Physiology and pathophysiology of incretins in the kidney

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\textbf{Purpose of review}  
Incretin-based therapy with glucagon-like peptide-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors is considered a promising therapeutic option for type 2 diabetes mellitus. Cumulative evidence, mainly from preclinical animal studies, reveals that incretin-based therapies also may elicit beneficial effects on kidney function. This review gives an overview of the physiology, pathophysiology, and pharmacology of the renal incretin system.

\textbf{Recent findings}  
Activation of GLP-1R in the kidney leads to diuretic and natriuretic effects, possibly through direct actions on renal tubular cells and sodium transporters. Moreover, there is evidence that incretin-based therapy reduces albuminuria, glomerulosclerosis, oxidative stress, and fibrosis in the kidney, partially through GLP-1R-independent pathways. Molecular mechanisms by which incretins exert their renal effects are understood incompletely, thus further studies are needed.

\textbf{Summary}  
The GLP-1R and DPP-4 are expressed in the kidney in various species. The kidney plays an important role in the excretion of incretin metabolites and most GLP-1R agonists and DPP-4 inhibitors, thus special attention is required when applying incretin-based therapy in renal impairment. Preclinical observations suggest direct renoprotective effects of incretin-based therapies in the setting of hypertension and other disorders of sodium retention, as well as in diabetic and nondiabetic nephropathy. Clinical studies are needed in order to confirm translational relevance from preclinical findings for treatment options of renal diseases.

\textbf{Keywords}  
DPP-4 inhibition, diabetes, diabetic nephropathy, GLP-1 receptor, hypertension, incretins, kidney, renal impairment

\section*{INTRODUCTION}

The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are secreted from intestinal cells in response to food intake. By enhancing insulin secretion from pancreatic beta cells and reducing glucagon secretion from alpha cells in a glucose-dependent manner, these endogenous peptide hormones lower plasma glucose and regulate postprandial glucose homeostasis [1]. \textit{In vivo}, GLP-1 and GIP are degraded by the serine protease dipeptidyl peptidase-4 (DPP-4) [2]. Apart from their actions on the pancreas, incretins also have systemic effects due to an expression of incretin receptors and DPP-4 in a variety of tissues such as kidney, heart, lung, the central and peripheral nervous system, and the digestive system [3,4].

Renal expression of GLP-1R and DPP-4 is described in several publications for rats, mice [5], pigs [6], cattle [7], and humans [8]. GLP-1R mRNA was found in the proximal tubular cells of rats [9] and pigs [6]. Immunostaining of human kidney cortex showed a GLP-1R signal predominantly in proximal tubular cells [6]. Real-time PCR of micro-dissected rat nephron segments showed GLP-1R mRNA expression in the glomerulus and proximal convoluted tubule [9]. Summarized information about the localization and physiology of DPP-4 and GLP-1R in the kidney are found in Table 1 and Fig. 1.

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The membrane-bound form of DPP-4 is present on many cell types, including kidney epithelial cells, endothelial cells, and T cells [22], where it functions as a binding protein and transmits intracellular signals [23]. The circulating, soluble form of DPP-4 in the plasma accounts for the degradation of GLP-1, and its inhibition leads to the glucose-lowering effect utilized with DPP-4 inhibitors in clinical use. Mentlein [12] found a high concentration of DPP-4 in the mammalian kidney where it is involved in the degradation of filtered proteins [13,14]. Expression in brush border microvilli of the proximal tubules and glomerular podocytes is described for rodents [11,24,25]. Jackson et al. [10] found expression of DPP-4 mRNA and protein in the preglomerular microvascular smooth muscle cells and glomerular mesangial cells of rats. The expression of DPP-4 is up-regulated in cultured human renal glomerular epithelial cells during inflammation [26] and in a rat model of type 2 diabetes mellitus (T2DM) [27]. Increased DPP-4 activity in the kidney or urine is said to be a hallmark for human glomerular diseases [28,29]. We recently detected DPP-4 immunoreactivity in glomeruli of patients with diabetic nephropathy and nephrotic syndrome, but not in healthy kidneys (unpublished data).

The data about DPP-4 and GLP-1R expression in the kidney are still incomplete and speculation of low specificity and sensitivity of existing antibodies gives reason for the discussion about the validity of immunostaining data [30,31]. However, cumulative evidence from functional and mechanistic studies supports a role of a renal incretin system in the modulation of sodium and water homeostasis and kidney function. The natriuretic and diuretic properties of GLP-1 were proved in infusion studies: in a rat model of salt sensitivity, chronic intravenous infusion of GLP-1 increased glomerular filtration rate (GFR), inhibited proximal tubular reabsorption, and increased urinary flow and sodium excretion [17]. The effect on GFR was not present in rats with denervated kidneys, which shows that renal GLP-1 signalling seems to be a complex mechanism, also depending on functional neurotransmission [18]. For healthy humans, it was shown that infusion of GLP-1 has a dose-dependent effect on urinary sodium excretion without changing GFR [19]. In obese, insulin-resistant men, there was a significant increase in urinary sodium excretion, a decrease in urinary H⁺ secretion, and a decrease in the GFR [20]. The role for GLP-1 in modulating the renal Na⁺/H⁺ exchange was therefore examined more closely in rat [21] and porcine [6] renal proximal tubule cells. GLP-1 infusion significantly reduced Na⁺/H⁺

### Table 1. Cellular localization of the DPP-4 and the GLP-1 receptor in the kidney

<table>
<thead>
<tr>
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<th>Preglomerular</th>
<th>Mesangium</th>
<th>Glomerulus/podocyte</th>
<th>Proximal tubule</th>
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<td>DPP-4</td>
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<td>Method</td>
<td>RT-PCR</td>
<td>RT-PCR</td>
<td>EM</td>
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<td>GLP-1R</td>
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DPP-4: dipeptidyl peptidase 4; EM: electron microscopy; GLP-1R: glucagon-like peptide 1 receptor; RT-PCR: real-time PCR.
exchanger isoform 3 (NHE3)-mediated bicarbonate reabsorption via a protein kinase A-dependent mechanism in rat renal proximal tubule, thus influencing renal hemodynamics. The down-regulation of NHE3 activity in the renal proximal tubule by GLP-1 infusion led to an increased renal plasma flow and GFR [9].

PHARMACOKINETICS OF GLUCAGON-LIKE PEPTIDE-1 ANALOGS AND DIPEPTIDYL PEPTIDASE-4 INHIBITORS IN RENAL FAILURE

It is known that GLP-1 and GIP are eliminated in the kidneys [32], which could affect the plasma levels of incretins in patients with impaired kidney function. Studies with small groups of patients showed that serum incretin levels were significantly increased in uremic patients in comparison with healthy persons [33,34]. However, the kidney does not seem to be of much importance for the degradation and inactivation of GIP and GLP-1. Apparently, intact incretin hormones are inactivated by hepatic DPP-4 and further degraded in peripheral tissues [35]. Thus, the initial DPP4-mediated degradation of both hormones is almost unaffected by an impaired renal function [35]. Meier et al. [35] showed that the kidneys are the major site for excretion of incretin metabolites like GIP (3–42) and GLP-1 (9–36). It is important to learn more about what physiologic effects incretin metabolites may have, given the increasing importance of GLP-1R agonist and DPP-4 inhibitor treatment in routine clinical routine [36]. In-vitro studies showed an antagonistic effect of GLP-1 (9–36) to the GLP-1R, but affinity is very low, and at the moment there is no evidence of an effect of GLP-1 (9–36) on insulin secretion [37]. Still, it has to be kept in mind that accumulation of metabolites because of decreased elimination in renal insufficiency could influence the binding of GLP-1 agonists to the GLP-1R. Therefore, special consideration has to be given when treating patients with impaired renal function with an incretin-based therapy. As the pharmacokinetics of incretin therapies differ greatly between drugs of the same class, no general guidance can be given.

Exendin-4 is contraindicated in patients with moderate renal impairment and above. Experiments with pigs showed it is solely cleared by glomerular filtration [38] and in the current available doses it is not suitable for patients with end-stage renal disease [39]. In patients with mild and moderate renal
endothelial function and inhibiting inflammatory progression of diabetic nephropathy by improving GLP-1R agonists may be effective in decelerating the causes of chronic kidney disease.

The effects of GLP-1 on the kidney and its function have been investigated in several studies with degradation-resistant GLP-1R agonists, such as exendin-4 or liraglutide. Many observations suggest that GLP-1 exerts pleiotropic actions via its receptor, independent of the glucose-lowering effects. Preclinical research with GLP-1R agonists focuses on animal models of hypertension and diabetes as leading causes of chronic kidney disease.

There is growing evidence that treatment with GLP-1R agonists may be effective in decelerating the progression of diabetic nephropathy by improving endothelial function and inhibiting inflammatory and profibrotic actions [49*,50,51]. It was shown that the expression of GLP-1 receptors in the glomeruli of diabetic mice is reduced [49*,50]. Treatment with exendin-4 led to an increased number of GLP-1R-positive cells in the glomeruli and ameliorated glomerular pathology. GLP-1R agonist treatment might influence diabetic nephropathy by increasing GLP-1R expression in the glomerulus, thus accounting for a restored GLP-1 protective action on the renal endothelium. In rats with insulin-deficient diabetes mellitus (T1DM), exendin-4 treatment led to reduced albuminuria, prevention of glomerular hypertrophy, macrophage infiltration in the glomeruli, and decreased glomerular and tubulointerstitial fibrosis without influencing blood glucose levels [51]. As microinflammation is a common mechanism in the pathogenesis of diabetic nephropathy, these effects might in part be explained by the in-vitro observed anti-inflammatory properties of exendin-4 via the GLP-1R and subsequent activation of the cyclic AMP pathway [51]. The activation of the GLP-1R pathway with both GLP-1R agonists – exendin-4 and liraglutide – led to a protection against oxidative stress and albuminuria in several rodent models of diabetes. A protein kinase A-mediated inhibition of renal NAD(P)H oxidase [52] and a protection against effects of advanced glycation end-products (AGEs) by reducing receptor for AGE (RAGE) expression [53*] may play a role. By increasing the expression of the oxidative defense gene heme oxygenase-1, exendin-4 showed protective effects against reactive oxygen species in a rat model of renal ischemia–reperfusion injury [54]. In-vitro studies showed renoprotective effects of exendin-4 on human mesangial cells, cultured in a high glucose medium. GLP-1R agonist treatment inhibited the overproliferation of high-glucose treated cells. The decreased mRNA and protein expression of transforming growth factor-β, a major fibrogenic growth factor, suggests a possibility to improve renal interstitial fibrosis in diabetic nephropathy with GLP-1R agonist treatment [55].

In-vitro [56] and in-vivo [49*] experiments showed a GLP-1-mediated inhibition of angiotensin 2 (Ang II) signaling and its proinflammatory action in glomerular endothelial cells. In Ang II-induced salt-sensitivity hypertensive mice, a rise in blood pressure was attenuated with exendin-4 treatment [57]. As Ang II-induced hypertension is caused by fluid retention via excess sodium reabsorption [58], and infusion of GLP-1 enhances sodium excretion [17], the observed effect of exendin-4 might be due to a regulation of sodium excretion. Treatment with a peptide analog of exendin-4 attenuated salt-induced hypertension, cardiac morbidity, insulin resistance, and renal dysfunction in Dahl salt-sensitive rats [59]. Micropuncture studies in hydropenic rats revealed vasodilatory effects leading to an increase in GFR following an infusion of exentanide. At the same time, reabsorption in the proximal tubule was inhibited [60].
PHARMACOLOGIC APPROACHES TARGETING THE RENAL INCRETIN SYSTEM: Dipeptidyl Peptidase-4 Inhibition

Expression of DPP-4 in renal glomeruli during inflammation [26] suggests a role of the enzyme in the development of glomerulosclerosis. Expression of DPP-4 in the kidney of rats treated with a high-fat diet and streptozotocin is increased [27]. DPP-4 deficiency in rats made them more resistant to streptozotocin-induced T1DM, but predisposed the animals to dyslipidemia and a reduction of GFR [61]. These observations suggest a role of DPP-4 in the development of diabetes and diabetic nephropathy. Treatment with DPP-4 inhibitors may thus be effective in slowing the progression of diabetic nephropathy. In fact, literature provides some data about renoprotective effects of DPP-4 inhibition in diabetic nephropathy. In rats with T1DM, a reduction of albuminuria and an improvement in the histological changes in the kidney were observed with vildaglaptin treatment [62]. Similar renoprotective effects of DPP4 inhibition were shown in rats with T2DM and sitagliptin [63]. Significant reduction in urinary albumin excretion was also seen in diabetic endothelial nitric oxide synthase knockout mice treated with linagliptin on top of Ang II receptor antagonism medication [64]. In humans, it was shown that the DPP-4 inhibitor linagliptin significantly reduced urinary albumin excretion after 24 weeks of treatment in patients with T2DM [65]. Another randomized trial, the MARLINA (Efficacy, Safety & Modification of Albuminuria in Type 2 Diabetes Subjects with Renal Disease with Linagliptin) study (ClinicalTrials.gov Identifier: NCT01792518) [66], has recently been initiated in order to specifically evaluate the albumin-lowering potential of linagliptin in patients with T2DM and renal impairment. The renal effects of DPP-4 inhibitor treatment might be indirect benefits from changes in blood glucose, insulin levels, or weight loss. Nonetheless, the observed effects in those studies could as well be attributed to GLP-1R-mediated, blood sugar-independent effects of the incretin therapy [67]. Moreover, there might be GLP-1R-independent mechanisms of DPP-4 inhibitor treatment, as the DPP-4 has a wide range of other substrates apart from GLP-1 and GIP. The enzyme also cleaves peptide hormones such as brain/atrial natriuretic peptides, neuropeptide Y, peptide YY, and stromal-derived factor 1α, thus exerting cardio-renal effects [68]. Another beneficial effect of DPP-4 inhibition might be a reduction of oxidative stress. In a very recent study, it was shown that DPP-4 inhibitor treatment led to a reduction of lipid and protein oxidation in a rat model of renovascular hypertension [68].

Preclinical studies suggest that DPP-4 inhibitors may enhance urinary flow and sodium excretion, and influence blood pressure [69]. Treatment with the DPP-4 inhibitor sitagliptin preserved the GFR in an animal model of heart failure, thereby preventing the development of cardio-renal syndrome [70]. DPP-4 is expressed in association with the NHE3 in the proximal tubular cells [71]. Therefore, DPP-4 inhibition may affect NHE3 activity and the excretion of sodium, bicarbonate, and water reabsorption in the proximal tubule. Pharmacologic DPP-4 inhibition reduced NHE3 activity in kidney proximal tubule cells in vitro [72]. Hence, the functional relationship between NHE3 and DPP-4 in the intact proximal tubule in vivo was investigated in rats. Inhibition of DPP-4 catalytic activity was associated with inhibition of NHE3-mediated NaHCO3 reabsorption in rat renal proximal tubule [15]. Rieg et al. [16*] used GLP1R-deficient mice to show that alogliptin-induced natriuresis and diuresis were in part independent of the GLP1R. In contrast to alogliptin, the natriuretic action of exendin-4 observed in wild-type mice was not detected in the GLP-1R-deficient mice.

CONCLUSION

Current literature suggests that GLP-1R agonists and DPP-4 inhibitors may reduce renal disease progression by directly acting on the kidney. Clinical studies addressing renal endpoints in patients with diabetic and nondiabetic kidney diseases are warranted to provide information whether the promising preclinical findings can be translated into clinical benefit for patients with renal diseases.

Acknowledgements

None.

Conflicts of interest

Our research work cited in this work (‘DPP-4 inhibition on top of angiotensin receptor blockade offers a new therapeutic approach for diabetic nephropathy’) was supported by Boehringer Ingelheim with money for the institution.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

**: of outstanding interest


15. Interestingly, the studies from Rieg et al. are indicating that aloglipin-induced natriuresis and diuresis are in part independent of the GLP-1 receptor. Arch Intern Med 2013; 49:306–315.


21. The authors found that renal protective effects of GLP-1 might be mediated via the inhibition of Ang II actions and diminished by diabetes because of PKCζ activation and the increased degradation of GLP-1R in the glomerular endothelial cells.


24. This study describes one possible mechanistic explanation for the known protective effects of GLP-1 receptor agonist against the development and progression of diabetic nephropathy.
64. Alter ML, Ott IM, von Websky K, et al. DPP-4 inhibition on top of angiotensin receptor blockade offers a new therapeutic approach for diabetic nephropathy. Kidney Blood Press Res 2012; 36:119–130. This experiment is important, as it indicates that linagliptin on top of an AngII receptor blocker may offer a new therapeutic approach for patients with diabetic nephropathy who do not adequately respond to angiotensin 2 receptor blocker treatment.