Editorial Comments

Cytochrome P450 metabolites of arachidonic acid: novel regulators of renal function

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Metabolism of arachidonic acid by cytochrome P450 enzymes

Recent studies indicate that in the kidney arachidonic acid (AA) is metabolized by cytochrome P450 (CYP450) enzymes to produce epoxyeicosatrienoic acids (EETs), dihydroxyeicosatetraenoic acids (DiHETEs), and 19- and 20-hydroxyeicosatetraenoic acids (19- and 20-HETE). CYP450 metabolites of AA play a major role in the regulation of renal vascular tone, tubuloglomerular feedback (TGF), and sodium transport [1,2]. Enzymes of the CYP450 4A and 4F families catalyse the formation of 20-HETE. CYP450 4A mRNA and protein are expressed in the renal arterioles, glomerulus, proximal tubule (PT), cortical and medullary thick ascending limb of the loop of Henle (TALH), and in the pericytes surrounding vasa recta capillaries [2,3]. The renal arterioles, PTs, and glomeruli avidly produce 20-HETE, EETs, and DiHETEs when incubated with AA, while in the TALH, 20-HETE is the primary metabolite of AA [2].

Enzymes of the CYP450 1A, 2B, 2C, 2D, 2E, and 2J families catalyse the formation of EETs [1,4]. In the kidney, protein for each of these enzymes is expressed; however, the specific enzymes primarily responsible for EETs production in different nephron segments have not been determined.

Numerous factors influence the expression of CYP450 enzymes. High levels of CYP450 4A1 and 4A3 mRNA and protein are detected in neonatal rat kidney, but the levels of these enzymes diminish into adulthood [5,6]. In PT cells, angiotensin II (Ang II) stimulates formation of EETs [7], whereas epidermal growth factor [8], dopamine [9], and parathyroid hormone (PTH) [10] stimulate the formation of 20-HETE. Vasopressin, Ang II, bradykinin, and calcium stimulate 20-HETE formation in the TALH [2,4]. The expression of CYP450 4A protein is downregulated in the kidney and renal vasculature when rats are fed a high salt diet [11–13] and this can be prevented when circulating Ang II concentrations are maintained at normal levels via an i.v. infusion of Ang II [2]. On the other hand, EETs formation has been reported to be elevated by increased dietary salt intake in some strains of rats [13–15]. Upregulated epoxygenase activity has been postulated to play a role in the chronic adaptation of animals to elevations in salt intake by enhancing sodium excretion.

Renal CYP450 4A activity is increased by mineralocorticoids, glucocorticoids, and progesterone [2]. Antilipidaemic agents like clofibrate induce the expression of CYP450 4A1 and 4A3 protein and increase the formation of 20-HETE in the kidney. A variety of vasoconstrictor agents, including Ang II, norepinephrine, vasopressin, and endothelin, stimulate phospholipases and increase 20-HETE formation in the kidney and peripheral vasculature [4]. In contrast, NO inhibits the formation of EETs and 20-HETE [16].

The expression of CYP450 4A enzymes in the kidney is altered in diabetes, pregnancy, hepatorenal syndrome, cyclosporin-induced nephrotoxicity, alcohol-induced liver disease, and in various animal models of hypertension [2]. However, the role that 20-HETE plays in mediating the changes in renal function associated with these conditions has not yet been defined. In rats, enzymes of the CYP450 4A and 2C families exhibit sexual dimorphism [17], but the physiological significance of this observation remains unknown.

CYP450 metabolites of arachidonic acid and the control of renovascular tone

Both EETs and 20-HETE are produced by renal arterioles when incubated with exogenous AA [2].
EETs are formed by vascular endothelial cells and are potent vasodilators of renal arterioles. EETs exert their vasodilatory effect by increasing the open-state probability of large conductance K_{Ca} channels, which hyperpolarizes vascular smooth muscle (VSM) cells [1,2]. As EETs are released by endothelial cells and activate K_{Ca} channels, they have been proposed to be an endothelial dependent hyperpolarizing factor (EDHF) that mediates the vasodilatory effects of acetylcholine and bradykinin following blockade of NO synthesis. 20-HETE is produced by VSM cells and is a potent vasoconstrictor that contributes to the autoregulation of renal blood flow and renal vascular tone. 20-HETE promotes calcium influx by depolarizing renal VSM cells secondary to blockade of K_{Ca} channels and by increasing the conductance of L-type Ca^{2+} channels [1]. There is also convincing evidence that 20-HETE plays an important role in the regulation of renal vascular tone. In this regard, inhibitors of 20-HETE formation block autoregulation of renal blood flow in vivo and attenuate the renal vasoconstrictor responses to vasopressin, Ang II, endothelin, and norepinephrine both in vivo and in vitro [2].

20-HETE also participates as a mediator or modulator of TGF responses in the kidney. The enzyme responsible for 20-HETE formation is expressed in both the macula densa and afferent arteriole [2] and 20-HETE constricts afferent arterioles. Addition of AA to fluid perfusing the loop of Henle potentiates TGF responses and CYP450 inhibitors block TGF responses [2]. Thus, available evidence supports the view that 20-HETE released by the macula densa may serve as a second messenger at the level of the afferent arteriole by transducing the vasoconstrictor response for some other macula densa-derived mediator.

**20-HETE–NO interaction**

Tonic NO release opposes renal vasoconstriction and TGF responses. Originally, the renal vasodilatory effects of NO were attributed to increased cGMP levels; however, there is now convincing evidence that NO inhibits the formation of 20-HETE in renal arterioles by forming an iron–nitrosyl complex at the catalytic haeme binding site of the CYP450 4A enzymes [2]. Recent studies indicate that NO selectively activates K_{Ca} channels in renal VSM and dilates renal arterioles via a cGMP-independent mechanism [18]. The effects of NO on K_{Ca} channels and vascular tone were prevented when 20-HETE was included in the bath solution to prevent the decrease in 20-HETE formation [18]. Collectively, these results suggest that a fall in 20-HETE formation contributes to the renal vasodilatory response to NO and that this cGMP-independent component is a result of activation of K_{Ca} channels in VSM cells.

**CYP450 metabolites of arachidonic acid and regulation of sodium transport**

CYP450 metabolites of AA play an important role in the regulation of sodium transport in the nephron. EETs and 20-HETE are produced in PT cells and 20-HETE inhibits Na^{+}/K^{+}-ATPase activity by stimulating PKC-induced phosphorylation of the z-subunit of Na^{+}/K^{+}-ATPase [19]. Other studies indicate that the inhibitory effects of dopamine and PTH on Na^{+}/K^{+}-ATPase activity and sodium transport in the PT involves activation of phospholipase A_{2} and an increase in 20-HETE levels [19]. There is also evidence that inhibitors of CYP450 metabolism prevent the effect of Ang II on sodium transport in the PT and that this is associated with augmented 5,6-EET formation which may affect translocation of Na^{+}/H^{+} exchangers to the apical membrane of PT cells [7]. In addition to effects in the PT, 20-HETE is the major metabolite of AA produced in TALH cells where it inhibits reabsorption of sodium and chloride [2].

**CYP450 metabolites of arachidonic acid and hypertension**

Given the important role of 20-HETE and EETs in the regulation of renal function and vascular tone, considerable attention has been focused on defining the role that CYP450 metabolites of AA play in the pathogenesis of hypertension. However, it is difficult to predict how changes in the metabolism of AA by CYP450 enzymes may affect blood pressure as this pathway has both pro- and anti-hypertensive properties. In the renal and peripheral vasculature, 20-HETE is a potent vasoconstrictor and has pro-hypertensive properties. EETs, on the other hand, are anti-hypertensive since they are potent vasodilators. At the level of the renal tubule, both 20-HETE and EETs inhibit sodium reabsorption, increase sodium excretion, and oppose the development of hypertension. However, 20-HETE elevates vascular tone in the afferent arterioles and augments TGF responses, which would lower glomerular filtration rate (GFR), promote volume retention, and elevate blood pressure.

Inhibitors of CYP450 reduce blood pressure and improve renal function in SHR, DOCA-salt, Ang II-induced, and Lyon hypertensive rats suggesting that enhanced CYP450 metabolism of AA may be pro-hypertensive in these models. In the SHR, the CYP450 4A2 gene is preferentially overexpressed in the kidney [11], renal production of 20-HETE is elevated [6,20], and agents that inhibit 20-HETE formation attenuate the development of hypertension [20,21]. 20-HETE may reset the pressure-natriuresis relationship in SHR by elevating renal vascular tone and enhancing TGF responses [22]. In DOCA-salt rats and Ang II-induced models of hypertension, CYP450 inhibitors also prevent the development of hypertension [2,23]. Finally, in Lyon hypertensive rats CYP450 inhibitors enhance...
pressure-natriuresis, reduce renal vascular tone, and increase renal blood flow and GFR [2]. These results suggest a role for CYP450 metabolites of AA in elevating renal vascular tone in this model of hypertension.

In contrast, the metabolism of AA by CYP450 enzymes is reduced in the Dahl salt-sensitive (Dahl S) model of hypertension [2]. The CYP450 4A2 genotype co-segregates with blood pressure in an F2 population of rats derived from a cross of Dahl S and Lewis rats [2]. The formation of 20-HETE and expression of CYP450 4A protein are reduced in the outer medulla of Dahl S rats [2]. Recent studies indicate that a deficiency in the formation of 20-HETE in the TALH contributes to elevation in chloride reabsorption in the loop of Henle of the Dahl S rat and the development of hypertension [2]. Induction of 20-HETE synthesis with fibrates improves pressure-natriuresis and lowers blood pressure in Dahl S rats [2]. Collectively, the available evidence suggests that the renal production of CYP450 metabolites of AA is altered in genetic and experimental models of hypertension and that this system contributes to the resetting of pressure-natriuresis and the development of hypertension in some models.

References

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