Eicosanoids and renal vascular function in diseases

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ABSTRACT

Arachidonic acid metabolites are vital for the proper control of renal haemodynamics and, when not properly controlled, can contribute to renal vascular injury and end-stage renal disease. Three major enzymatic pathways, COX (cyclo-oxygenase), CYP450 (cytochrome P450) and LOX (lipoxygenase), are responsible for the metabolism of arachidonic acid metabolites to bioactive eicosanoids. These eicosanoids can dilate or constrict the renal vasculature and maintain vascular resistance in the face of changing vasoactive hormones. Renal vascular generation of eicosanoids is altered in pathophysiological conditions such as hypertension, diabetes, metabolic syndrome and acute renal failure. Experimental evidence supports the concept that altered eicosanoid metabolism contributes to renal haemodynamic alterations and the development and progression of nephropathy. The possible beneficial renal vascular actions of enzymatic inhibitors, eicosanoid analogues and receptor antagonists have been examined in hypertension, diabetes and metabolic syndrome. This review highlights the roles of renal vascular eicosanoids in the pathogenesis of nephropathy and therapeutic targets for renal disease related to hypertension, diabetes, metabolic syndrome and acute renal failure.

INTRODUCTION

Eicosanoids refer to C20 metabolites generated by three major enzymatic pathways: COX (cyclo-oxygenase), LOX (lipoxygenase) and CYP450 (cytochrome P450). The major biological eicosanoids are derived from arachidonic acid released from membrane phospholipids by the action of phospholipases. The arachidonic acid metabolites formed are determined by factors, including species, cell types, hormonal levels, diets and disease states. Once formed, these arachidonic acid metabolites regulate function at the cellular and organ levels. With regards to renal vascular function, biologically active eicosanoids derived from endothelial cells and VSMCs (vascular smooth muscle cells) operate in concert to regulate renal haemodynamics [1–3]. Unfortunately, in disease states the regulation of these arachidonic acid enzymatic pathways is not properly controlled and renal vascular injury can progress to ESRD (end-stage renal disease) [1,3,4]. These findings have led to investigations that focus on eicosanoids as therapeutic targets for renal vascular diseases. This review will spotlight emerging concepts related to the control of renal haemodynamics by eicosanoids in various disease states.

Key words: cyclo-oxygenase (COX), cytochrome P450 (CYP450), epoxyeicosatrienoic acid (EET), hypertension, kidney, metabolic syndrome, obesity.

Abbreviations: BP, blood pressure; COX, cyclo-oxygenase; CYP450, cytochrome P450; DOCA, deoxycorticosterone acetate; dTGR, double transgenic rat harbouring both the human renin and human angiotensinogen genes; EDHF, endothelium-derived hyperpolarizing factor; EET, epoxyeicosatrienoic acid; EH, epoxide hydrolase; EP receptor, PGE2 receptor; GFR, glomerular filtration rate; HEET, hydroxyEET; HETE, hydroxyeicosatetraenoic acid; IP receptor, PGI2 receptor; IP3, inositol trisphosphate; KCa channel, calcium-activated K+ channels; LOX, lipoxygenase; LT, leukotriene; LX, lipoxin; NO, nitric oxide; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; SHR, spontaneously hypertensive rat; TGF-β, transforming growth factor-β; TP receptor, TXA2 receptor; TX, thromboxane; VSMC, vascular smooth muscle cell.

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**OVERVIEW OF RENAL HAEMODYNAMIC ACTIONS OF EICOSANOIDS**

COX enzymes in the kidney vascular structures are responsible for the conversion of arachidonic acid into PG (prostaglandin) G/H₂ [2,3]. The kidney constitutively expresses both the COX-1 and COX-2 isofoms, and each of these isofoms contributes to the regulation of vascular function [2,5]. The second step in the enzymatic reaction occurs through the actions of PG and TX (thromboxane) synthases that convert PGH₂ into biologically active metabolites. These prostanoids can subsequently act on G-protein-coupled receptors to control renal haemodynamics [2,6] (Figure 1). TXA₂, PGE₂ and PGI₂ (prostacyclin) are the primary COX metabolites that contribute to the regulation of renal blood flow and GFR (glomerular filtration rate) [2,3,5,6].

PGE₂ is the major renal COX-derived metabolite in the kidney and acts on EP receptors (PGE₂ receptors) to influence renal vascular resistance [6,7]. Although PGE₂ has been demonstrated to increase renal blood flow and GFR, EP₁ and EP₃ receptor activation can result in constriction of the renal vasculature [2,6–10]. The vasoconstrictor EP₁ receptors act via the IP₃ (inositol trisphosphate), DAG (diacylglycerol) and PKC (protein kinase C) pathway, and EP₁ receptors decrease cAMP levels [6,7]. Activation of either EP₂ or EP₃ receptors results in an increase in renal vascular cAMP levels and results in relaxation [6,11,12]. In addition to direct actions on renal VSMC EP receptors, PGE₂ is a stimulator of renin release from juxtaglomerular cells [6,13,14]. PGE₂ stimulates renin release via activation of the EP₂ and EP₄ receptors [14]. Renal haemodynamic interactions between PGE₂ and angiotensin have been studied extensively. Angiotensin increases the production of PGE₂ by the kidney and PGE₂ buffers the angiotensin-evoked renal vasoconstriction [2,15–19]. Investigation of kidney PGE₂ and renal vascular EP receptor regulation is of extreme interest, because the vascular effects of PGE₂ are altered in renal disease states.

PGI₂ exerts its vascular actions via stimulation of the IP receptor (PGI₂ receptor) that is found throughout the kidney [6]. IP receptors are G-protein-coupled receptors that increase the generation of intracellular cAMP in pregglomerular vessels [20]. As would be expected, PGI₂ and PGI₂ analogues dilate the glomerular vasculature [2,6]. The renal vasodilation response to PGI₂ is not as effective as the relaxation evoked by PGE₂ activation of EP₂ and EP₄ receptors [6]. Like PGE₂, PGI₂ activation of IP receptors has been shown to stimulate renin release from the juxtaglomerular cells [13]. Renal vasoconstrictor responses are also attenuated by PGI₂ and IP receptor activation [2,18,20]. PGI₂ contributes to vascular homoeostasis and can oppose platelet aggregation in the circulation [21,22]. Alterations in renal vascular PGI₂ production in disease states could contribute to the progression of kidney damage. In particular, changes in the balance between the vasoconstrictor and pro-aggregatory COX metabolite TXA₂ and PGI₂ appear to contribute to renal vascular pathology in disease states [23–25].

TXA₂ is a renal vasoconstrictor that is produced at low levels in the kidney under normal physiological conditions [2,26]. Glomerular mesangial cells and podocytes are the major kidney cell types that synthesize TXA₂ [2]. Once produced, TXA₂ acts on TP receptors (TXA₂ receptors) to activate PLC (phospholipase C), resulting in elevated intracellular IP₃ levels and mobilization of calcium from intracellular stores and activation of L-type calcium channels [27,28]. Afferent arteriolar constriction in response to the TXA₂ mimetic, U-44169, is dependent on activation of L-type calcium channels [29]. TP receptor activation of L-type calcium channels contributes to renal vasoconstriction in response to angiotensin [2,18]. Additionally, TXA₂ is pro-aggregatory, stimulates mesangial cell matrix production and has been associated with progressive glomerular damage [25]. TXA₂ and TP receptors have been implicated in the glomerular renal vascular alterations that occur during various renal disorders [3,18,25]. Thus TXA₂ and TP receptors are viable therapeutic targets for the treatment of renal vascular dysfunction.

CYP450 enzymes are expressed in vascular and tubular structures in the kidney and have varied functional consequences that depend on the arachidonic acid product [1,2,30,31]. Two families of CYP450 enzymes are present in the kidney and account for the generation of EETs (epoxyeicosatrienoic acids) and HETEs (hydroxyeicosatetraenoic acids) (Figure 1). The production...
of the epoxide metabolites is primarily via the CYP2C gene family, whereas the CYP4A and CYP4F families are the major pathways for synthesis of the hydroxylase metabolites [1,2,30–32]. CYP450 enzyme expression varies among the kidney cell types and enzymatic regulation of this pathway impacts on renal haemodynamics [1,30].

20-HETE is the major CYP4A metabolite of arachidonic acid and this eicosanoid plays a central role in the regulation of vascular resistance and growth [1,2]. This hydroxylase metabolite increases renal vascular resistance and can influence the actions of NO (nitric oxide) on VSMCs [1,33]. 20-HETE constricts the afferent arteriole by closing large-conductance K_Ca channels (calcium-activated K^+ channels), resulting in membrane depolarization and a sustained increase in intracellular calcium [1,2,34,35]. The contribution of 20-HETE to the renal vasodilator response to NO has also been demonstrated. NO can bind to the haem moiety of CYP4A enzymes, inhibit 20-HETE generation and decrease the influence of 20-HETE to inactivate K_Ca channels [33]. In addition to these actions on VSMCs, 20-HETE appears to play a significant role in renal blood flow autoregulatory responses [1,2]. The regulation of renal vascular CYP4A enzymes and 20-HETE have also been implicated in the development of essential hypertension and diabetes complications [1,2,31].

The epoxygenase metabolites counteract the renal vasoconstrictor actions of 20-HETE. EETs are produced in endothelial cells and activate VSMC K_Ca channels, resulting in hyperpolarization [36,37]. These EET actions are consistent with their identification as EDHFs (endothelium-derived hyperpolarizing factors). Although all four regioisomeric EETs have been demonstrated to dilate the renal vasculature, 11,12-EET and 14,15-EET appear to be the major epoxides that mediate afferent arteriolar dilation [38,39]. These epoxygenase metabolites also contribute to the bradykinin and acetylcholine renal dilator responses [40,41]. Other vascular properties of the EETs include anti-inflammatory actions, profibrinolytic effects and inhibition of VSMC migration [1,30,31,42]. These favourable EET properties could protect the kidney and renal vasculature from the progression of damage associated with diabetes and hypertension.

LOX enzymes metabolize arachidonic acid to generate LTs (leukotrienes), HETEs and LXs (lipoxins) (Figure 1). Expression of LOX enzymes in vascular tissue is highly localized with sources including platelets (12-LOX), monocytes/macrophages (12/15-LOX) and neutrophils (5-LOX) [43–46]. Glomerular mesangial cells and endothelial cells express LOX enzymes and produce LT_A_4, 12-HETE and 15-HETE [2,18,46]. These LOX metabolites have inflammatory actions, VSMC growth effects and regulate renal haemodynamics [2,46]. 12-HETE and 15-HETE constrict renal vessels and glomerular mesangial cells [2,47]. Likewise, 12-HETE decreases renal blood flow and GFR when infused into the renal artery [48]. LTs can increase glomerular and peritubular capillary permeability, and increased LT production can contribute to proteinuria and interstitial nephritis [2,46,49]. Therefore LOX-derived metabolites could contribute to the renal glomerular and vascular damage that is associated with hypertension and diabetes.

LXs are LOX-derived metabolites that counteract the renal vascular and inflammatory actions of 12-HETE, 15-HETE and LTs. LXA_4 has been demonstrated to oppose the decrease in renal blood flow and GFR mediated by intrarenal infusion of LTD_4 [50]. This action of LXA_4 is partially due to activation of peptidoLT receptors [2,49]. The generation of LXs and aspirin-triggered LXs can switch the glomerular cellular response from inflammation in favour of resolution and inhibition of monocyte recruitment [49]. Interestingly, structural analogues of LXA_4 show therapeutic potential for the treatment of acute renal failure [49,51].

**RENA** **L VAS** **CULAR** **EICO** **SAN** **OID** **S** **AND** **HYPERTENSION**

The influence of arachidonic acid metabolites on renal haemodynamics and the long-term control of arterial BP (blood pressure) has been recognized for a number of years [1–3,31]. Additionally, regulation of kidney COX, LOX and CYP450 enzymes has been associated with renal haemodynamic alterations that could possibly contribute to the development of hypertension [3,31]. The diversity of biological actions of the arachidonic acid metabolites produced by these enzymes and interactions between the eicosanoid products has made it difficult to sort out the contribution of these enzymatic pathways to BP regulation. In any case, hypertension-induced renal vascular and glomerular damage has also been partially attributed to inappropriate regulation of eicosanoids [1,3,31] (Figure 2). Consequently, investigators have manipulated kidney eicosanoids to determine their possible antihypertensive and renal vascular protective effects.

COX enzymatic products can have antihypertensive and prohypertensive properties depending on the profile of prostanoids produced and the model of hypertension. COX inhibitors may actually elevate BP and antagonize the effects of antihypertensive medication [3,5]. Long-term use of NSAIDs (non-steroidal anti-inflammatory drugs) and COX-2 inhibitors has been limited, because of increased arterial BP, oedema and congestive heart failure in a significant proportion of patients [3,5]. Likewise, decreased production of PGs may contribute to increased renal vascular resistance in hypertension. The inability of vasodilator PGs to buffer the renal vascular response to angiotensin appears to contribute to the increased vascular resistance in angiotensin hypertension and the SHR (spontaneously hypertensive rat) [12,20,52].
Renal and cardiovascular diseases are associated with altered kidney and vascular arachidonic acid enzymatic pathways. Inappropriate eicosanoid generation contributes to increased vascular and endothelial dysfunction, BP and glomerular injury.

On the other hand, TXA2 and TP receptor activation contribute to the increase in renal vascular resistance and BP in angiotensin hypertension [53–56]. This type of heterogeneity in the biological actions of the COX metabolites and receptor activation has made the net effect of COX inhibition on BP control in hypertension unpredictable.

The participation of COX-1 and COX-2 enzymes and specific PGs and their receptors to renal vascular resistance and hypertension has been made possible by the development of selective agonists and antagonists and transgenic mice. Qi et al. [57] assessed the BP response to angiotensin in COX-1- and COX-2-deficient mice and found that these COX enzymes had opposing effects on the angiotensin pressor response. COX-2-deficient mice or mice infused with a COX-2 inhibitor had an enhanced response, whereas COX-1-deficient mice had an attenuated BP response to angiotensin [57]. COX-2 is expressed in the renal cortex and medulla and appears to be involved in maintaining sodium excretion, glomerular filtration and renal blood flow [2,5,18]. Renal medullary, but not cortical, blood flow is significantly reduced by COX-2 selective inhibition [5,58]. This is consistent with mounting evidence that COX-2 is responsible for synthesis of dilator PGE2 and PGF2 and COX-1 mediates synthesis of TXA2 [5]. The renal EP2 and EP4 receptors have been demonstrated in EP-receptor-deficient mice to mediate the PGE2 dilator response [6–9]. Interestingly, IP-receptor-deficient mice had significantly attenuated elevations in BP following induction of two-kidney, one-clip hypertension [59]. This finding suggests that IP receptors do not oppose but actually contribute to the elevated BP in renovascular hypertension. The contribution of the IP receptor to this model of renovascular hypertension was related to the critical role for COX-2-derived PGF2 in regulating renal renin release [59]. Likewise, COX-2-derived PGE2 and activation of the EP1 receptor contributes to the development of renal vascular injury in the stroke-prone SHR [60]. Studies in TP-receptor-deficient mice demonstrate that the slow pressor response to angiotensin is attenuated by the lack of this receptor [54,56]. The attenuated BP response to angiotensin in TP-receptor-deficient mice was partially due to a decrease in renal vascular resistance [54,56]. These findings highlight the varied regulation and actions of COX metabolites and the difficulties in predicting their contribution to renal vascular resistance and BP control.

Like COX metabolites, CYP450 arachidonic acid products can dilate or constrict the renal vasculature and have prohypertensive and antihypertensive properties. Accumulating evidence suggests that CYP4A synthesis of 20-HETE in the renal vasculature acts in a prohypertensive manner and that tubular 20-HETE generation increases sodium excretion and can lower BP [1,31]. Increased renal vascular 20-HETE production was first described in the SHR and led several investigators to explore the role of 20-HETE in the development of hypertension [1,61]. 20-HETE could contribute to the increase in BP by elevating renal vascular resistance. In support of this view, studies demonstrated that inhibition of 20-HETE production decreased renal medullary vascular resistance and BP in the SHR [1,31]. Likewise, inhibition of renal microvascular CYP4A by antisense oligonucleotides directed against CYP4A1 and CYP4A2 lowered BP in SHRs [62]. On the other hand, it does not appear as if mutations in CYP4A play a causal role in the development of hypertension in the SHR. Genetic co-segregation analysis on an F2 cross of the SHR and Brown Norway rat indicates that the CYP4A gene is not the primary cause of hypertension in the SHR [63]. Even with this being the case, CYP450 inhibitors that decrease 20-HETE formation have been demonstrated to increase renal blood flow and lower BP in angiotensin hypertension and the Lyon hypertensive rats [1,31]. Intriguingly, disruption of the CYP4a14 gene produced hypertension in male, but not female, mice [64]. Male CYP4a14−/− mice actually had increased 20-HETE production that was associated with a compensatory increase in CYP4a12 expression [64]. Afferent arteriolar diameter was reduced and impaired myogenic responses were observed in these hypertensive CYP4a14-deficient mice [64]. Overall, studies conducted in rats and mice demonstrate that renal vascular CYP4A-derived 20-HETE synthesis contributes to blood flow and BP control in hypertension.

The prohypertensive aspects of 20-HETE produced by the renal vasculature and antihypertensive actions associated with renal tubular 20-HETE generation in rodent studies is also supported by studies in humans. An impaired renal natriuretic response to furosemide is observed in salt-sensitive essential hypertensive
patients and correlates with decreased urinary 20-HETE excretion [65]. In addition, Laffer et al. [66] demonstrated that 20-HETE is regulated by dietary salt loading and that salt-sensitive hypertensive patients administered a salt load are unable to increase renal 20-HETE levels properly. A functional variant of a CYP4A hydroxylase has also been demonstrated to be associated with essential hypertension [67]. This variant of CYP4A11, a homologue of the murine Cyp4a14, encodes for a mono-oxygenase with reduced 20-HETE synthase activity and has a greater prevalence in hypertensive compared with normotensive Caucasians [67]. On the other hand, there is also evidence for increased 20-HETE levels and vascular dysfunction in humans with hypertension. Urinary 20-HETE levels positively correlated with BP in women and an impaired forearm blood flow response to nitroglycerine was observed in males and females with increased 20-HETE levels [68]. A positive correlation between urinary 20-HETE levels and markers of oxidative stress, such as isoprostanes, has also been demonstrated in human hypertensive subjects [69]. Although there is still a great deal more that needs to be evaluated in human hypertensive patients, the clinical studies strongly suggest that findings concerning the regulation of 20-HETE in animal models of hypertension will provide important information that can possibly be translated to therapy in humans.

CYP450-derived epoxygenase metabolites are involved in renal blood flow regulation and long-term arterial BP control [1,2,30,31]. CYP2C-derived EETs are vasodilators and can be converted into inactive diols by the soluble epoxide hydrolase enzyme [70,71]. A number of reports have suggested a significant role for CYP2C and epoxide hydrolase enzymes in the regulation of endothelial cell function and BP control [36,37,72,73]. Interestingly, polymorphisms in the human epoxide hydrolase enzyme (EPHX2) are associated with coronary artery calcification in African-Americans and hypercholesterolaemia in Utah kindred at risk for coronary artery disease [74,75]. Evidence for an association of an epoxide hydrolase polymorphism and BP control in humans has not yet been identified. As for the actions of epoxygenase metabolites, they vasodilate blood vessels, but can also act on tubules to cause natriuresis to lower BP [1,31]. Acutely elevating 11,12-EET levels abolished the enhanced afferent arteriolar reactivity to angiotensin in hypertensive rats [76]. Increasing epoxygenase metabolite levels chronically lowers BP and protects the kidney from hypertension-induced injury [72,73,77–79]. Treatment with the PPARα (peroxisome-proliferator-activated receptor α) activator fenofibrate resulted in increased kidney CYP2C23 expression, lower BP and protection from renal injury in hypertensive dTGRs (double transgenic rats harbouring both the human renin and human angiotensinogen genes) [78,79]. Fenofibrate increased EET production and the epoxygenase metabolite product of 20-HETE, HETEs (hydroxyEETs) in dTGRs [79]. The contribution of HETEs to the protection of the kidney in hypertension and their renal vascular actions remains to be explored. Other studies have increased epoxygenase levels in hypertension by inhibiting the epoxide hydrolase conversion of the epoxides into diols [72,73,77,78]. Epoxide hydrolase inhibition lowers BP in SHRs and angiotensin hypertension [72,73,77,78]. Afferent arteriolar responses are also improved in angiotensin hypertensive animals treated with an epoxide hydrolase inhibitor [73,77]. Taken together, these studies demonstrate that increasing epoxygenase metabolites lowers BP, improves renal vascular responses and protects the kidney from hypertension-induced injury.

The arachidonic acid pathway that has received less attention with regards to renal vascular alterations in hypertension is the LOX pathway. Although less studied, the importance of the LOX pathway and the metabolite 12(S)-HETE as a significant component in cardiovascular and kidney pathologies has been clearly established [45,46,81]. SHRs and patients with essential hypertension have elevated LOX pathway or 12(S)-HETE levels [82,83]. Increased vascular 12-LOX protein and mRNA expression and 12(S)-HETE production has also been observed in different models of angiotensin-dependent hypertension [84–86]. These findings are consistent with the actions of 12(S)-HETE in the kidney. 12(S)-HETE decreases renal blood flow, constricts renal vessels and contributes to the afferent arteriolar response to angiotensin [2,17,47]. Likewise, 12(S)-HETE enhances angiotensin contraction through increased intracellular calcium signalling in the aorta of SHRs [87]. Previous studies have shown that treatment with non-specific LOX inhibitors phenidone, baicalein or CDC (cinnamyl-3,4-dihydroxycinnaminate) lowers BP in SHRs and reduces angiotensin-dependent hypertension [81,84,85]. More recently, findings from 12/15-LOX-deficient mice have suggested that LOX and NO signalling pathways interact and impact on vascular and BP control. BP was decreased in 12/15-LOX knockout mice that were chronically infused with angiotensin [88]. 12/15-LOX-/- mice also had reduced vasoconstrictor responses to angiotensin, and increased eNOS (endothelial NO synthase) expression and NO bioavailability [88]. This is in agreement with the finding that 12/15-LOX can result in catalytic consumption of NO and prevent NO-mediated soluble guanylate cyclase activation [89]. Taken as a whole, it appears as if 12(S)-HETE and the 12/15-LOX pathway modulate angiotensin-induced vascular responses and contribute to BP regulation in hypertension.

Although there is clear evidence for the COX, CYP450 and LOX pathways in renal vascular responses and long-term BP control, the possible interactions between these arachidonic acid metabolic pathways in hypertension...
is less well understood. One consideration is that any time that one arachidonic acid enzymatic pathway is inhibited, more substrate is available for the generation of products by the other enzymatic pathways. Another possibility is that metabolism of arachidonic acid by multiple enzymatic pathways results in a substance that has vascular actions. For example, the CYP450 hydroxylase metabolite 20-HETE can be metabolized in the renal vasculature by COX enzymes yielding PG-like dilators or TX-like constrictors [90]. Intriguingly, the renal vasodilation in response to the CYP450 inhibitor in the Lyon hypertensive rat was dependent on COX enzymatic activity [91]. In this case, the renal vasodilation induced by indomethacin was due to increased CYP450 epoxygenase metabolites [91]. Examples of interacting arachidonic acid pathways have been demonstrated numerous times and can complicate experimental interpretation. A challenge for future studies will be to determine the interactions between various eicosanoid enzymatic pathways and their contribution to renal haemodynamics and regulation of BP.

**RENAL VASCULAR EICOSANOIDS IN DIABETES AND METABOLIC SYNDROME**

The major cause of kidney failure in the world is diabetic nephropathy, and obesity-related metabolic syndrome often contributes to the development of Type II diabetes [92,93]. Hyperglycaemia, obesity and elevated BP, along with early hyperfiltration, are major risk factors that contribute to the progression of renal damage in diabetes [4,93,94]. This progression of diabetic nephropathy includes an initial increase in GFR, thickening of the glomerular basement membrane, expansion of the mesangium and eventually a decline in GFR [4,93,94].

Type I and Type II diabetes are also associated with endothelial dysfunction that has been linked to changes in eicosanoid metabolism [4,93]. Renal vascular and glomerular COX, CYP450 and LOX metabolites change during diabetes and partially contribute to the decline in renal function [4,5,46,58] (Figure 2). The renal vascular beneficial aspects of manipulating the arachidonic-acid-metabolizing pathways are actively being pursued.

Several studies have linked diabetes with enhanced kidney COX-2 generation of prostanoids. COX-2 protein expression is increased in streptozocin-induced diabetic rats and obese Zucker rats [5,58,95–100]. In addition, diabetic rats with DOCA (deoxycorticosterone acetate)-salt hypertension have enhanced COX-2 expression at the macula densa and cortical thick ascending loop of Henle cells [58]. Dey et al. [98,99] observed increased renal cortical and vascular COX-2 expression in obese Zucker rats. COX-2 expression is increased and NO bioavailability is decreased in endothelial cells treated with high glucose [101]. RAGE (receptor for advanced glycation end-products) in monocytes appears to up-regulate COX-2 activity through NF-κB (nuclear factor κB) activation [102,103]. COX-2 expression is also dramatically increased in monocytes of diabetic patients [46,102,103]. These results suggest that COX-2 may be a key inflammatory mediator in the pathogenesis of vascular dysfunction in diabetes. On the other hand, a number of studies have found no differences in renal cortical or vascular expression of COX-1 in Type I or Type II diabetes [4,58,104]. Thus the contribution of COX-2 and arachidonic acid metabolites generated by this enzyme to renal damage in diabetes has been extensively explored.

Renal vascular and glomerular PG and TXA₂ production have been implicated in the progression of diabetic nephropathy [4,46,58]. Enhanced production of vasodilatory PGs by glomeruli has been associated with an increased GFR in streptozocin-induced diabetes [5,46,58]. Other studies in Type I and Type II diabetes and obesity animal models have demonstrated increased urinary and glomerular TXA₂ production [4,46,58,105]. Increased TXA₂ production contributes to the progression of diabetic nephropathy by its vasoconstrictor action and induction of glomerular matrix proteins [58,106–108]. Likewise, COX generation of the platelet-activating metabolite PGI₂ is increased in Type I and Type II diabetes [24,25,58,107]. PGI₂ measured as the urinary metabolite PGF₁α, acts as a renal vasodilator and anti-aggregatory metabolite that counteracts the injurious TXA₂ effects [25]. One consistent finding in diabetic patients and animal models has been a decreased urinary 6-keto PGF₁α/TXB₂ ratio [23,24]. Thus the decrease in the urinary 6-keto PGF₁α/TXB₂ ratio has been linked to the progression of renal damage in obese and diabetic rats as well as diabetic patients.

A number of studies have evaluated the ability of COX inhibitors and TX synthase inhibitors to retard the progression of diabetic nephropathy [4,58,98]. Non-selective COX inhibitors acutely decrease the early stage hyperfiltration in diabetes and decrease proteinuria [58,109,110]. The renal vascular effects of selective COX-2 inhibitors have been studied in streptozocin-induced diabetes [95,111,112]. COX-2 inhibitors reduce proteinuria, glomerular extracellular matrix deposition and improve renal blood flow and glomerular filtration in this rat model of Type I diabetes [95]. Defective renal blood flow and afferent arteriolar auto-regulatory responses are also improved by COX inhibition [113]. Administration of the COX-2 inhibitor rofecoxib also provides renal vascular protection in obese Zucker rats [98]. Likewise, TX synthase inhibitors slow the progression of renal damage in diabetic rats. Diabetic rats treated with TX synthase inhibitors have decreased proteinuria and preserved renal blood flow [23,24,114,115]. Diabetic patients administered the TXA synthase inhibitor oza- grel had decreased urinary TXB₂ levels and decreased urinary protein and albumin levels [23,114,115]. Even
although these studies have demonstrated promise for the use of COX-2-selective and TX synthase inhibitors in patients with diabetic nephropathy, enthusiasm for their use has recently been dampened by the withdrawal of some COX-2 inhibitors from the market.

Even though the COX-2-selective inhibitors have been removed from the market, because of an increased incidence of myocardial infarction and thrombotic stroke, adverse renal haemodynamic effects are also evident in patients treated with COX-2-selective inhibitors. Studies in animals were the first to suggest that constitutively expressed COX-2 in the kidney could be important for the regulation of renal haemodynamics and fluid volume regulation. COX-2 was initially localized to the macula densa and thick ascending loop of Henle epithelial cells in rodents [116]. COX-2 expression has also been found in a number of vascular and epithelial tissues within the kidneys of rodents, rabbits and canines [5, 117]. A number of these studies determined that COX-2 was important for the maintenance of renal haemodynamics in states of volume depletion [5, 117]. Humans also constitutively express COX-2 in the kidney [118, 119]. COX-2 expression has been found in the macula densa of elderly patients and in the VSMCs and glomerular podocytes of normal human kidneys [118, 119]. This would explain why selective COX-2 inhibitors have demonstrated the same risks as traditional NSAIDs with respect to adverse renal fluid and electrolyte events [5, 117]. Decreased sodium excretion and fluid retention that can result in oedema in the lower extremities has been reported in a significant portion of patients treated with NSAIDs or selective COX-2 inhibitors [5, 117]. Animal and clinical studies have also found evidence for increased BP following treatment with selective COX-2 inhibitors [5]. Thus patients with volume depletion, heart failure, hepatic cirrhosis, lupus nephritis and renal artery stenosis are susceptible to the deleterious renal haemodynamic and electrolyte effects of COX-2 inhibitors. One approach to decrease these adverse effects has been to develop combined COX inhibitors with NO donors [120]. Other issues that are not always properly addressed when inhibiting COX are the possibilities of shunting arachidonic acid to another enzymatic pathway or the decrease in conversion of LOX and CYP450 metabolites by COX. As a case in point, COX can convert 20-HETE into the renal vasodilator 20-OH-PGE2 and constrictor 20-OH-PGH2 [90]. As for COX-2 inhibition and the renal protective effects in diabetes, therapeutic approaches such as the combined COX inhibitor and NO donor or specific PG receptor agonists and antagonists could be more beneficial in reducing renal injury in diabetes and lack the deleterious cardiovascular and renal side effects associated with NSAIDs and COX-2-selective inhibitors.

Recent studies have also implicated alterations in CYP450 metabolites as contributing to renal damage in obesity and diabetes [4, 98, 99]. Streptozotocin-induced diabetic rats have elevated CYP4A expression and 20-HETE generation in renal microsomes that can be reversed by insulin treatment [121–124]. This finding is consistent with studies that have demonstrated increased hepatic CYP4A protein expression in streptozotocin diabetic rats [121]. Likewise, obese Zucker rats have increased renal vascular and hepatic CYP4A protein levels [98, 99, 121]. On the other hand, rats fed a high-fat diet have decreased renal tubular CYP4A expression, but unaltered renal microvessel 20-HETE generation [123]. This difference in renal tubular and vascular CYP4A regulation in the obese Zucker rats and rats fed a high-fat diet could be contributing to the elevated BP in each of these animal models. Decreased renal tubular 20-HETE production would impair pressure natriuresis in rats fed a high-fat diet and increase BP, whereas increased renal vascular 20-HETE generation would increase vascular resistance in the obese Zucker rat. Interestingly, obese patients with hypertension have decreased urinary 20-HETE excretion that is associated with increased plasma insulin levels [125]. These studies suggest that insulin has an inhibitory effect on CYP4A expression. A recent study [126] has demonstrated that 20-HETE inhibition can decrease the enhanced carotid artery endothelin-mediated constrictor response in streptozotocin-induced diabetic rats. In any event, the exact contribution of vascular 20-HETE generation to renal damage that occurs in diabetes and obesity awaits investigation.

Renal and vascular regulation of the CYP450 epoxygenase enzymes is also an active area of research, because epoxygenase metabolites are EDHFs and endothelial dysfunction occurs in diabetes and metabolic syndrome [2, 36, 127]. In general, CYP2C epoxygenase enzymes are decreased in diabetes [98, 99, 123, 127]. In addition, high-fat-fed rats that develop insulin resistance also have decreased renal CYP2C23 levels [123]. Renal and mesenteric vascular CYP2C11 and CYP2C23 expression is decreased in obese Zucker rats [127]. Interestingly, the conversion of EETs into biologically less active diols may be increased in the mesenteric artery, since epoxide hydrolase protein expression is increased in obese Zucker rats [127]. Obese Zucker rats and diabetic rats have impaired endothelium-dependent dilator responses that can be attributed to decreased NO and EET bioavailability [4, 46, 127]. EETs or epoxide hydrolase inhibition could potentially slow the progression of diabetic nephropathy by two mechanisms. Elevating kidney and vascular EET levels would improve endothelial dilator function and exert anti-inflammatory actions and, thereby, inhibit the nephropathy associated with metabolic syndrome and diabetes.

LOX enzymes and the generation of HETEs have been observed in animal models of diabetes and diabetic patients. The increased expression of LOX enzymes has been implicated in the vascular pathogenesis and
renal injury associated with diabetes [46,128–130]. The 5-LOX enzymes and LTE₄ generation have been associated with diabetes [131]. Streptozotocin-induced diabetic rats have increased urinary excretion of LTE₄ that is decreased by insulin treatment [131]. The 5-LOX enzyme is abundantly expressed in atherosclerotic lesions, but the contribution of 5-LOX to diabetic nephropathy remains unknown [46]. As with renal injury in hypertension, the 12/15-LOX is the primary LOX enzymatic pathway implicated in vascular and renal injury associated with diabetes and metabolic syndrome. The 12/15-LOX product 12-HETE is increased in the urine of diabetic patients with early kidney disease [46,131]. Increased 12/15-LOX mRNA and protein expression parallel increases in fibronectin and other key contributors to diabetic nephropathy [131]. In addition to producing 12-HETE and 15-HETE, the 12/15-LOX enzyme can oxidize LDL (low-density lipoprotein) and appears to contribute to the pathogenesis of atherosclerosis [132,133]. A number of studies utilizing ribozyme targeted to rat 12/15-LOX- and 12/15-LOX-gene-deficient mice have substantiated further the important role for this pathway in neointimal thickening and atherosclerosis [134–138]. High glucose levels also increase the 12/15-LOX in cultured endothelial cells, VSMCs, mesangial cells and glomeruli of diabetic rats [131,139,140]. The LOX pathway is also involved in high glucose-induced adhesion of monocytes to endothelial cells [140,141]. 12(S)-HETE is hypertrophic in cultured VSMCs under high glucose conditions [46,142]. Diabetic db/db mice have increased endothelial cell 12/15-LOX and increased binding of monocytes [143]. 12/15-LOX expression is also markedly increased in obese Zucker rats and is associated with vascular inflammation [144,145]. Interestingly, 12/15-LOX is one of a few genes that are increased in the pancreatic β-cell of the insulin-resistant prediabetic Zucker diabetic fatty rat [145]. The LOX inhibitor masoprocol has been studied in insulin resistance and Type II diabetes. Masoprocol reduces triacylglycerols (triglycerides), non-esterified fatty acids (free fatty acids) and improves insulin sensitivity in Type II diabetes and fructose-fed and fat-fed rats [146,147]. The promise of LOX inhibitors for renal vascular injury and nephropathy in diabetes and metabolic syndrome will undoubtedly be a primary focus of future experimental studies.

The peroxidation of arachidonic acid by oxygen radicals and the generation of isoprostanes can also contribute to the renal vascular injury that occurs during diabetes [4,5,46,58]. Oxygen radicals and isoprostanes decrease renal blood flow and GFR and can result in renal vascular injury [2,18,148]. Along this line, isoprostane synthesis is increased in diabetic patients and animal models of diabetes [98,149,150]. High glucose also increases isoprostane synthesis in cultured glomerular endothelial and mesangial cells [149]. Furthermore, incubating glomerular cells with isoprostane simulated TGF-β (transforming growth factor-β) generation [149]. TGF-β is a prosclerotic growth factor that has been shown to increase glomerular matrix formation and contribute to diabetic nephropathy [46,149]. Obese Zucker rats also have increased urinary isoprostane levels that have been associated with glomerulosclerosis [98]. Interestingly, treatment with the COX-2 inhibitor rofecoxib decreased urinary 8-isoprostanate levels, renal inflammation and glomerular injury in this model of metabolic syndrome [98]. On the whole, the contribution of isoprostanes and the enzymes that may be responsible for their generation in diabetes and metabolic syndrome is not well characterized.

**ACUTE RENAL FAILURE AND VASCULAR EICOSANOIDS**

Acute renal failure is defined as an abrupt decrease in GFR that results in tubular necrosis [151,152]. Although there is a large body of evidence on the eicosanoids in the progression of nephropathy associated with hypertension and diabetes, less is known about these arachidonic acid metabolites in renal vascular alterations that occur during acute renal failure. COX metabolites have been implicated because NSAID and COX inhibitor treatment has been linked to acute renal failure in patients [3,5,151]. In general, rat models of acute renal failure demonstrate increased levels of renal constrictor and decreased levels of dilator PGs [5,151,153–155]. As with diabetes, the kidney PGI₂/TXB₂ ratio is decreased in antibiotic-induced acute renal failure [153,155]. Treatment with a TXA₂ synthase inhibitor or the PGI₂ analogue iloprost can attenuate the decreases in GFR and renal tubular injury that occur during antibiotic- or glycerol-mediated acute renal failure [155,156]. Angiotensin and endothelin renal vascular constriction and interactions with arachidonic acid metabolites also contribute to acute renal failure. COX metabolites positively influence endothelin constriction in glycerol-induced acute renal failure [157]. On the other hand, CYP450 and LOX metabolic pathways demonstrate approximately equal contribution to the enhanced renal vascular angiotensin constrictor response in this model of renal failure [157]. CYP450 hydroxylase metabolites most probably account for a portion of the enhanced renal vascular response to angiotensin. Interestingly, CYP450 epoxides of linoleic acid may be cytotoxic to renal tubular cells in acute renal failure [158]. CYP1 and CYP2 gene families can generate epoxides of linoleic acid that disrupt mitochondrial function without causing oxidative stress in rabbit renal proximal tubule cells [158]. The ability of CYP450 inhibitors to attenuate renal damage during acute renal failure has not yet been elucidated.

Although the LOX enzymes have been implicated in the renal constriction and inflammation in acute renal
failure, synthetic analogues of LOX-derived LXs are beneficial as a treatment for ischaemic acute renal failure [49,51]. As mentioned previously, LXA₄ and aspirin-triggered LXs produced by the LOX pathway can oppose the LT-mediated renal constrictor and inflammatory responses [49]. Recent studies have demonstrated that the stable LX analogue, 15-epi-16-(FphO)-LXA₄-Me [15-epi-16-(p-fluorophenoxyl)-lipoxin A₄ methyl ester], is protective in acute renal failure in mice [51]. Administration of this LX analogue prior to ischaemia preserved renal epithelial integrity and attenuated chemokine and cytokine responses [51]. The effect of this treatment on renal blood flow remains to be determined. In any case, initial studies support the concept that LX analogues may be therapeutically beneficial in acute renal failure and other inflammatory glomerular diseases.

CONCLUSIONS

Eicosanoids generated by the COX, CYP450 and LOX pathways play a vital role in the normal functioning of the renal vasculature and regulation of these enzymes occurs during the pathogenesis of nephropathy. The study of these arachidonic-acid-metabolizing pathways is complex and each enzymatic pathway is capable of generating metabolites that contribute to renal disease. These same enzymatic pathways also produce renal vascular eicosanoids that provide protection from nephropathy. It is the balance between eicosanoids that increase renal blood flow and glomerular filtration and those that act as constrictors that determines renal haemodynamics. Renal vascular eicosanoids can influence other aspects of vascular control during disease states. Arachidonic acid metabolites are involved in vascular inflammatory responses and contribute to oxidative stress. A number of studies have investigated the beneficial actions of COX, CYP450 and LOX inhibitors because of the vital role that these eicosanoid pathways play in kidney function during disease states. The limitation of this therapeutic approach has been that these enzymatic inhibitors not only decrease eicosanoids that contribute to nephropathy, but also inhibit the generation of metabolites that provide protection from renal injury. Lessons have been learnt from the recent problems with the therapeutic use of COX-2 inhibitors in the human population. The main lesson learned was that inhibiting an arachidonic acid enzymatic pathway changes the metabolism of more than one metabolite and can have adverse effects. The findings that COX-2 inhibitors suppressed PGI₂ formation, but did not inhibit vascular TXA₂ production, in mice and humans could explain the increased incidence of myocardial infarction and thrombotic stroke in patients administered COX-2 inhibitors [159]. On the other hand, NSAIDs that non-selectively inhibit COX-1 and COX-2 enzymes decrease both PGI₂ and TXA₂ vascular generation. Thus COX-2 inhibitors tipped the vascular prostanooid profile to one that might be expected to elevate BP, accelerate atherogenesis and increase thrombosis. Fortunately, this has intensified research to identify eicosanoid receptors and determine their renal and vascular actions. In recent years, therapeutic tools have been developed to target specific eicosanoid metabolites and the receptors upon which eicosanoids act. This approach has led to the development of promising therapies for the treatment of nephropathy associated with hypertension, diabetes and acute renal failure (Table 1). Undoubtedly, future investigation will identify additional renal vascular eicosanoid targets and therapies for renal damage.

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REFERENCES

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72 Jung, O., Brandes, R. P., Kim, I. H. et al. (2005) Soluble epoxide hydrolase is a major effector of angiotensin II-induced hypertension. Hypertension 45, 759–765


74 Sato, K., Emi, M., Ezura, Y. et al. (2004) Soluble epoxide hydrolase acts as a peroxisome proliferator-activated receptor-α activator and reduces the plasma total cholesterol and triglyceride content in familial hypercholesterolemia: intramural association study in an eight-generation hyperlipidemic kindred. J. Hum. Genet. 49, 29–34


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131 Kang, S. W., Adler, S. G., Nast, C. C. et al. (2001) i nit h er a t .  R188–R196


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Does gentamicin induce acute renal failure by increasing renal TXA2 synthesis in rats? Prostaglandins Leukotrienes Essent. Fatty Acids 45, 131–136


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