–p70S6K and PI3K-induced autophagy (not shown). The metabolic effects of apelin receptor activation have been proposed to be mediated via  $G_{\alpha_i}$  and  $G_{\alpha_q}$ . Signalling via  $\beta$ -arrestin leads to apelin receptor desensitization and internalization. The internalized receptor is targeted for degradation or recycled to the cell surface.  $\beta$ -Arrestin-mediated signalling may also contribute to the actions of apelin. DAG, diacylglycerol; eNOS, endothelial nitric oxide synthase; ERK1, extracellular signal-regulated kinase 1; GRK $\beta$ ; G protein coupled receptor kinase- $\beta$ ; IP $_3$ , inositol tris-phosphate; MEK1, mitogen-activated protein kinase 1; NO, nitric oxide; pAKT, phosphorylated AKT; PIP $_2$ , phosphatidylinositol 4,5-bisphosphate; p70S6K, p70 ribosomal S6 kinase.

To date, only one crystal structure has been reported for the apelin receptor in complex with a 17-amino-acid non-endogenous apelin agonist<sup>80</sup>. Substantial mutation was required for successful crystallization of the receptor. However, using this structure in combination with molecular dynamic simulations and molecular modelling, the study demonstrated a two-site peptide–ligand binding mode. This ‘message–address’ concept of peptide binding was also suggested by earlier studies, including one in which amino acids in the N-terminal tail and first extracellular loop were shown to be important both for folding of the receptor protein and binding of apelin peptides<sup>81</sup>. In another structural study, the upper face of the apelin receptor binding site was visualized using 3D homology models, and the interactions of basic residues in the N-terminal RPRL of [Pyr<sup>1</sup>]apelin-13 with acidic residues in extracellular loops I and II as

**‘Message–address’ concept of peptide binding**  
The concept that agonists contain two distinct parts: one determines receptor efficacy and activation (the ‘message’); the other influences receptor selectivity (the ‘address’).

well as the interface between extracellular loop III and transmembrane VII were confirmed using site-directed mutagenesis<sup>82</sup>.

The ELA peptides show little sequence similarity to apelin although the predicted isoforms ELA-32, ELA-21 and ELA-11 bind to the apelin receptor in human heart; ELA-32 bound with subnanomolar affinity, ELA-21 with nanomolar affinity (comparable to that of [Pyr<sup>1</sup>]apelin-13) and the shortest isoform, ELA-11, bound with affinity an order of magnitude lower than that of the other peptides<sup>56</sup>. An alanine scan and mutagenesis analysis suggested that apelin and ELA peptides engage with different residues in rat and human apelin receptors<sup>83</sup>.

The apelin receptor couples to pertussis toxin-sensitive inhibitory G proteins ( $G_{\alpha_i}$ ) with subsequent activation of extracellular-regulated kinases (ERKs) and phosphoinositide 3-kinase (PI3K)–AKT (also known as protein kinase B (PKB)) signalling cascades<sup>57,84–87</sup> (FIG. 2). These cascades lead to a broad range of physiological effects that are dependent on the type of cell that is activated. All putative apelin and ELA isoforms elicit canonical  $G_i$ -mediated inhibition of adenylate cyclase, which results in inhibition of cAMP<sup>45,56,67,72,84</sup>. Similar to other GPCRs, the apelin receptor can engage with other heterotrimeric G proteins, particularly  $G_q$ , resulting in downstream stimulation of phospholipase C (PLC) and AMP-activated protein kinase (AMPK) pathways<sup>88,89</sup>.

Following activation, GPCRs can be uncoupled from their G proteins and internalized via recruitment of  $\beta$ -arrestins to the receptor. The extent and kinetics of apelin receptor-mediated internalization may be isoform specific, at least in vitro, with apelin-36 suggested to produce more prolonged internalization than [Pyr<sup>1</sup>]apelin-13 (REFS<sup>72,90–92</sup>). For successful translation of apelin receptor agonists to the clinical setting, the finding that GPCR ligands may preferentially activate a subset of the signalling repertoire of a GPCR or indeed stimulate some signalling pathways and inhibit others, so called ‘biased signalling’, is an important discovery<sup>93</sup>. Compared with [Pyr<sup>1</sup>]apelin-13, longer apelin ligand isoforms such as apelin-36, apelin-17, ELA-32 and ELA-21 have been shown to exhibit some bias towards  $\beta$ -arrestin recruitment<sup>45,56,73</sup>. The in vivo relevance of these observations to apelin receptor physiology or pathophysiology remains to be determined.

Ligand-independent signalling through the apelin receptor has also been reported<sup>19,94</sup> and may be pathologically important. Phosphorylation of ERK1 and ERK2 downstream of Ang II-mediated activation of AT $_1$  receptor was inhibited in cells that co-expressed the apelin receptor and this inhibition was abolished by apelin in a pertussis toxin-sensitive manner<sup>19</sup>. Subsequently, genetic loss of the apelin receptor was demonstrated to protect against cardiac hypertrophy and heart failure in a chronic pressure overload mouse model, whereas loss of apelin had no effect<sup>94</sup>. This discrepancy could potentially be explained by the later discovery of ELA. However, isolated cardiomyocytes from apelin receptor-knockout mice exhibited a reduced response to stretch, implying that the apelin receptor can act as a mechanosensor even in the absence of ligand. This stretch-mediated response



was abrogated by knockdown of  $\beta$ -arrestins and blunted by the addition of apelin<sup>94</sup>. The apelin receptor might therefore have pro-hypertrophic effects via  $\beta$ -arrestin signalling in the absence of ligand and anti-hypertrophic effects via  $\beta$ -arrestin-independent pathways when activated by apelin.

### Clinical targeting of the apelin system

As apelin peptides have a half-life of only a few minutes in humans<sup>95–97</sup>, and rapid receptor desensitization via coupling to  $\beta$ -arrestins has been reported<sup>98</sup>, clinical studies of the apelin system are challenging. These issues have led to efforts to produce apelin receptor agonists and antagonists that may be used as pharmacological probes to explore the role of the system in health and disease. Clear understanding of the relevant signalling pathways is essential to enable the development of appropriately targeted therapeutic agents.

Apelin analogues with enhanced biological activity compared with the endogenous peptides and resistance to degradation have now been developed<sup>99,100</sup>. In a human forearm blood flow study, a cyclic apelin peptide mimetic, MM07, which has an extended half-life, showed biased agonism to beneficial G protein pathways and enhanced vasodilatation compared with [Pyr<sup>1</sup>]apelin-13 (REF.<sup>97</sup>). Similarly, another apelin mimetic peptide bound to anti-serum albumin domain antibodies to extend its half-life had in vivo potency, lowering blood pressure and increasing cardiac contractility, stroke volume, heart rate and cardiac output in rodents<sup>75</sup>.

From a clinical perspective, the ideal agent would likely be a small-molecule compound biased towards G protein signalling that does not effectively recruit  $\beta$ -arrestins and, therefore, limits receptor desensitization and subsequent downregulation with continued clinical use<sup>97,101</sup>. One such small molecule, CMF-019, has been developed. This molecule exhibits high affinity for the apelin receptor and has in vivo functional activity with a bias towards G protein signalling<sup>101</sup>. However, its use as an oral agent may be limited by its poor solubility.

Two small-molecule agonists of the apelin receptor, AMG-986 and AMG-8123, with extended plasma half-lives (>2–4 h in rats and dogs) have also been developed. These molecules have a high affinity for the apelin receptor and were shown to be functional in vivo and in animal models of heart failure<sup>102</sup>. Other small-molecule apelin agonists are also in development<sup>28</sup>.

Although several compounds have been suggested to act as apelin antagonists, contradictory studies suggest that some also have agonist activity. The field would benefit from the identification of well-characterized antagonists. If successful, the development of apelin agonists and antagonists could lead to a new era of research and therapeutics based on the apelin system<sup>28</sup>.

### Apelin in cardiovascular physiology

The apelin system is a broad regulator of physiology (FIG. 3). In the cardiovascular system, apelin has roles in control of vascular tone, blood pressure, inotropy, angiogenesis and haemostasis.

#### Vascular function and blood pressure

Apelin has well-described effects on the vasculature. Preclinical studies established that in healthy vessels, apelin acts on the endothelium to promote vasodilatation through an increase in nitric oxide (NO)<sup>21,103</sup> (FIG. 2). By contrast, in endothelium-denuded vessels, apelin acts directly on vascular smooth muscle cells to promote vasoconstriction<sup>36,45</sup>. Various animal models have demonstrated that multiple apelin peptides lead to a rapid, transient and dose-dependent reduction in blood pressure, with variable effects on heart rate<sup>33,45,72,104,105</sup>. The blood pressure-lowering action of apelin in vivo is also NO dependent<sup>21,105</sup>.

In humans, infusions of [Pyr<sup>1</sup>]apelin-13, [Pyr<sup>1</sup>]apelin-13<sub>(1–12)</sub> and apelin-36 led to reproducible, dose-dependent and NO-dependent arteriolar vasodilatation in the forearm<sup>45,95,97</sup>. In keeping with in vitro data that show slower dissociation of apelin-36 than [Pyr<sup>1</sup>]apelin-13 from the apelin receptor, this peptide has a more prolonged action in humans<sup>57,95</sup>. Systemic studies in healthy volunteers demonstrated that apelin reduces blood pressure; [Pyr<sup>1</sup>]apelin-13 led to a 5–10% fall in blood pressure, reduced peripheral vascular resistance and caused a small increase in heart rate that was most likely compensatory<sup>96</sup>. The effects of apelin on venous tone are less consistent. One study found that [Pyr<sup>1</sup>]apelin-13 reversed noradrenaline-induced pre-contraction of dorsal hand veins, whereas another showed no effect<sup>95,97</sup>. However, this discrepancy may be explained by the ~30-fold difference in peptide dose in these studies.

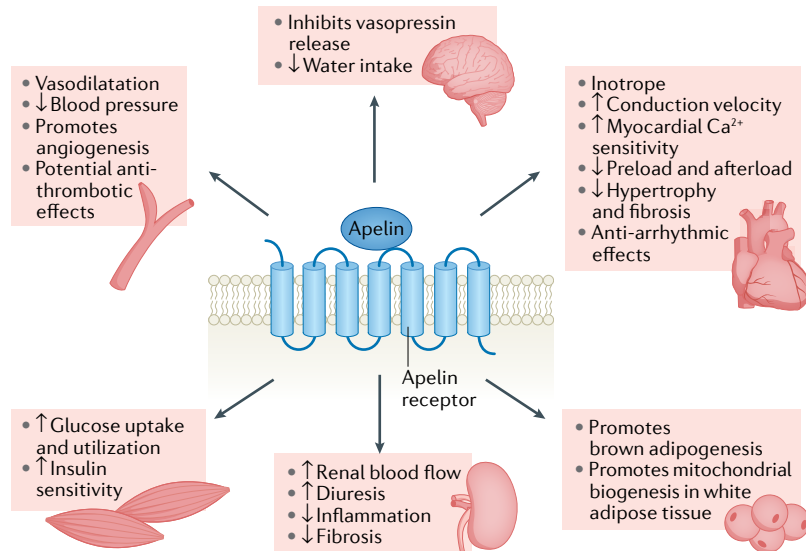


Fig. 3 | **Physiological actions of the apelin system in different organs and tissues.**

Activation of the apelin receptor results in a broad range of physiological effects in central and peripheral tissues. Within the vasculature, the apelin system promotes vasodilatation, subsequent lowering of blood pressure and angiogenesis and might also have antithrombotic effects. Apelin is a potent inotrope that increases myocardial  $\text{Ca}^{2+}$  sensitivity and reduces cardiac preload and afterload. It also increases the conduction velocity within cardiomyocytes, has anti-arrhythmic effects and reduces myocardial hypertrophy and fibrosis. In the kidney, activation of the apelin system increases renal blood flow and diuresis and reduces inflammation and fibrosis. The apelin system also has a central role in the regulation of fluid homeostasis: apelin inhibits hypothalamic vasopressin release and reduces water intake. Finally, the apelin system has a range of metabolic effects. It promotes brown adipogenesis and mitochondrial biogenesis in white adipose tissue, increases muscle glucose uptake and utilization and increases insulin sensitivity.

Box 1 | **Apelin in cancer**

Apelin is overexpressed in one-third of human solid organ malignancies and may act in a paracrine or autocrine manner to promote angiogenesis<sup>179,180</sup>. Hypoxia has been shown to upregulate apelin expression in tumour cell lines<sup>179,181</sup>. However, cancer biology is unique to the type of malignant cell. Studies in colorectal cancer cell lines found that apelin was anti-apoptotic, and the apelin receptor antagonist F13A reduced cell proliferation<sup>180</sup>. Genetic overexpression of apelin in mouse non-small cell lung cancer (NSCLC) cells promoted tumour growth, and loss of apelin in models of breast and lung cancer reduced tumour growth and angiogenesis and increased survival<sup>182,183</sup>. In addition, loss of apelin or treatment with the biased apelin receptor ligand MMS4 had synergistic antitumour effects with the tyrosine kinase inhibitor sunitinib in the MMTV-*NeuT* mouse model of mammary cancer<sup>183</sup>. Although previously considered to be an apelin receptor antagonist, MMS4 has been shown to act as a partial agonist and to promote G protein signalling pathways<sup>184</sup>.

Studies in models of glioblastoma, the most common primary adult brain tumour, suggest that endothelium-derived apelin sustains glioblastoma stem-like cells, which have been implicated in initiation and facilitation of tumour growth as well as treatment resistance. MMS4 inhibited tumour growth and prolonged survival in intracranially xenografted mice<sup>185</sup>. Interestingly, bevacizumab, a monoclonal antibody to vascular endothelial growth factor (VEGF), can reduce apelin expression in biopsy samples from patients with glioblastoma and is known to increase cell invasion<sup>186</sup>. F13A reduced cell invasion and tumour angiogenesis and showed synergistic anti-angiogenic effects when combined with VEGF inhibition in a mouse model of glioblastoma<sup>186</sup>.

Elabela/toddler (ELA) is downregulated in human renal cell carcinoma (RCC), and activation of the apelin receptor by ELA reduced tumour cell survival, proliferation and motility in cultured renal cancer cells and in mouse models of RCC. Overexpression of ELA in renal cancer cells induced apelin receptor expression, and the tumour-suppressing effect of ELA was enhanced *in vivo* in the presence of sunitinib<sup>187</sup>.

Intriguingly, a functional apelin receptor is necessary for tumour T cell effector function and successful cancer immunotherapy; however, this effect may be ligand independent<sup>188</sup>. In RCC, apelin receptor expression is inversely associated with tumour grade and the presence of metastases, and lower apelin receptor expression is an independent predictor of shorter cancer-specific and overall survival<sup>189</sup>. In NSCLC models, elevated levels of apelin protein are also independently associated with poorer survival<sup>182</sup>. Overall, the available data suggest that targeting the apelin system may offer therapeutic promise in several cancers.

ELA also promotes vasodilatation, although its mechanism of action may differ from that of apelin. In preclinical studies, ELA relaxed pre-constricted mouse aortic rings in a dose-dependent manner and induced carotid artery vasodilatation in the rat<sup>70,71</sup>. *In vivo* data confirm that systemic infusions of both ELA and a bioactive analogue, ELA(19–32), reduce blood pressure in rodents<sup>56,99</sup>. In contrast to apelin, ELA-induced vasodilatation is NO independent and only partly dependent on the endothelium<sup>71</sup>. The effects of ELA have not yet been investigated in humans.

**Inotropy and conduction**

Apelin acts via several signalling pathways and is the most potent endogenous inotrope discovered to date<sup>36,88,106,107</sup> (FIG. 2). Mice with knockout of apelin develop a progressive reduction in cardiac contractility that can be restored by apelin infusion<sup>108</sup>. Apelin administration accentuated sarcomere shortening in healthy rat cardiomyocytes and resulted in a dose-dependent increase in contractility in isolated perfused rat hearts<sup>88,106,109</sup>. Consistent with this finding, chronic [Pyr<sup>1</sup>]apelin-13 infusion increased cardiac output *in vivo* in mice<sup>110</sup>.

A number of apelin peptides have been shown to promote inotropy with subnanomolar potency in human paced atrial strips<sup>36,45</sup>. In a clinical study in

healthy volunteers, intracoronary boluses of apelin-36 and systemic infusions of [Pyr<sup>1</sup>]apelin-13 and apelin-36 increased cardiac output<sup>36,45,96</sup>. This effect was maintained in the setting of renin–angiotensin system (RAS) activation owing to either salt depletion or Ang II co-infusion<sup>24</sup>.

Clear evidence exists of antagonism between the apelin system and the RAAS. In addition to impaired contractility, apelin-deficient mice have reduced ACE2 expression, and inhibition of AT<sub>1</sub> receptors or infusion of Ang(1-7) rescued cardiac function and restored ACE2 expression in these mice<sup>111</sup>. In addition, Ang II-induced cardiac dysfunction is enhanced by apelin deficiency<sup>112</sup>. Many inotropic agents result in LVH and subsequently increased mortality; however, apelin does not cause cellular hypertrophy in animal studies<sup>110</sup>. This difference is likely a consequence of the systemic haemodynamic effects of apelin, which are in contrast to the effects of other inotropic agents and increase its appeal as a therapeutic agent.

ELA is also a positive inotrope in preclinical models. In isolated adult rat hearts, ELA-32 increased cardiac contractility<sup>70</sup>. This was replicated in a second study that also showed ELA(19–32) to be inotropic<sup>99</sup>. Further *in vivo* studies confirmed that in rats, ELA-32 increases cardiac contractility, ejection fraction and cardiac output<sup>56</sup>.

The apelin system has also been shown to influence cardiac conduction. Preclinical studies demonstrate that apelin increases conduction velocity within cardiomyocytes and influences the cardiac action potential<sup>109,113</sup>. For example, apelin shortens the action potential in atrial myocytes via effects on multiple ion currents<sup>113</sup>.

**Angiogenesis**

Apelin is necessary for normal vascular development during embryogenesis<sup>114,115</sup>. Embryos with knockout of apelin initially show reduced-calibre blood vessels<sup>115</sup>. Other factors seem to be able to ‘rescue’ these vessels later in development, although these remain undefined. However, studies of ocular blood vessel formation show that apelin-knockout mice have impaired retinal vascularization and reduced responsiveness to pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (REF.<sup>116</sup>). Preclinical studies confirm that ELA also promotes angiogenesis by acting on the apelin receptor<sup>55,71</sup>.

Apelin expression is induced by hypoxia both *in vitro* and *in vivo*, and hypoxia-inducible factor 1α (HIF1α) promotes apelin gene transcription<sup>114,117,118</sup>. Interestingly, hypoxia-stimulated endothelial cell proliferation is prevented by inhibition of the apelin pathway, suggesting that the apelin system contributes to physiological and pathophysiological regulation of blood vessel growth<sup>117</sup>.

In addition to its physiological role in vascular development, apelin might have a pathological role in promoting angiogenesis in various cancers (BOX 1).

**Haemostasis**

Both apelin and the apelin receptor are expressed on human platelets. Apelin-13 inhibits platelet aggregation induced by low (but not high) concentrations of

**Inotrope**

A substance that alters the force of contraction of a muscle. This term is normally used to refer to effects on the myocardium and, unless otherwise specified, implies increased contractility.

thrombin or collagen *in vitro* but does not seem to influence platelet activation by other stimuli such as thromboxane A<sub>2</sub> or ADP<sup>119</sup>. The effects of apelin on platelets are mediated through inhibition of calcium mobilization as well as the generation of NO<sup>119</sup>, which promotes platelet production of cGMP. The NO–cGMP pathway has a biphasic effect with low concentrations activating platelets and high concentrations being inhibitory<sup>120</sup>. Thrombin increases cGMP levels within platelets, promoting their activation. The addition of apelin further increases cGMP levels, leading to inhibition of platelet activity<sup>119</sup>.

Animal data confirm the antithrombotic effect of apelin *in vivo*. Apelin-deficient mice are pro-thrombotic, with reduced tail bleeding time, enhanced platelet aggregation and faster thrombotic occlusion of venules compared with wild-type controls. Infusion of apelin-13 prolongs bleeding time in both wild-type and apelin-deficient mice<sup>119</sup>. Whether apelin also has an antithrombotic effect in humans has not yet been investigated. Current antiplatelet therapies focus on inhibition of cyclooxygenase 1 (using aspirin) or antagonism of the ADP P2Y<sub>12</sub> receptor (using clopidogrel, prasugrel or ticagrelor). Thrombotic events can still occur in patients who are receiving antithrombotic combination therapies, and an agent that provides broader cardiovascular protection while reducing the risk of thrombo-occlusive events would be very desirable.

#### Apelin in cardiovascular disease

The apelin system is dysregulated in cardiovascular disease, and beneficial effects of its activation have been shown in disease models. The apelin receptor is therefore an appealing target for pharmacological intervention.

#### Vascular actions of apelin in disease

CKD is characterized by medial vascular calcification and the development of arterial stiffness. This change in vascular structure alongside endothelial injury leads to endothelial dysfunction. Importantly, preclinical and clinical studies show that the vasodilatory effect of apelin persists even in states of endothelial dysfunction, and this effect seems to be independent of NO<sup>36,103</sup>. In this setting, apelin might act via a prostanoid-dependent mechanism. However, the available data are inconclusive, perhaps owing to the use of different cyclooxygenase inhibitors and their differential effects on downstream signalling<sup>36,103</sup>.

In clinical studies, patients with heart failure and healthy volunteers had equivalent dose-dependent vasodilatation of forearm vessels in response to [Pyr<sup>1</sup>]apelin-13 (REF.<sup>96</sup>). In a mouse model of immune-mediated vascular injury after heart transplantation, apelin promoted vascular repair and endothelial cell differentiation and reduced immune cell adhesion<sup>121</sup>. This finding could have exciting therapeutic potential for similar immune-mediated vascular injury following kidney transplantation.

The vascular endothelium is an important regulator of coagulation. Endothelial release of tissue plasminogen activator promotes fibrinolysis, and its activity is regulated by plasminogen activator inhibitor 1 (PAI1).

In states of endothelial dysfunction, fibrinolysis may be impaired. A reciprocal relationship exists between expression of the apelin system, the AT<sub>1</sub> receptor and PAI1 in patients with atrial fibrillation and thrombosis<sup>122</sup>. In left atrial appendages from these patients, expression of apelin and the apelin receptor was reduced and expression of AT<sub>1</sub> receptors and PAI1 was increased compared with those from patients with sinus rhythm or with atrial fibrillation without thrombosis. In healthy mice, 3 weeks of treatment with Ang II promotes *PAI1* gene expression, whereas similar exposure to apelin downregulates *PAI1* gene expression in preclinical models<sup>23</sup>. This finding may provide an additional mechanism by which apelin regulates haemostasis.

#### Atherosclerosis

The apolipoprotein E (*ApoE*)-knockout mouse is a widely used model of atherosclerosis. Mice that are deficient in both ApoE and apelin develop more atheromatous lesions than *ApoE*-knockout mice, which in turn develop more atheromatous lesions than *ApoE* and apelin receptor-double-knockout mice<sup>123,124</sup>. The reasons for these contrasting data are unclear but it is noteworthy that mice that lack the apelin receptor show increased prenatal death, and those that do survive have cardiovascular malformations that likely contribute to accelerated atherosclerosis. The apelin receptor might have ligand-independent effects on the development of atherosclerosis, but such effects have not been noted in studies using apelin receptor-knockout mice. In the *ApoE*-knockout mouse, treatment with apelin alone had no effect on the development of atheromatous lesions<sup>123</sup>. However, administration of Ang II resulted in an increase in atherosclerotic lesions that was abrogated by coadministration of apelin, providing further evidence of functional interaction of the RAS and the apelin system *in vivo*<sup>123</sup>.

#### Heart failure

The available data suggest that the apelin system is upregulated early in heart failure and downregulated as the disease progresses<sup>125</sup>. Plasma apelin concentrations show a similar pattern<sup>22,125–128</sup>. Evidence exists of functional crosstalk between the apelin system and the RAS in heart failure. In animal models, Ang II infusion results in downregulation of cardiac apelin mRNA and this effect can be inhibited by an angiotensin receptor blocker (ARB). Similarly, in animals with heart failure, downregulation of the apelin system can be restored by an ARB<sup>22,129</sup>. Administration of L-N<sup>G</sup>-monomethylarginine (L-NAME), an inhibitor of NO synthesis, abrogated the beneficial effects of an ARB (olmesartan) on cardiac function and the apelin system in a heart failure model<sup>129</sup>. These findings together with the inotropic effect of apelin have led to sustained enthusiasm regarding the potential therapeutic role of apelin in heart failure.

Preclinical studies have demonstrated that apelin has inotropic effects in models of heart failure<sup>109,130</sup>. Moreover, systemic infusion of [Pyr<sup>1</sup>]apelin-13 resulted in a modest fall in blood pressure and systemic vascular resistance and a ~10% increase in cardiac index

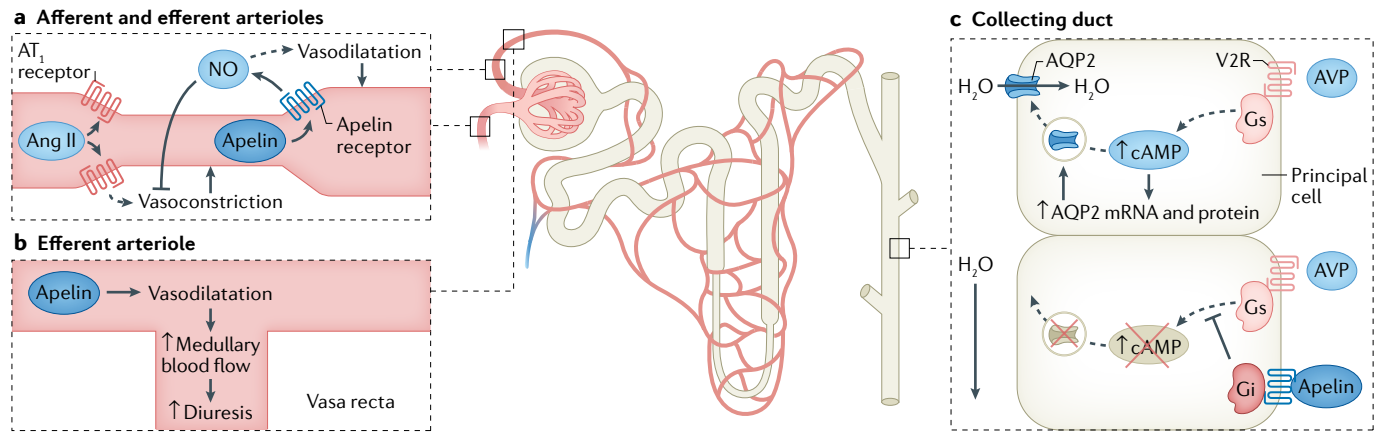


Fig. 4 | **The actions of apelin in the nephron.** **a** | Apelin acts at the afferent and efferent arterioles to promote vasodilatation via production of nitric oxide (NO), opposing the action of angiotensin II (Ang II). **b** | Increased vasodilatation at the efferent arteriole directly increases blood flow through the vasa recta, leading to increased medullary blood flow and promoting diuresis. **c** | The action of apelin counteracts vasopressin signalling in the kidney tubules. In the principal cells of the collecting duct, apelin prevents vasopressin-induced translocation of aquaporin 2 (AQP2) channels to the apical membrane and therefore prevents water reabsorption. AT<sub>1</sub> receptor, type 1 angiotensin II receptor; AVP, arginine vasopressin; Gi, inhibitory G protein  $\alpha$ -subunit; Gs, stimulatory G protein  $\alpha$ -subunit; V2R, vasopressin v2 receptor.

in patients with stable New York Heart Association Class II–IV heart failure symptoms and a left ventricular ejection fraction of <40% (or shortening fraction <20%) who were receiving optimized treatment<sup>96</sup>. Further studies found this improvement in cardiac output to be sustained during prolonged apelin infusion (6 h), with an associated increase in ejection fraction of ~10%<sup>24</sup>. At present, the utility of apelin in heart failure is limited by the lack of long-acting preparations.

**Myocardial infarction**

Studies in several preclinical models have demonstrated that apelin protects against ischaemic myocardial injury. Apelin-knockout mice show enhanced susceptibility to injury, with larger infarct size, increased ventricular dilatation and increased mortality compared with wild-type controls<sup>100</sup>. Administration of apelin or an apelin analogue had protective effects in models of myocardial infarction, including improved functional recovery and a reduction in infarct size. These effects were most likely due to promotion of NO production and angiogenesis<sup>100,131</sup>.

Following any ischaemic injury, angiogenesis is stimulated to promote oxygen delivery to tissues. In a mouse model of myocardial infarction, overexpression of HIF1 $\alpha$  reduced infarct size and the development of heart failure<sup>132</sup>. Apelin signalling is critically important in angiogenesis, *APLN* may be upregulated by HIF1 $\alpha$ , and apelin-deficient animals have impaired angiogenic responses to ischaemia–reperfusion injury (IRI)<sup>100,118</sup>. Currently, following myocardial infarction, patients are treated with RAS blockers to reduce the detrimental effects of Ang II. Importantly, a preclinical study showed that combination treatment with apelin and the ARB losartan reduced infarct size by ~50%, compared with a ~30% reduction when either treatment was used individually<sup>133</sup>. This finding holds promise for future clinical studies in this area.

**Arrhythmias**

Clinical studies have shown that plasma apelin levels are reduced in patients with supraventricular tachycardias, including atrial fibrillation<sup>134,135</sup>. Moreover, apelin levels predict the recurrence of atrial arrhythmias<sup>136</sup>. Patients with atrial fibrillation have reduced atrial apelin levels, and apelin administration completely prevented the induction of atrial arrhythmias in mice with increased susceptibility owing to chronic intense exercise<sup>137</sup>. Atrial fibrillation is associated with a high risk of ischaemic stroke and is common in patients with CKD. The prevalence of atrial fibrillation increases as kidney function declines, reaching ~20% in patients not requiring kidney replacement therapy<sup>138,139</sup>. Decisions on anticoagulation in this patient group are complex as bleeding risk also increases as kidney function declines<sup>140</sup>. If targeting of the apelin system is able to protect against arrhythmia, this strategy could be particularly advantageous for patients with CKD.

**Apelin in kidney physiology**

Although widely expressed in human kidney, studies of the role of apelin in human kidney physiology are lacking. Animal studies suggest that apelin has direct actions on the kidney that contribute to the regulation of renal haemodynamics and fluid homeostasis.

**Glomerular haemodynamics**

Glomerular blood flow is under the tight control of the RAS, which regulates both glomerular perfusion and GFR, and crosstalk exists between the RAS and the apelin system (FIG. 4a). By increasing intracellular calcium levels, Ang II vasoconstricts both the afferent and efferent arterioles, with a preferential effect on the efferent arteriole. In rats, apelin reverses Ang II-induced vasoconstriction of the afferent and efferent glomerular arterioles and causes a rapid fall in intracellular calcium levels. This vasodilatory action of apelin is dependent on an intact



endothelium and NO production<sup>141</sup>. As the vasa recta are supplied by the efferent arteriole, apelin-induced vasodilatation might promote diuresis through an increase in renal medullary blood flow (FIG. 4b).

### **Tubular function**

Apelin contributes to fluid homeostasis by opposing the actions of vasopressin, with crosstalk between the systems. Apelin, the apelin receptor and vasopressin co-localize within the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus, and clear reciprocal regulation of the peptides exists<sup>142–144</sup>. Clinical studies in healthy volunteers support these findings, with changes in plasma osmolality accompanied by parallel reciprocal changes in vasopressin and apelin levels<sup>145</sup>.

Both central and peripheral injections of apelin peptides and ELA promote diuresis in animals<sup>68,141,143,144,146</sup>. In addition, apelin acts directly on the renal tubule to promote a dose-dependent aquaresis without affecting renal sodium or potassium handling<sup>141,146</sup>. In vitro and in vivo studies suggest that this aquaresis is due to inhibition of aquaporin 2 channel insertion into the apical plasma membrane of the collecting duct<sup>146,147</sup> (FIG. 4c). Within the rodent kidney, ELA is more abundantly expressed than apelin and predominates in the medullary collecting ducts<sup>69</sup>. The specific mechanism of ELA-induced diuresis has not yet been defined. However, given that expression of ELA is restricted to the vascular endothelium and kidney in adults, a direct renal action would be expected.

### **Apelin in kidney disease**

Several in vitro and in vivo studies have investigated the effects of the apelin system in kidney pathology. Apelin is protective in a broad range of diseases owing to its anti-inflammatory and anti-fibrotic effects and might also have important roles in certain kidney diseases.

### **Acute kidney injury**

Acute kidney injury (AKI) affects 8–17% of hospitalized patients and >50% of those who are admitted to intensive care<sup>148,149</sup>. Moreover, AKI is strongly associated with adverse outcomes including the development of CKD, kidney failure and death, although the mechanisms are poorly defined<sup>150</sup>. Studies in models of AKI suggest early upregulation of the apelin system that is followed by downregulation as injury progresses<sup>151,152</sup>. This dynamic change is similar to that seen in heart failure.

Both in vitro and in vivo data support a direct protective effect of apelin treatment in kidney IRI, which is a widely used model of AKI<sup>151,153</sup>. Following IRI in rats, twice-daily administration of apelin-13 for 3 days protected against tubular injury, the hallmark of AKI, and also limited functional kidney impairment<sup>151</sup>. Apelin-13 also influenced injury-induced alterations in gene transcription both in vitro and in vivo, preventing downregulation of apelin mRNA and upregulation of HIF1 $\alpha$  and transforming growth factor  $\beta$  (TGF $\beta$ ), which are important mediators of progressive injury. Upregulation of inflammatory markers such as intracellular adhesion molecule 1 and monocyte chemokine protein 1 was also reduced, as was apoptosis<sup>151</sup>. Importantly, apelin was

protective when administered before IRI<sup>153</sup>, suggesting potential clinical applications in settings where AKI might be anticipated, including kidney transplantation or cardiothoracic surgery. Renoprotective effects of apelin have also been reported in other models of AKI, such as cyclosporin A-induced tubular injury, suggesting that apelin might protect against various renal insults, including drug-induced injury<sup>154</sup>.

ELA also protects against kidney IRI. Treatment with either ELA-32 or ELA-11 inhibited IRI-induced inflammation, apoptosis and fibrosis and preserved kidney function in a mouse model of unilateral ureteric obstruction<sup>155</sup>. Like apelin, specific ELA fragments have different signalling effects, with the shorter fragment, ELA-11, being more protective than ELA-32 both in vitro and in vivo. The mechanism of action is not clear, as doses of peptide that conferred protection did not induce a change in intracellular cAMP, which would be expected with apelin receptor activation<sup>155</sup>. Apelin-13 and ELA therefore seem to have different mechanisms of action and a synergistic effect has been suggested, with combination treatment offering greater protection against in vitro cell death<sup>155</sup>. Whether this finding could translate to synergy in humans and greater protection against AKI is unclear, but should be explored in clinical studies.

### **Diabetic kidney disease**

Diabetic kidney disease (DKD) is the most common cause of kidney failure worldwide<sup>156</sup>. Hyperglycaemia alters glomerular haemodynamics, leading to glomerular hypertrophy, damage to the filtration barrier and the development of albuminuria. Preclinical studies suggest that expression of the apelin system is altered in DKD. Cultured mouse podocytes have been shown to downregulate *APLNR* mRNA<sup>157</sup> but upregulate apelin receptor expression<sup>158</sup> in response to a high-glucose environment. Ang II administration further increased *APLNR* mRNA downregulation in these cells<sup>157</sup>. Some studies in animal models of T1DM and T2DM have shown reduced kidney expression of the apelin receptor and pre-pro-apelin, whereas others have shown the opposite<sup>157,159,160</sup>.

Definition of the role of the apelin system in DKD is limited by a lack of clinical studies and conflicting animal data<sup>161</sup>. In cultured mouse podocytes, apelin-13 had anti-apoptotic effects<sup>157</sup>. In the Akita mouse model of T1DM, apelin-13 preserved glomerular architecture, reduced proteinuria and inhibited renal inflammation<sup>162</sup>. The researchers found that this protection was due, in part, to an inhibitory effect of apelin on diabetes-induced histone hyperacetylation. Similar protection was observed in Ove26 mice with T1DM in which apelin-13 was found to have antioxidant effects and prevent the loss of megalin expression in the proximal tubule, resulting in maintenance of albumin resorption<sup>159</sup>.

Conversely, in the KK-Ay mouse model of T2DM, apelin contributed to disease progression, worsening of albuminuria and creatinine clearance via detrimental effects on renal blood flow, podocyte apoptosis and autophagy<sup>158,160,163,164</sup>. In this model, F13A conferred protection, which was purported to reflect antagonism of the apelin receptor. However, whether this compound

**Box 2 | Apelin in diabetic retinopathy**

Approximately 7% of patients with diabetes mellitus have proliferative diabetic retinopathy<sup>190</sup>. Preclinical studies have shown that apelin and vascular endothelial growth factor (VEGF) are upregulated in diabetic retina, and a high-glucose environment upregulates apelin in human retinal pigment epithelial cells<sup>191,192</sup>. Vitreous apelin levels are also increased in patients with proliferative diabetic retinopathy, although data regarding circulating apelin levels are conflicting<sup>193,194</sup>.

Intravitreal administration of the apelin receptor antagonist F13A in diabetic rats reduced retinal gliosis and restored VEGF expression<sup>191</sup>. This finding should be interpreted with caution as F13A is reported to also have agonist activity at the apelin receptor. However, apelin knockdown using a small interfering RNA approach suppressed pathological angiogenesis in a model of ischaemic retinopathy<sup>195</sup>. Overall, apelin seems to promote neovascularization in diabetic retinopathy. Further studies are needed to investigate whether apelin receptor antagonism could be clinically useful in these patients.

is a true antagonist is unclear. Overall, the available preclinical data suggest that apelin is protective in DKD. However, systemic application of apelin in patients with diabetes could potentially be limited by adverse effects such as the promotion of diabetic retinopathy (BOX 2).

**Disorders of fluid homeostasis**

The relationship between the apelin system and vasopressin is disrupted in disorders of fluid homeostasis. Syndrome of inappropriate antidiuretic hormone (SIADH) results in water retention due to vasopressin excess. Plasma copeptin is a reliable marker of vasopressin secretion and the apelin to copeptin ratio indicates relative activation of these systems. Patients with SIADH have substantially elevated plasma vasopressin levels and modest increases in plasma apelin. The apelin to copeptin ratio is lower than anticipated in ~90% of these patients<sup>165</sup>.

Disruption of apelin–vasopressin balance is also seen in patients with polyuria and polydipsia syndromes. Parallel decreases in plasma apelin and copeptin levels occur in primary polydipsia and cranial diabetes insipidus, whereas parallel increases in these levels occur in nephrogenic diabetes insipidus, resulting in alterations in the apelin to copeptin ratio<sup>166</sup>. Alterations in circulating apelin are suggested to be adaptations to restore the balance of diuresis and antidiuresis; however, they seem to be unable to fully compensate. Kidney function may be impaired in disorders of fluid homeostasis as a direct consequence of the cause of the disorder (such as lithium toxicity or nephrogenic diabetes insipidus) or as a consequence of hypovolaemia. The impact of reduced kidney function on circulating apelin levels is currently unclear.

**Polycystic kidney disease**

Autosomal dominant polycystic kidney disease (ADPKD) is the most common human monogenic disease. Vasopressin has an important role in the pathogenesis of ADPKD, and vasopressin antagonists are the first licensed treatment for this disease. Patients

with ADPKD have higher plasma copeptin and lower plasma apelin concentrations than healthy individuals<sup>167</sup>. Moreover, in ADPKD, plasma apelin concentration falls as kidney function declines and correlates negatively with TGF $\beta$ 1, which is a marker of kidney fibrosis<sup>167,168</sup>. Although noteworthy, this finding does not enable conclusions to be drawn more broadly regarding apelin levels in CKD given the close relationship between apelin and vasopressin. Little is known about the renal expression of the apelin system in ADPKD. However, the inhibitory effect of apelin on aquaporin 2 channel insertion in the collecting duct<sup>146,147</sup> suggests that treatment with apelin might be a potential alternative approach to use of the vasopressin 2 receptor antagonist tolvaptan. An aquaretic with direct anti-fibrotic effects on the kidney and beneficial cardiovascular effects would be a very attractive treatment option for this at-risk patient group.

**Kidney fibrosis**

The development of glomerulosclerosis and interstitial fibrosis is the final common end point of many CKDs. In the early stages of kidney injury, upregulation of inflammatory and pro-fibrotic mediators occurs. Left unchecked, these mediators promote progressive fibrosis and a reduction in GFR. The RAS promotes kidney fibrosis through the action of Ang II on the AT<sub>1</sub> receptor, and blockade of the system is beneficial in CKD<sup>169–171</sup>.

In vitro studies demonstrate that expression of the intrarenal RAS is regulated by the apelin system. For example, in collecting duct-derived M1 cells, ELA-32 reduced expression of the (pro)renin receptor, prorenin and renin<sup>172</sup>. In mice, knockout of the (pro)renin receptor led to increased levels of apelin and ELA mRNA<sup>172</sup>. The impact of (pro)renin receptor knockout on the apelin receptor was dependent on the location of the knockout: nephron-specific knockout resulted in reduced medullary apelin receptor mRNA and protein, whereas collecting duct-specific knockout had no effect. Animal models confirm reciprocal regulation of the RAS and apelin systems. For example, in Dahl salt-sensitive rats, ELA-32 infusion prevented high-salt diet-induced upregulation of renin, AT<sub>1</sub> receptor and AT<sub>2</sub> receptor mRNA<sup>172</sup>.

Apelin has anti-fibrotic effects<sup>173</sup>, and ELA overexpression or ELA-32 peptide administration reduced renal fibrosis and inflammation in salt-sensitive rats<sup>172,174</sup>. In the unilateral ureteric obstruction model of kidney fibrosis, the apelin system is upregulated in the obstructed kidney with evidence of activation of the AKT–endothelial nitric oxide synthase (eNOS) pathway, which is stimulated by apelin<sup>175</sup>. In this model, losartan, which blocks AT<sub>1</sub> receptors, reduced kidney fibrosis and promoted activation of the AKT–eNOS pathways and expression of apelin mRNA. Intriguingly, inhibition of the apelin system using either an apelin receptor antagonist or L-NAME abrogated the anti-fibrotic effect of losartan. Administration of L-NAME together with losartan increased fibrosis compared with controls<sup>175</sup>. In addition, the AT<sub>1</sub> receptor blocker, telmisartan, restored apelin receptor and pre-pro-apelin mRNA expression in a mouse model

of T2DM<sup>157</sup>. These observations suggest that the anti-fibrotic effects of ARBs are partly mediated through crosstalk with the apelin system, probably through NO generation. It would be of great interest to investigate whether combined treatment with an ARB and apelin can act synergistically in the kidney, as has been seen in a model of myocardial IRI<sup>133</sup>. If translated to clinical studies, such synergistic benefits would represent a remarkable therapeutic advance for patients with CKD.

## Conclusions

CKD is a global health problem and new treatments that delay progression to kidney failure are urgently needed. Targeting the apelin system is a promising strategy given its beneficial renal and cardiovascular effects. In addition, apelin positively affects lipid metabolism and improves glycaemic control by enhancing insulin sensitivity and promoting glucose utilization<sup>176,177</sup>. These protective effects on metabolic health are particularly attractive for patients with DKD. However, many unanswered questions remain. At present, no clinical

studies exist of the effects of apelin on renal physiology in either health or disease. In addition, the blood pressure-lowering and inotropic effects of apelin have been robustly demonstrated in healthy individuals and in patients with heart failure, but whether apelin has similar effects in patients with CKD who commonly have multiple comorbidities is unclear. Studies are now underway to answer these questions<sup>178</sup>. Moreover, current therapeutic use of apelin in CKD would require systemic administration. The endogenous apelin system appears to have tissue-specific expression with paracrine or autocrine signalling. Systemic application might have undesirable off-target effects, such as promotion of pathological angiogenesis, which is an important consideration for patients with multimorbidity. Overall, the apelin system clearly warrants further investigation and has exciting potential as a therapeutic target that could offer multi-system protection to improve outcomes for patients with kidney disease.

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