Prokineticin receptor 1 (PKR1) signalling in cardiovascular and kidney functions

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Abstract

Prokineticins (PK1 and PK2) are peptide hormones that exert their biological activity via two common G-protein-coupled receptors: prokineticin receptor (PKR) 1 and 2. Their physiology was originally explored mostly in the context of angiogenic actions in the reproductive tract and gut motility. Since autocrine and paracrine loops have been established between PKR1 and PKR2 in the heart, in this review we focus on the PK2/PKR1 signalling in the functions of the heart and kidney. PKR1 signalling is required for cardiomyocyte survival and angiogenesis. In the mouse model of myocardial infarction, intracardiac transient PKR1 transfection protects the structure and function of the heart. Gain- and loss-of-function studies reveal that PKR1 in mouse heart up-regulates its own ligand and PK2, which in turn acts as a paracrine signal and promotes epicardin-positive progenitor cell differentiation into a vasculogenic cell type. Transgenic mice over-expressing PKR1 in cardiomyocytes exhibit increased neovascularization. Loss of PKR1 causes structural and functional changes in the heart and kidney. In isolated epicardin-positive progenitor cells from the kidney, PK2, acting via PKR1, stimulates differentiation of these progenitor cells into endothelial and smooth muscle cells. Taken together, these data show that PK2/PKR1 is involved in postnatal cardiac and renal neovascularization. The knowledge gained from these studies should facilitate the discovery of therapeutic interventions in heart and kidney diseases targeting PKR1.

Keywords

Receptor • Prokineticin • Cardiomyopathy • Renal physiology • Progenitor cell

1. Introduction

Prokineticins (PK1 and PK2) are structural homologues of amphibian or reptilian peptide toxins that were first identified in the gastrointestinal tract1 as potent agents mediating muscle contraction, and have been isolated from bovine milk.2 They comprise two classes: prokineticin 1 (PK1), originally called endocrine gland-derived vascular endothelial growth factor (EG-VEGF)1 and prokineticin 2 (PK2, also called Bv8). PK1 and PK2 are approximately 50% homologous to each other and contain carboxyl terminal cysteine-rich domains that form five disulfide bridges.3 The N-terminal hexapeptide (AVITGA) and cysteine residues in the carboxyl terminal domain are crucial for their biological activities. PK1 is encoded by a gene mapped on chromosome 1 (NCBI Gene ID: 84432). PK2 is localized on chromosome 3p13 and contains four exons (NCBI Gene ID: 60675). The most active form of PK2, with 81 amino acids, is encoded by the exons 1, 2 and 4; however, a long form of the PK2 peptide, PK2L, with 21 additional amino acids, is encoded by all four exons of PK2. Prokineticins and their receptors are widely distributed in mammalian tissues.4 Regulators for the prokineticins have been described in reproductive tract, neurons and macrophages.

In reproductive tract, PK1 expression was found to be up-regulated by estrogen, progesterone, and human chorionic gonadotrophin, as well as hypoxia-inducible factor 1α.5,6 In olfactory bulbs, PK2 expression was elevated by two proneural basic helix–loop–helix factors (neurogenin1 and Mash1) and repressed by homeobox transcriptional factors (distal-less homeobox 1 and 2).7,8 Transcription factor activating protein-1 is involved in regulation of the expression of the Bv8 homologue in amphibians.9 PK2 was shown to be positively regulated in CD11b+ Gr1+ myeloid cells, specifically by granulocyte colony stimulating factor.10 Although this information does not explain the prevalence of distribution of prokineticins in tissues and the stimuli that regulate their expression, it is likely that circadian stimuli11 in the central nervous system and remodelling stimuli12 in the heart regulate the expression of PK2.

Prokineticins have been shown to modulate cell survival,13–17 cell motility,18–20 cell excitability,21–24 and other more complex behaviours,25–27 as summarized in Table 1.

Prokineticins bind to two cognate 7-transmembrane G-protein-coupled receptors. Prokineticin receptor 1 (PKR1) and PKR2 share about 85% amino acid identity and are encoded within distinct chromosomes in both mice and humans.1 PKR1 is located on...
Table 1 Prokineticins are involved in diverse cellular activities, including cell survival, motility and excitability, and complex behaviours

| Role              | Cell type/function                  |
|-------------------|------------------------------------|
| Cell survival     | Endothelial cells, 13, 14 Neuronal cells, 15 Haematopoietic cells, 16 Cardiomyocytes, 12, 17 |
| Cell motility     | Angiogenesis, 18 Neurogenesis, 15 Haematopoiesis, 19 Neovascularogenesis, 20 |
| Cell excitability | Gut spasmyogen, 21 Pain sensitization, 22 Circadian rhythm, 23 Sleep, 24 |
| Complex behaviours| Feeding, 25 Drinking, 26 Anxiety, 27 |

Prokineticins are involved in diverse cellular activities, including cell survival, motility and excitability, and complex behaviours. Prokineticins serve as mitogens and survival factors for corpus luteum-derived endothelial cells and bovine aortic endothelial cells. Both PK2 and PKR1 are expressed in the H9c2 cardioblast cell line derived from rat heart tissue, as well as in HSV capillary endothelial cells derived from mouse heart tissue, and in human and mouse hearts. Prokineticins are involved in diverse cellular activities, including cell survival, motility and excitability, and complex behaviours.

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2. The PK2/PKR1 signalling pathway is an important cardiovascular regulatory pathway

PK2 appears to be an ideal hormonal candidate to regulate cardiovascular function. Prokineticins are potent angiogenic factors that have beneficial effects on cardiac tissue repair, possibly by inducing angiogenesis to improve coronary circulation. Prokineticins utilize mainly Gq protein signalling for their biological effects. Gq/Gα11 signalling is an essential pathway to induce cardiac development and hypertrophy. Moreover, Gq/Gα13 signalling is involved in vessel formation, as observed in VEGF knock-out mice. Prokineticins can act as cytokines, inducing differentiation of murine and human bone marrow cells into the monocyte/macrophage lineage and activation of monocyte proliferation and differentiation.

3. Prokineticin receptor 1 expression in cardiovascular and renal tissues and cells

Both PK2 and PKR1 are expressed in the H9c2 cardioblast cell line derived from rat heart tissue, as well as in HSV capillary endothelial cells derived from mouse heart tissue, and in human and mouse hearts. In situ hybridization analysis of mouse heart and aorta samples has shown that PKR1 is expressed in the ventricular wall of the heart and aortic endothelial cells, but not in smooth muscle cells of the aorta. Endothelial cells from bovine aorta have also been shown to express PKR1. Immunohistochemical analysis confirmed the presence of PKR1 protein in myocardium and epicardium, in agreement with the PKR1 transcript expression profile. Together, these data show that PKR1 and its ligand are expressed postnatally in cardiovascular tissues and cells and might be functional in the heart (Figure 1A). In the kidney, glomeruli and blood vessels were strongly labelled with an antibody directed against PKR1. PKR1 is expressed in endothelial cells within the glomeruli, but not in podocytes and mesangial cells (Figure 1B). PKR1 expression was also detected in the tubular structures. In situ hybridization confirmed PKR1 expression in glomerular and tubular structures.

4. Prokineticin receptor 1 signalling in cardiovascular cells

Prokineticins serve as mitogens and survival factors for corpus luteum-derived endothelial cells and bovine aortic endothelial cells. PKR1, acting via Gq11, activates both mitogen-activated protein kinase and Akt signalling pathways to stimulate proliferation, migration, and angiogenesis in capillary endothelial cells derived from heart (HSV cells). Activation or over-expression of PKR1 in isolated cardiomyocytes prevents the apoptosis induced by oxidative stress by inducing Akt phosphorylation. Gq-coupled receptor activation in isolated cardiomyocytes could promote a survival pathway by activating Akt signalling. PKR1 activation in isolated cardiomyocytes has been shown to induce hypertrophy. Increased Gq protein activity in cardiomyocytes induces anti-apoptotic signals by transactivating epidermal growth factor receptor and by Akt activation independent of the ability of Gq signalling to elicit hypertrophy. These data show that survival and...
hypertrophic responses to Goαq are mediated by different signalling pathways. Given the known functions of Akt, these data are consistent with Akt having a central role in PKR1 signalling for protection of cardiomyocytes against apoptosis.

5. Prokineticin signalling in myocardial infarction

Myocardial infarction (MI) triggers inflammation, scar formation, and scar remodelling, which are crucial for tissue repair. Following MI, chemokines induce migration of the circulating blood monocytes into the infarcted myocardium, where they can differentiate into macrophages. Macrophage infiltration into the left ventricle (LV) promotes phagocytosis, wound debridement, fibroblast activation and proliferation, collagen metabolism, and angiogenesis, which have a major role in the wound-healing process. Macrophages are involved in removal of necrotic cardiac myocytes and apoptotic neutrophils; secretion of cytokines, chemokines, and growth factors; and modulation of the angiogenic response. Macrophage depletion impairs wound healing and increases LV remodelling following myocardial injury in mice. Indeed, it has been shown in early clinical trials that inhibition of the inflammatory component with methylprednisolone drastically increased mortality due to LV rupture.

Prokineticins are described as chemokines/cytokines for monocytes and macrophages. Prokineticins and their receptors are highly expressed in inflamed tissues associated with infiltrating cells. In addition, PK2 is up-regulated at the site of rupture of abdominal aortic aneurysms, and in peripheral monocytes and neutrophils in response to granulocyte colony-stimulating factor. Interestingly, expression of PK2 and its receptors was significantly increased within 48 h and remained elevated 1 week after coronary ligation in a mouse MI model (Nebigil laboratory, C. G. Nebigil and K. Urayama, unpublished observations). Moreover, a 30% increase of phosphorylated Akt correlated with increased levels of PK2 in ischaemic hearts, initializing the endogenous wound-healing process via prokineticin signalling. Intra-cardiac PKR1 gene transfer utilizing adenovirus after MI in mice further increased PKR1 levels by approximately four times within 24 h. PKR1 gene transfer resulted in a reduction of the MI size, improvement of left ventricular performance, and consequently, a reduction of mortality compared with untreated control mice. Neither VEGF protein levels nor VEGF A transcripts 164 and 188 were altered in PKR1-treated hearts compared with vehicle-treated hearts after MI, consistent with the observation in cultured endothelial cells following prokineticin 2 treatment. These data clearly show that PKR1 gene therapy induces capillary network growth without increasing VEGF levels. The cardioprotector, Akt, was found to be increased by 60% in vivo in the PKR1-treated hearts and PK2-treated cardiomyocytes. These data thus suggest that transient PKR1 gene therapy has beneficial effects on recovery from myocardial infarction.

What is the mechanism of the cardioprotective effect of PKR1 signalling in the infarcted heart? Transient gene therapy with PKR1 enhanced angiogenesis and reduced apoptosis after MI. Prevention of apoptosis in cardiomyocytes is a possible mechanism of PKR1-mediated cardioprotective effects. A second mechanism to preserve myocardial function is to promote collateral vessel formation in order to overcome insufficient tissue oxygenation. Therefore, PKR1-mediated angiogenesis may be important to maintain sufficient numbers of living cardiomyocytes and to allow successful cardioprotection following its early phase anti-apoptotic effect. A third mechanism could be that the inflammation itself might contribute to PKR1-mediated angiogenesis in infarcted hearts. In fact, PKR1 signalling is involved in inflammation, monocyte activation, and macrophage differentiation, and furthermore, PKR1 knock-out mice exhibit lack of inflammation in response to PK2 stimulation. However, the number of infiltrated cells in the scar area after the myocardial infarction was not significantly different between the PKR1 gene-transfected and control mice following MI, arguing against an indirect effect of PKR1 through the inflammatory response. A fourth mechanism for PKR1 to preserve myocardial function, through the induction of progenitor cell differentiation, remains to be investigated.

6. Prokineticin receptor 1 in cardiovascular and renal pathophysiology

Recently, it has been demonstrated that conditional disruption of the PKR1 gene in mice provokes heart and kidney disorders, including...
cardiomegaly, interstitial fibrosis and cardiac dysfunction in stress conditions, renal tubular dilatation, abnormal glomerular capillaries, an increase in urinary phosphate excretion, and proteinuria.\textsuperscript{43} PKR1-null mutant mice displayed morphological abnormalities in both hearts and kidneys at the neonatal stage, eliminating the possibility of a cardiorenal syndrome.\textsuperscript{56} Several angiogenic factors have been shown to regulate the function of the heart and kidney. Mice lacking platelet-derived growth factor B display renal and cardiovascular abnormalities.\textsuperscript{57,58} VEGF has been shown to be involved in heart development\textsuperscript{38} and in glomerulogenesis and tubulogenesis in the kidney.\textsuperscript{59} Fibroblast growth factor is also involved in heart development and function,\textsuperscript{60} and in glomerulus formation.\textsuperscript{61} 

PKR1-null mutant mice exhibited swollen mitochondria with few cristae and increased apoptosis in both heart and kidneys. Impaired capillary formation in mutant heart and kidneys at early ages creates a hypoxic environment that up-regulates hypoxia-inducible factor-1α and pro-angiogenic factors as a compensatory mechanism to restore capillary perturbation at the adult stage. This compensatory recovery of capillary formation was only observed in the extraglomerular area; however, a small number of epicardial and glomerular capillary networks remained unaltered, because of increased apoptosis and reduced progenitor cell numbers found in both tissues. In summary, the loss of PKR1 causes renal and cardiac structural and functional changes because of deficits in survival signalling, mitochondrial, and progenitor cell functions in both the heart and the kidneys.\textsuperscript{43}

7. Prokineticin receptor 1 signalling in progenitor cells derived from heart and kidney

Cardiac epicardin positive (epicardial derived) progenitor cells (EPDCs or EPPCs) are characterized as the endogenous progenitor cells in the heart.\textsuperscript{62} Moreover, adult EPDCs represent a bona fide source of cardiovascular progenitor cells, which have the capacity to respond to multiple cues. A combination of myocardial VEGF and fibroblast growth factor signalling promotes EPDC differentiation into endothelial cells. Platelet-derived growth factor, transforming growth factor β, or bone morphogenetic protein 2 are involved in differentiation of EPDCs into smooth muscle cells.\textsuperscript{63} Only two factors have been identified as capable of stimulating adult EPDCs into vasculogenic cell types. Thymosin β4 has been described as a factor that is capable of activating adult EPDCs, resulting in the differentiation of EPDCs into endothelial cells, smooth muscle cells, and fibroblasts.\textsuperscript{64} Prokineticin 2 is also able to stimulate adult EPDCs;\textsuperscript{20} however, prokineticin/PKR1 signalling reprogrammes adult EPDCs to differentiate only into endothelial and smooth muscle cells, but not into fibroblasts. Transgenic mice over-expressing PKR1 in the heart displayed an increased number of epicardin-positive EPDCs, with an increase of capillary density and coronary vessels. This mouse model demonstrated a novel PK2/PKR1 signalling pathway that is involved in communication between cardiomyocytes and EPDCs, thereby promoting cardiac neovascularization.\textsuperscript{20} Interestingly, epicardin-positive progenitor cells in the PKR1-null mutant epicardium, subepicardium, and glomerulus were significantly lower than those in wild-type tissues.\textsuperscript{43} PK2 promoted differentiation of renal epicardin-positive progenitor cells into platelet endothelial cell adhesion molecule-1-positive endothelial and α-smooth muscle actin-positive vascular smooth muscle cells, and this effect was blocked in the PKR1-null mutant renal epicardin-positive progenitor cells.\textsuperscript{65} Taken together, these data demonstrate that PKR1 is involved in regulating postnatal neovascularogenesis in the heart and the kidney (Figure 2). It is noteworthy that epicardin-positive progenitor cells resident in both the heart and the kidney express PKR1. Recently, a relationship between the epicardium and the glomerulus was identified, originating in a similar progenitor tissue,\textsuperscript{65} and expressing similar genes, such as podoplanin, a podocytic marker, the T-box gene Tbx18, and Wt1.\textsuperscript{66} Figure 3 summarizes the potential role of epicardin-positive progenitor cells derived from pro-epicardium in glomerular and epicardial angiogenesis.

8. Chronic inactivation of PKR1 in mice produces a phenotype similar to adverse effects of anti-angiogenic therapy

Prokineticins are implicated in development of the polycystic ovary syndrome,\textsuperscript{67} and of neuroblastoma,\textsuperscript{27} colorectal, testicular and prostate cancers (summarized in Table 2). It has recently been demonstrated that prokineticin 2 secreted from myeloid cells strongly induces tumour angiogenesis. Moreover, anti-prokineticin 2 treatment significantly decreases tumour-infiltrating myeloid cell mobilization, thereby inhibiting tumour growth.\textsuperscript{10} Thus, the use of immunoneutralizing antibodies or antagonists of prokineticin 2 and
its receptors may be a useful strategy for cancer treatment. However, anti-angiogenesis treatments targeting platelet-derived growth factor, VEGF and its receptors or receptor tyrosine kinase signalling have been associated with clinical renal and cardiac toxicity.68–70 These conditions include cardiac impairment, proteinuria, hypertension, haemorrhage, and thrombosis, as well as impairment of wound healing and tissue repair. Although several hypotheses have been put forward regarding the aetiology of angiogenesis inhibition-related cardiovascular and renal adverse effects, many of the underlying pathophysiological mechanisms remain to be elucidated. Some of the adverse effects of anti-angiogenic drugs could be due to an off-target effect of the drugs.

Do PKR1-null mutant mice display heart and kidney disorders reminiscent of anti-angiogenic therapy-mediated adverse effects? Cardiomyopathy, proteinuria, renal disorders, and impaired angiogenesis following rebound neovascularization were evident in PKR1-null mutant mice. These mice exhibit progressive fibrosis in their remodelling hearts and kidneys. On the contrary, anti-VEGF anti-angiogenic therapy reduces fibrosis and collagen deposits in the lungs and the liver by an unknown mechanism.71 It is not evident that this reduction in collagen deposits is due to an inhibition of VEGF signalling, because administration of VEGF had a clear therapeutic benefit, slowing the development of renal fibrosis.72 Neonatal and 3-week-old PKR1 mutant mice have fewer than normal numbers of capillaries in their hearts and kidneys, establishing hypoxic conditions with impaired mitochondrial function, as seen in humans and mice treated with anti-angiogenic drugs.73 This defect in angiogenesis was compensated by an increase in hypoxia-inducible factor 1α production, accompanying an increase in pro-angiogenic factors in the mutant heart and glomerular zone. The activation and/or up-regulation of other pro-angiogenic signalling pathways as a form of ‘evasive resistance’ to angiogenesis inhibitors has been shown to circumvent anti-angiogenic treatment, triggering neovascularization in due course.74 Restoration of the angiogenic pathways

### Table 2 Involvement of prokineticins in human diseases

| Domain          | Role/expression/mutation in human diseases                                                                 |
|-----------------|------------------------------------------------------------------------------------------------------------|
| Reproduction    | Endometrial vascular function76 Mid- to late luteal phase77 Ectopic endometrium in endometriosis6 Host implantation during early pregnancy78 Ectopic pregnancy79 Idiopathic recurrent pregnancy loss80 Development of the olfactory system and reproductive axis in humans81 Mutation in Kallman syndrome and idiopathic hypogonadotrophic hypogonadism81 |
| Behaviour       | Mood disorders in the Japanese population82 Methamphetamine dependence in the Japanese population84 |
| Cancer          | Prostate malignancy85 Neuroblastoma progression86 Angiogenesis in pancreatic disease87 Human tumours and inflammatory disorders88 |
| Cardiovascular  | Abdominal aortic aneurysms83 Human end-stage cardiac failure82 |

**Figure 3** The relationship between glomerulus and epicardium has been postulated via pro-epicardial cells. Epicardium has an essential modulating role in the differentiation of the compact ventricular layer of the myocardium and the development of cardiac vessels. Moreover, the epicardial progenitor cells have been shown to differentiate into the pronephric external glomerulus (PEG), a structure composed of capillary networks, mesangial cells, and podocytes in vertebrates.
in the PKR1 mutant heart and kidneys could not reverse the damage, because of defective survival pathways, such as reduced Akt activity, increased caspase-3 activity, and impaired common progenitor cell function in the neonatal mutant hearts and kidneys. Interestingly, a compensatory mechanism restored the extraglomerular capillary network without an alteration in the number of glomerular capillaries in the mutant kidneys. Interstitial cells and the tubular epithelium also provide signals such as VEGF for maintenance of the peritubular capillary network, independent of the glomerular capillary network. Our findings clearly demonstrated that the level of VEGF in the glomerulus was not altered, but VEGF was indeed increased in mutant interstitial cells and tubular epithelium, possibly restoring extraglomerular angiogenesis.

9. Perspectives

Taken together, these data show that PK2, acting via PKR1 signalling, has important actions on heart and kidney physiology and pathophysiology. Clearly, the main avenues for future studies will be to investigate cell-specific inactivation of PKR1 in a mouse model to further and fully elaborate the common mechanism underlying pathology in PKR1 mutant heart and kidneys. More specifically, whether impaired homing of epicardin-positive progenitor cells and/or endothelial cell dysfunction in PKR1-null mice leads to heart and kidney pathology remains to be further investigated (Figure 4). The role of prokineticins in the pathophysiology of human heart and kidney diseases remains to be explored. Table 2 summarizes the involvement of prokineticin in these diseases.76–90

PKR1 is involved in postnatal cardiac and renal vascularization by activating organ-specific progenitor cells. The identification of factors that stimulate endogenous cardiac and renal stem or progenitor cells to induce neovascularization and cardiomyocyte/tubular cell replacement should lead towards therapeutic intervention in heart and kidney diseases. In this context, prokineticins hold promise for use as angiogenic factors and for progenitor cell-based therapy in ischaemic hearts and kidneys. The race is on to facilitate drug discovery for targets acting on cardiomyocytes or epicardin-positive progenitor cells to invoke new coronary vessels, and cardiac and renal tissues as a significant step towards cardioprotection, and cardiovascular and renal regeneration.

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