The Apelin–APJ system: Its role in renal physiology and potential therapeutic applications for renal disease

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Abstract

Introduction

Apelin is a vasoactive peptide isolated as a selective endogenous ligand of orphan receptor, APJ, which was genetically identified to have closest identity to the angiotensin II type 1 (AT-1) receptor. Subsequent studies elucidated the roles of the apelin–APJ system in human physiology, including the regulation of cardiovascular function and fluid homeostasis. In spite of the high homology between APJ receptor and AT-1 receptor, the apelin–APJ system has been found to exert opposing actions to angiotensin II (Ang II)-AT-1. Recent reports have also shown the roles of apelin–APJ in renal physiology, including the maintenance of water balance in the kidney. In addition, the renoprotective effect of the apelin–APJ system in renal diseases has been reported in the context of renal fibrosis, renal ischemia/reperfusion (I/R) injury and diabetic nephropathy, suggesting that the apelin–APJ system may become a new therapeutic target for renal diseases. The aim of this critical review is to discuss the apelin–APJ system, its role in renal physiology and potential therapeutic applications for renal diseases.

Conclusion

The functional counter-regulatory role of apelin–APJ in Ang II-AT1 and AVP action is critically important when considering the action of the apelin–APJ system in the regulation of human physiology. Regarding kidney function, recent reports have shown the direct action of apelin–APJ to the kidney in the context of fluid homeostasis, and also its roles in renal fibrosis, renal I/R injury and diabetic nephropathy, along with its potential use as a therapeutic target.

Discussion

The authors have referenced some of their own studies in this critical review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

Distribution of apelin and APJ receptor in the kidney

Apelin is synthesized as a 77-amino-acid pre-propeptide, and is cleaved to a mature peptide. Different isoforms of apelin, such as apelin-36 (apelin-42-77), apelin-17 (apelin-61-77), apelin-13 (apelin-65-77) and apelin-13 in its pyroglutaminated form [(Pyr^1)apelin-13], i.e. with N-terminal glutamate residue, have been identified and are thought to exist in vivo. Pre-proapelin mRNA is abundantly expressed in the human CNS and in the placenta, and moderately in the kidneys, heart, lungs and mammary glands. In the kidney, apelin-like immunoreactivity was detected in endothelial cells from human small intrarenal vessels.

Studies regarding the tissue distribution of APJ receptor in rats, mice and humans have shown that it is abundantly expressed in the CNS, as well as in a variety of tissues, including rat and human kidneys. In situ hybridization histochemical studies in rats revealed patch-like labelling in the kidney cortex corresponding to APJ receptor mRNA expression. Labelling was noted in approximately 40% glomeruli (41/108) along with the pronounced labelling of cells along the vasa recta in the inner stripe of the outer medulla. Labelled cells were less frequently observed in the outer stripe of the outer medulla, and isolated cells in the cortex also contained moderate amounts of APJ receptor mRNA. In another study, APJ receptor mRNA was quantified by real-time reverse transcription-polymerase chain reaction (RT-PCR)
in renal zones and along the nephron. In renal zones, APJ receptor mRNA expression was highest in the inner stripe of the outer medulla, followed by the outer stripe of the outer medulla and then the inner medulla, and lowest in the cortex. Along the nephron, the expression of APJ receptor mRNA was very high in the glomerulus and was more moderate in other nephron segments from the proximal convoluted tubule to collecting duct from the inner medulla. Furthermore, in situ hybridization experiments revealed specific labeling for the APJ receptor mRNA in the vascular wall of glomerular arterioles, in both endothelial and vascular smooth muscle cells.

Increased expressions of apelin and APJ receptor mRNAs and proteins were also reported in several disease models of the kidney. In a mouse model of unilateral ureteral obstruction (UUO)-induced renal fibrosis, mRNA expressions of both apelin and APJ receptor in the kidney, as assessed by real-time RT-PCR, increased following ureteral ligation.

In adriamycin-induced nephrotic rats, apelin protein, which was immunohistochemically detected along the glomerular basement membrane in the kidney, was significantly increased following adriamycin injection. In a mouse model of diabetic nephropathy, treatment with apelin resulted in the restoration of decreased mRNA and protein expressions of APJ receptor, which was localized in the glomeruli and blood vessels of the kidney, to the control level.

Interactions of the apelin–APJ and renin–angiotensin systems

In spite of the high homology between APJ receptor and AT-1 receptor, i.e. 115 amino acids (30%) of the total sequence and 86 amino acids (54%) in transmembrane regions, as well as similar patterns of tissue expression for both receptors, angiotensin II (Ang II) does not bind to APJ receptor and apelin does not bind to AT-1 receptor. Furthermore, previous reports have shown opposing actions between the apelin–APJ and Ang II–AT-1 systems mainly in regulating the pathophysiology of cardiovascular function. Ang II-induced vasoconstriction was attenuated by apelin treatment and, an increased vasopressor response to Ang II was observed in APJ knockout (APJ–/–) mice. This vasodilator effect of apelin has been thought to be nitric oxide (NO)-dependent, because it was abrogated in the presence of a nitric oxide synthase (NOS) inhibitor. Functional counter-regulation of apelin and Ang II has also been reported in a rat model of progressive heart failure. In this model, apelin and APJ receptor mRNAs were markedly down-regulated in the heart failure stage, although no significant change was observed in the compensatory left ventricular hypertrophy (LVH) stage. When rats were treated with angiotensin receptor blocker (ARB), matrix metalloproteinase inhibitor or β-blocker from the LVH stage, restoration of cardiac apelin and APJ expression was observed only in the ARB group, although the functional improvements were similar among the three treated groups. Furthermore, in Ang II-infused rats, cardiac apelin mRNA was decreased and its restoration was achieved by treatment with ARB. These results established a direct counter-regulatory influence of Ang II in apelin and APJ expression in the context of heart failure. Protective effects of apelin against cardiovascular fibrosis have also been shown in a model of Ang II-induced cardiovascular fibrosis and during treatment with ARB. In the kidney, the effects of apelin on renal hemodynamics counteracting Ang II and the role of apelin in ARB-induced alleviation of renal fibrosis have been reported.

Recent reports also have shown direct interactions between the apelin–APJ and renin-angiotensin systems at both molecular and transcriptional levels. Apelin inhibits Ang II signalling pathways, including extracellular signal-regulated kinase phosphorylation and activation of transcriptional targets, such as nuclear factor (NF)-κB, and the Rho kinase pathway. Ang II also appears to counteract the apelin–APJ system in the context of apelin and APJ receptor gene expressions. Furthermore, AT-1 and APJ receptors can form heterodimers and can physically associate, presumably on the cell membrane, and may influence downstream signalling in a stoichiometric fashion.

Involvement of the apelin–APJ system in the maintenance of water balance in the kidney

Previous reports have shown that apelin and vasopressin (AVP) are conversely regulated in the CNS. Apelin and APJ receptor mRNAs are widely distributed in the brain, particularly in the supraoptic and paraventricular hypothalamic nuclei, and colocalize with AVP on neurosecretory magnocellular neurons. This strongly suggested that there may be an interaction between apelin and AVP in response to osmotic and volemic stimuli. In lactating rats, which are characterized by an increase in both the synthesis and release of AVP to preserve the hydraulic content for maximal milk production, injection of apelin-17 into the third ventricle inhibited the phasic electrical activity of AVP neurons, reduced the plasma AVP level and increased diuresis. Moreover, water deprivation, which increases systemic AVP release and causes the depletion of hypothalamic AVP stores, decreased plasma apelin and induced hypothalamic accumulation of apelin. These data indicated that apelin and AVP are oppositely regulated to maintain body fluid homeostasis in the CNS.

In addition to its central action, the apelin–APJ system might act directly on the kidney. Intravenous injection of increasing doses of apelin-17 to

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lactating rats also progressively increased diuresis, suggesting that apelin might act as an aquaretic peptide, not only by a central action, but also by modulating the action of renal V2 receptors. The direct action of apelin on the kidney to increase diuresis was also shown in APJ–/– mice. Water deprivation significantly reduced urine volume and increased osmolality in wild-type but not in APJ–/– mice; however, the baseline plasma AP concentration increased comparably in both wild-type and APJ–/– mice following dehydration. Although peripheral administration of a V2 agonist, desmopressin, induced an increase in urine osmolality also in APJ–/– mice, this increase was not as great as that seen in wild-type mice, indicating the attenuation of V2 receptor-associated pathways in APJ–/– mice 24. Thus, developing non-peptidic agonists of the APJ receptor could be an alternative approach to V2 receptor antagonists.

Apelin also regulates renal hemodynamics through pre- and post-glomerular microvasculature 8. In juxtamedullary afferent and muscular efferent arterioles of microdissected rat kidneys, apelin-17 abolished the increases in intracellular calcium concentrations as well as vasoostriction induced by Ang II. This inhibitory effect of apelin on Ang II action was not observed in the presence of L-NAME, a NOS inhibitor, or in endothelium-denuded arterioles, indicating that the action of apelin is mediated by endothelium-derived NO. Furthermore, apelin-17 alone caused significant increases in intracellular calcium concentrations both in afferent and efferent arterioles with significantly higher amplitude in afferent arterioles. These results show that apelin has complex effects on the pre- and post-glomerular microvasculature regulating renal hemodynamics counteracting Ang II. Nevertheless, taking into consideration that APJ receptor mRNA is highly expressed in a highly vascularized zone of the outer medulla, and that apelin induces vasorelaxation against Ang II action in glomerular arterioles, the increased renal medullary microcirculation induced by apelin might possibly contribute to apelin-induced diuresis in addition to having a direct tubular effect in lactating rats.

**Involvement of the apelin–APJ system in renal diseases**

**Renal fibrosis**

Involvement of the apelin–APJ system in the progression of tissue fibrosis has been shown in the context of liver disease 25,26. In contrast, antifibrotic actions of apelin that counteract Ang II have been reported in models of cardiac fibrosis 27,28 and renal fibrosis 29. In a mouse model of UUO-induced renal fibrosis, the apelin–APJ system was shown to contribute to the alleviating effect of ARB on renal fibrosis 10. In this model, both apelin and APJ receptor mRNAs in the kidney were upregulated following ureteral ligation, and treatment with losartan resulted in the further upregulation of apelin mRNA. One of the major actions of apelin via the APJ receptor is NO production in the endothelium through the Akt/endothelial nitric oxide synthase (eNOS) pathway, which causes vasodilation 19. Previous studies also have shown the antifibrotic tissue-protective effects of NO in the UUO kidney 27,28. In this context, the phosphorylation of both Akt and eNOS in the UUO kidney was markedly increased following treatment with losartan along with alleviated renal interstitial fibrosis, decreased myofibroblast accumulation, and a decreased number of interstitial macrophages. Co-treatment with F13A, a specific antagonist of the APJ receptor, as well as with L-NAME, a NOS inhibitor, completely abrogated all these effects of losartan 10. These results suggested that increased NO production through the apelin/APJ/Akt/eNOS pathway may, at least in part, contribute to the alleviating effect of losartan on UUO-induced renal fibrosis. A recent report also provided evidence that apelin inhibits the TGF-β-stimulated activation of cardiac fibroblasts through a reduction in sphingosine kinase 1 (SphK1) activity 20. These results raise the possibility of therapeutic options targeting apelin/APJ as well as the renin–angiotensin system for the treatment of renal fibrosis.

**Renal ischemia/reperfusion injury**

A recent report showed the renoprotective effects of apelin against renal ischemia/reperfusion (I/R) injury. In a rat model of I/R injury, intraperitoneal administration of apelin-13 for three consecutive days prior to the surgical procedure resulted in decreased blood urea and creatinine levels and a higher glomerular filtration rate as well as attenuated renal histological damage 30. These findings raised the potential for the therapeutic use of apelin in ischemic renal diseases.

**Diabetic nephropathy**

A recent report also showed the renoprotective effect of apelin in diabetic nephropathy 12. In Ove26 diabetic mice, protein and mRNA expressions of the APJ receptor were significantly reduced compared to control NJ mice; however, daily subcutaneous injections of apelin-13 for 2 weeks restored both of these APJ protein and mRNA expressions to the control level. Progression of renal and glomerular hypertrophy in diabetic mice and renal inflammation including monocyte chemoattractant protein 1 and vascular cell adhesion molecule 1 expression, NF-κB activation and monocyte infiltration, as well as the downregulation of the antioxidant enzyme catalase, were restored by both 2 weeks and 14 weeks of apelin treatment. The degree of albuminuria in diabetic mice was reduced at 6 months of age (after 14 weeks of apelin treatment), but not at 3 months of age.
(after 2 weeks of apelin treatment). This effect of apelin on albuminuria seemed to be due to the restored proximal tubular reabsorption of albumin, rather than by the restoration of podocyte loss, because the expression of megalin in proximal tubules, but not the podocyte number, was restored by apelin treatment. Ang II and AT-1 receptor protein expressions in the kidneys of diabetic mice were not affected by apelin treatment. Thus, the renoprotective effect of apelin was independent of the renin–angiotensin system activation, but correlated with upregulation of the antioxidant enzyme catalase. This study suggested that apelin might be a novel therapeutic tool for diabetic nephropathy.

**Conclusion**

Since the discovery of the apelin–APJ system, its roles in human physiology and pathology, and its potential as a new therapeutic target have been thoroughly investigated. Previous studies mainly targeted and elucidated its roles in cardiovascular function and fluid homeostasis; however, the distribution of the apelin–APJ system in a variety of tissues suggests its roles in various organs and tissues. The functional counter-regulatory role of apelin–APJ in Ang II-AT1 and AVP action is also critically important when considering the action of the apelin–APJ system in the regulation of human physiology. Regarding kidney function, recent reports have shown the direct action of apelin–APJ on the kidney in the context of fluid homeostasis, and also its roles in renal fibrosis, renal I/R injury and diabetic nephropathy, along with its potential use as a therapeutic target (summarized in Table 1). These effects of apelin were considered not only to depend on, but also to be independent of, the regulation of Ang II-AT1 or AVP actions. Further studies regarding the role of the apelin–APJ system in various pathological settings in the kidney will provide insights into the potential therapeutic option of targeting apelin–APJ for renal diseases.

**References**


### Table 1 Effects of apelin on renal disease models

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Abbreviations: UUO, unilateral ureteral obstruction; I/R injury, ischemia/reperfusion injury; iv, intravenous administration; ad, addition; po, oral administration; ip, intraperitoneal administration; sc, subcutaneous administration; ARB, angiotensin receptor blocker; AVP, arginine vasopressin; Ang II, angiotensin II; NO, nitric oxide.

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Critical review