Roles of the cytochrome P450 arachidonic acid monooxygenases in the control of systemic blood pressure and experimental hypertension

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Studies of the cytochrome P450 arachidonic acid (AA) monooxygenase, now established as a major pathway for the bioactivation of this physiological important fatty acid, have uncovered new and important roles for this enzyme system in the regulation of kidney function, including renal hemodynamics and tubular ion transport. Associations between genetically controlled alterations in blood pressure and the activity and/or transcriptional regulation of the kidney Cyp2c AA epoxygenases and Cyp4a ω-hydroxylases revealed a role for these enzymes in the pathophysiology of hypertension, a leading cause of cardiovascular, cerebral, and renal morbidity and mortality. Furthermore, analysis of associations between genetic variants of human CYP4A11 and hypertension suggest a potential role for this gene as a determinant of polygenic blood pressure control in humans. These results are providing conceptually novel approaches for studies of the molecular basis of human hypertension that could lead to new strategies for the early diagnosis and clinical management of this devastating disease.

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Cytochrome P450s (P450) are best known for their roles in the metabolism of toxic chemicals, drugs, and endogenous substrates, such as steroids and cholesterol.[1,2] More recently, evidence has accumulated suggesting a functional role for these enzymes as participants in the arachidonic acid (AA) metabolic cascade.[3–9] P450s belong to a complex superfamily of genes with a common evolutionary origin, centered around a conserved peptide that provides them with a cysteine heme ligand and the ability to deliver an active form of atomic oxygen to ground state carbons.[1,2,6,10] Several mammalian P450s are expressed in a gender-, age-, and organ-specific manner, and their levels are regulated by hormones, cytokines, diet, fasting, drugs, or are altered during the course of diseases, such as obesity, diabetes, and hypertension.[11–13] Based on nucleotide sequence identity, P450s are organized into gene families ($\geqslant$40% identity) and subfamilies ($\geqslant$60% identity), with approximately 57 genes identified in the human genome.[10] The biochemical, biophysical, and structural properties of these enzymes, as well as their pharmacological and toxicological roles have been extensively documented.[1,2,10] In contrast, our knowledge of the physiological and/or pathophysiological significance of these proteins is still limited. Ongoing studies of the roles of P450 in AA bioactivation in water and electrolyte homeostasis and systemic blood pressure control are providing new avenues for the definition of their functional significance and roles in renal physiology and pathophysiology.

The P450 *AA monooxygenase* catalyzes the nicotinamide adenine dinucleotide phosphate-dependent oxidation of the fatty acid to four regioisomeric epoxyeicosatrienoic acids (EETs)(5,6-, 8,9-, 11,12-, and 14,15-EET)(*AA epoxygenase branch*) and/or 19- and 20-hydroxyeicosatetraenoic acids (19- and 20-HETE)(*AA ω hydroxylase branch*)[3,6,7] (Figure 1). The identification of EETs and 20-HETE, as products of the *in vivo* metabolism of AA by rodent and human tissues,[3,4,6,7] established the AA monooxygenase as a formal metabolic pathway, and suggested a biological role for its metabolites.[3–9] After a slow start, the list of the citations dealing with different aspects of the AA monooxygenase system has grown significantly, with nearly 1200 PubMed (http://www.ncbi.

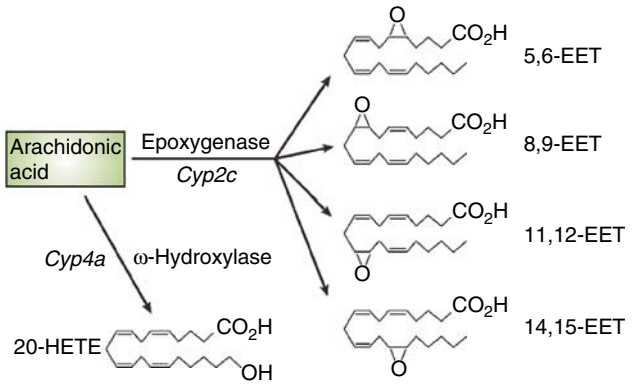

Figure 1 | Reactions catalyzed by cytochrome P450 AA monooxygenases.

nlm.nih.gov) citations in the last 10 years. Efforts to identify the P450 isoforms responsible for these reactions were complicated by the complexity of the enzyme system in which, different proteins can share significant sequence homology, metabolize AA to similar products, and, in many cases, show common immunological determinants.[3,6,10] It is now accepted that members of the *CYP2C* and *CYP4A* gene subfamilies are the predominant and functionally relevant kidney AA epoxygenases and ω-hydroxylases, respectively[3,4,6–9] (Figure 1). However, roles for CYP2J and CYP4F isoforms as AA epoxygenase and ω-hydroxylases, respectively, have been reported.[14,15]

The synthesis of most P450 AA metabolites facilitated their functional analysis, and generated an extensive list of *in vitro* biological activities suggestive of a functional role for the P450s involved in their biosynthesis.[4,5,8,9] Many of these studies have targeted the kidney since: (a) AA and its metabolites have long documented roles in renal function; (b) the early reports of the effects of EETs and 20-HETE on distal sodium reabsorption and the activity of the kidney Na^+/K^+-ATPase, respectively,[3–6,8] were suggestive of an involvement in blood pressure regulation;[4] (c) the organ shows high CYP4A and CYP2C expression, and *in vivo* EET and 20-HETE biosynthesis;[3–6] (d) kidney offered an opportunity to correlate P450 expression with the nephron functional segmentation;[4,5] and (e) available animal models of kidney dysfunction permitted associations between renal function and P450 expression and/or enzymatic activity.[3–6,8] It was this type of an approach that led John McGiff and collaborators to suggest a role for the renal AA monooxygenase in the pathophysiology of experimental hypertension.[4,16] This original proposal opened new opportunities to study the physiological and/or pathophysiological significance of the P450 AA pathway, and has served as a powerful stimuli for some of the most important advances in this area. From insightful integrations of the functional responses to P450 metabolites and correlations between their biosynthesis, the expression of CYP4A and CYP2C isoforms and blood pressure changes in rodent models of genetically controlled hypertension, pro- and anti-hypertensive roles were proposed for the products of the AA ω-hydroxylase and epoxygenase, respectively.[4,16] Notwithstanding the clinical importance of hypertension, this proposal offered novel approaches for studies of the molecular basis of this disease, and it identified kidney dysfunction and blood pressure as distinct end point phenotypes for studies of the functional consequences of genetic and/or experimental manipulations of P450 expression and/or enzyme activity.

PRO- AND ANTI-HYPERTENSIVE ROLES FOR 20-HETE HAVE BEEN IDENTIFIED IN RAT MODELS OF GENETICALLY DETERMINED HYPERTENSION

In spontaneously hypertensive rats (SHR rats), blood pressure increases between 5 and 10 weeks of age while, under identical conditions, comparable (but not isogenic) Wistar–Kyoto rats remain normotensive. Based on: (a) biochemical and temporal correlates of kidney CYP4A AA ω-hydroxylase expression and activity with the development of hypertension in SHR rats;[4,16] (b) the prevention of hypertension in SHR rats by $SnCl_2$-mediated depletion of renal P450s;[4,16] (c) the normotensive effects of $SnCl_2$ administration to hypertensive SHR animals,[4,16] and (d) the functional effects of 20-HETE,[4–6,8,16] a pro-hypertensive role was identified for the CYP4As and 20-HETE.[4,5,8,16] Subsequently, anti-sense nucleotide inhibition of gene expression identified *CYP4A1* and *4A2* as the pro-hypertensive 20-HETE synthases in the rat kidney.[17] In agreement with its proposed pro-hypertensive action, 20-HETE was shown to be a potent, *in vitro*, renal vasoconstrictor,[4,5,8] and to inactivate smooth muscle calcium-sensitive K^+ channels.[5] Furthermore, 20-HETE is formed by vascular smooth muscle cells,[18] and it potentiates the activity of vasoconstrictors, such as angiotensin II, endothelin, and serotonin.[18] In contrast, studies with the Dahl rat model of salt-sensitive hypertension identified an anti-hypertensive role for 20-HETE and CYP4A2 based on: (a) the *in vitro* natriuretic properties of 20-HETE;[5] (b) CYP4A inhibitor studies;[5] (c) differences between Dahl salt-sensitive (DS) and salt-resistant rats (DR) in the expression of CYP4A2 and 20-HETE biosynthesis;[5] and (d) normalization of Cl^- transport in DS rats by 20-HETE.[5] Furthermore, CYP4A inducers have normotensive effects on DS rats and on sexually immature Sprague–Dawley rats.[5,19] This apparent duality in CYP4A2 and 20-HETE functional role illustrates the importance that the nephron segment-specific expression of selected P450 isoforms is likely a determinant of physiological and/or pathophysiology significance. Lastly, roles for angiotensin II on renal AA metabolism and EET and 20-HETE biosynthesis, as well as for these eicosanoids in the renal and vascular effects of the hormone have been reported.[5,20–24]

ANTI-HYPERTENSIVE ROLE FOR THE EETs HAS BEEN IDENTIFIED IN A RAT MODEL OF SALT-SENSITIVE HYPERTENSION

The inhibition of distal sodium reabsorption and potassium excretion by EETs,[25] and the upregulation of the rat kidney CYP2C23 epoxygenase expression and urinary EET excretion

by excess dietary salt intake[26] pointed to an anti-hypertensive role for the AA epoxygenase and the EETs.[3–8] Furthermore: (a) inhibition of the rat kidney epoxygenase reduces urinary EET levels, and induces salt-sensitive hypertension;[27] and (b) on a high salt diet, Dahl salt-resistant rats increase their renal EET biosynthesis and remained normotensive, whereas, under similar conditions, salt-loaded DS rats show comparably lower EET biosynthetic activity, and develop severe salt-sensitive hypertension.[27] In contradistinction to 20-HETE[4,5,8] and in support of their anti-hypertensive roles, the EETs were shown to participate in the bradykinin-induced, endothelium-dependent dilation of the afferent arteriole.[28] The EETs activate vascular smooth muscle calcium-sensitive K^+ channels,[29,30] and 11,12-EET has been identified as an endothelium-derived hyperpolarizing factor (EDHF).[9,29,30] Moreover, EET synthesis and release from endothelial cells is stimulated by vasodilators, such as methacholine and bradykinin.[30] Among the EETs, 11,12-EET is the most active renal vasodilatory epoxide, and the predominant regioisomer generated by a rat CYP2C23 present in isolated renal microvessels.[28] These facts underscored some of the anti-hypertensive properties identified for the CYP2C epoxygenase pathway, as well as their relevance to the kidney responses to changes in dietary salt intake.

Studies with the SHR/Wistar–Kyoto and Dahl salt-resistant/DS rat models of genetic hypertension yielded several important correlations between selected AA epoxygenase and ω-hydrolase isoforms, renal function, and systemic blood pressure.[3–5,8,16] However, the circumstantial nature of these associations, the questionable selectivity of many of the inhibitor approaches reported,[3–5,16,27] and the complexity and multi-genetic nature of the animal models studied precluded unequivocal interpretations of the accumulated data and thus, the role(s) of P450 in blood pressure regulation remained uncertain, and mostly unrecognized. On the other hand, advances in gene-targeting techniques have facilitated their application and, in the last few years, gene disruption has become a method of choice for studies of gene-determined phenotypes and the analysis of physiological roles.[31–33] Although not without some limitations, the unsurpassed selectivity of these methods has contributed to the uncovering of the function, mechanism of action, and the pathophysiological roles of an increasing number of genes and encoded proteins.

Based on the extensive list of biological activities attributed to 20-HETE, including pro-hypertensive properties,[4,5,8,16] the limited number of murine Cyp4a isoforms,[10,34] and the roles reported for the CYP4A 20-HETE synthases in SHR hypertension,[4,16] the murine *Cyp4a* gene subfamily was selected to initiate the development of monogenic models of P450 dysfunction. The mammalian CYP4A proteins show high degrees of sequence and functional evolutionary conservation, and the rat and mouse proteins share extensive sequence identity (Figure 2).[10] The murine *Cyp4a* gene subfamily is composed of three highly homologous genes, *Cyp4a10*, *Cyp4a12*, and *Cyp4a14* (Figure 2),[10,34] and two additional pseudo-genes (not transcribed or coding for non-functional proteins).[10] All three *Cyp4a* genes are expressed in the male kidney, although Cyp4a12 is nearly undetectable in the female kidney.[34,35] Studies with recombinant proteins showed that Cyp4a12 is an active AA ω-hydroxylase, Cyp4a10 shows a substantially lower activity, and Cyp4a14 is inactive.[35–37] Prior publications also demonstrated the initial phenotype characterization of the knockout mice by disruption of the *Cyp4a10* and *Cyp4a14* genes by non-homologous recombination with targeting vectors in which segments of the genes coding sequences were deleted.[35,36] It is of interest that both *Cyp4a14*(−/−) and *Cyp4a10*(−/−) mice are hypertensive, although the mechanisms involved in their hypertensive phenotypes are quite different.[35,36] Paradoxically, in both these models, the targeted genes are not directly responsible for the hypertensive phenotypes but rather, they control the expression of surrogate pro- or anti-hypertensive P450 genes.[35,36] Moreover, these studies further illustrated the importance that organ/tissue-specific expression and transcriptional regulation have as key determinants of the physiological and/or pathophysiological roles played by AA epoxygenases and ω-hydroxylase and their metabolites.

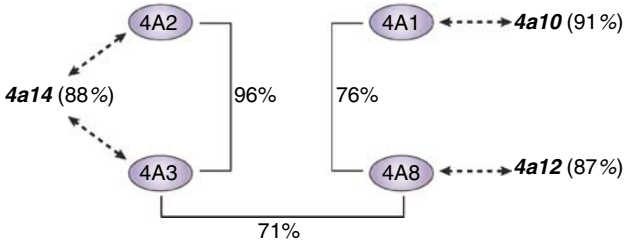

Figure 2 | Amino-acid sequence identities between members of the rat and mouse CYP4A gene subfamilies. As detailed in Nebert *et al.*,[10] gene subfamilies names are capitalized, except for the mouse subfamilies.

DISRUPTION OF THE MURINE Cyp4a14 GENE CAUSES SEXUALLY DIMORPHIC, ANDROGEN-SENSITIVE HYPERTENSION

Phenotypic analysis of *Cyp4a14*(−/−) mice showed that adult male mice were hypertensive (Figure 3a), whereas comparable females showed a less severe phenotype (mean arterial blood pressures of 145 ± 3 and 115 ± 2 for male and female Cyp4a14(−/−) mice, respectively. $n \geqslant 20$).[35] These earlier results provided direct evidence of a role for the P450 enzymes in the regulation of systemic blood pressure.[35] The sexual dimorphic nature of this phenotype suggested a role for sex hormones in *Cyp4a14*(−/−) hypertension, a suggestion confirmed after castration and/or androgen administration.[35] Castration lowered blood pressure of male *Cyp4a14*(−/−) mice (Figure 3a),[35] and testosterone or 5α-dihydroxy-testosterone administration restored the hypertensive phenotype of castrated *Cyp4a14*(−/−) mice.[35] Importantly, these androgens raised blood pressure, regardless of mouse gender or *Cyp4a14* genotype,[35] and had similar, pro-hypertensive, effects when administered to rats.[38] A more detailed study of *Cyp4a14* (−/−) mice showed that the gene disruption raised male plasma androgens levels, upregulated kidney expression of the

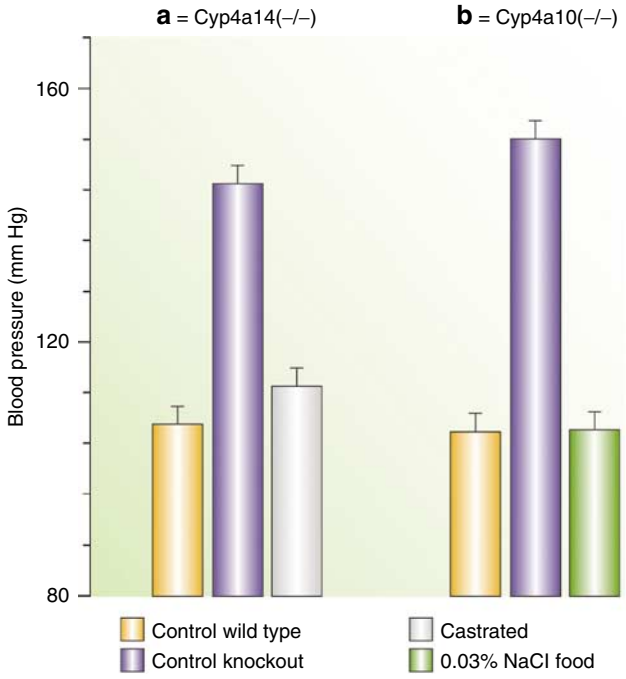

Figure 3 | The lack of a functional Cyp4a10 or Cyp4a14 gene raises systemic blood pressures in mice. (**a**) The mean arterial blood pressures of conscious wild-type and of non-castrated *Cyp4a14*(−/−) (control) and castrated *Cyp4a14*(−/−) adult male knockout mice were measured by means of a carotid artery catheter exactly as described in Holla *et al.*[35] The blood pressures of wild-type and *Cyp4a14*(−/−) mice were significantly different ($P<1\times10^{-4}$), whereas those of wild-type and castrated *Cyp4a14*(−/−) mice were not. Values are averages calculated from measurement carried out with 38 wild-type, 40 *Cyp4a14*(−/−), and 16 castrated *Cyp4a14*(−/−) mice ±s.e.m. (**b**) The systolic blood pressures of conscious wild-type and *Cyp4a10*(−/−) adult male mice fed diets containing either 0.3% (normal salt) or 0.05% NaCl (low salt) for 3 weeks were measured by the tail cuff method exactly as described in Imig *et al.*[28] Values are averages calculated from measurements carried out on at least 15 wild-type (control wild type) and 15 *Cyp4a14*(−/−) (control knockout) mice on normal salt, and 14 *Cyp4a10*(−/−) mice on low salt ±s.e.m. The blood pressures of control wild type and control *Cyp4a10*(−/−) mice were significantly different ($P<1\times10^{-3}$), whereas those of control wild type and *Cyp4a10*(−/−) mice on low salt diets were not. (Data from Holla *et al.*[35] and Nakagawa *et al.*[36])

Cyp4a12 AA ω-hydroxylase, and increased the renal biosynthesis of pro-hypertensive 20-HETE.[35] On the other hand, the normotensive effects of castration on the *Cyp4a14*(−/−) mice were accompanied by reductions in kidney Cyp4a12 expression and 20-HETE biosynthetic capacity.[35] Conversely, the pro-hypertensive effects of androgen administration to wild-type male or female mice were associated with increases in kidney Cyp4a12 expression and 20-HETE biosynthesis.[35] Importantly, and in analogy to *Cyp4a14(−/−)* mice, castration reduces by approximately 50 mm Hg the blood pressure of hypertensive SHR rats,[39] and hypertension is also more severe in male SHR rats.[40] These similarities between the androgen-sensitive components of SHR hypertension and that of *Cyp4a14*(−/−) mouse supports the original proposal that the CYP4A P450s contribute to the development of high blood pressure in adult SHR rats.[4,16]

An important subset of human hypertension is sexual dimorphism, that is, more severe in males,[41–43] and the involvement of an altered renal microvasculature in human hypertension has been reported.[41,44] As mentioned, 20-HETE is a powerful vasoconstrictor,[4,5,8] and its effects on renovascular tone served as a basis for its proposed pro-hypertensive role.[4,5,8] Functional analysis showed that male *Cyp4a14*(−/−) mice have an impaired renal microvascular autoregulatory efficiency and increased renal vascular resistance, presumably due to increased 20-HETE biosynthesis.[35] Impaired afferent arteriolar autoregulatory capacity and increased renal vascular resistance have been demonstrated in a number of hypertensive animal models.[45–47] Thus, an increased afferent arteriole resistance may compromise the excretory capacity of the *Cyp4a14*(−/−) kidney and be the determinant factor of their systemic high blood pressure. In summary, Cyp4a14-dependent, androgen-mediated, transcriptional and hemodynamic components are at the center of the hypertensive phenotype of *Cyp4a14*(−/−) mice.

DISRUPTION OF THE MURINE Cyp4a10 GENE CAUSES DIETARY SALT-SENSITIVE HYPERTENSION

Measurements of blood pressure showed that, at difference with the *Cyp4a14*(−/−) animals, adult male and female *Cyp4a10*(−/−) mice were equally hypertensive,[36] and that lack of a functional *Cyp4a10* gene had no effect on plasma androgens.[36] Furthermore, an analysis of kidney Cyp4a12 expression and AA ω-hydroxylase activity showed that hypertensive *Cyp4a10*(−/−) and normotensive *Cyp4a10*(+/+) mice had similar 20-HETE biosynthetic activities.[36] These results demonstrated that *Cyp4a10*(−/−) hypertension was unrelated to changes in overall renal 20-HETE biosynthesis and the hemodynamic effects of this metabolite.[36] Paradoxically, mass spectral quantifications showed that the levels of urinary epoxygenase metabolites in the *Cyp4a10*(−/−) mice were lower than those in wild-type mice, pointing to a reduced epoxygenase activity in the *Cyp4a10*(−/−) kidney.[36] A role for dietary salt in *Cyp4a10*(−/−) hypertension was identified after feeding *Cyp4a10*(+/+) and *Cyp4a10*(−/−) mice diets containing high and low salt (either 8 or 0.05% NaCl, w/w, respectively).[36] As reported, *Cyp4a10*(−/−) mice on a high salt diet become severely hypertensive,[36] whereas, as shown in Figure 3b, those on a low salt diet become normotensive.[36] Reduced urine volumes and sodium excretion, as well as an increased free fluid volume, suggested impairments in sodium excretion and compensatory increases in plasma volume as potential causative agents for the *Cyp4a10*(−/−) salt-sensitive hypertensive phenotype.[36]

HYPERTENSIVE Cyp4a10(−/−) MICE SHOW INCREASES IN THE GATING ACTIVITY OF THE RENAL EPITHELIAL SODIUM CHANNEL

The decreased levels of urinary epoxygenase metabolites and the salt-sensitive nature of the animal's blood pressure, suggested a potential role for the EETs in *Cyp4a10*(−/−)

hypertension. Previous studies showing that the luminal addition of EETs to perfused rabbit cortical collecting ducts (CDs) reduces sodium reabsorption and potassium excretion,[25] pointed to the CD epithelial sodium channel (ENaC) as a target for a putative EET natriuretic, anti-hypertensive, role. This hypothesis was corroborated by electrophysiology studies using dissected rat CDs and showing that: (a) AA and 11,12-EET inhibit ENaC activity;[48] (b) the AA-dependent inhibition of ENaC requires metabolism to 11,12-EET;[48] and (c) the CYP2C23 epoxygenase is expressed in the rat CD, and this segment makes 11,12-EET.[48] The effects of the epoxygenase metabolite on ENaC gating were unique, as indomethacin, a cyclooxygenase inhibitor, had no effects on the AA elicited responses, nor did 20-HETE mimic the EET effect.[48] Although its contribution to overall tubular sodium reabsorption is limited, the CD participates in the hormonal-mediated fine-tuning of plasma and interstitial sodium concentrations, and ENaC is the rate limiting step in this physiologically important function.[49,50] The importance of ENaC in the control of systemic blood pressure is demonstrated by human disorders in which gain of function mutations in this channel cause severe hypertension.[49–52]

Three important pieces of evidence identify a role for the *Cyp4a10* gene and the EETs in the *in vivo* regulation of ENaC gating activity, distal sodium reabsorption, and the control of systemic blood pressures. Firstly, patch clamp studies of CDs from *Cyp4a10*(+/+) and *Cyp4a10*(−/−) mice showed that a disrupted *Cyp4a10* gene causes constitutive ENaC activation and increases inward sodium currents.[36] This is similar to what is observed in Liddle's syndrome, a Mendelian form of human hypertension in which a hyperactive ENaC increases sodium reabsorption and causes hypertension.[49,51,52] Only in CDs from *Cyp4a10*(−/−) mice, does the addition of AA have no effect on ENaC activity, suggesting that in these animals the CD has a reduced 11,12-EET synthase capacity.[36] Importantly, 11,12-EET inhibited ENaC activity, regardless of mouse genotype,[36] confirming that disruption of the *Cyp4a10* gene does not alter ENaC intrinsic properties but, rather, the regulation of its gating activity.[36] As mentioned, *Cyp4a10*(−/−) show reductions in urinary EETs,[36] however, although EET urine levels give an estimate of their overall kidney biosynthesis, they do not provide information regarding their segmental biosynthesis or origin. Technical and mass spectrometry sensitivity issues prevents a direct quantification of EETs present in mouse CDs. Secondly, ligands for the peroxisomal proliferator-activated-α nuclear receptor have been reported to upregulate the expression, of the CYP2C23 AA epoxygenase in the rat kidney,[24] a functional and structural homolog of the murine Cyp2c44 epoxygenase.[10,53] Treatment of hypertensive *Cyp4a10*(−/−) mice with Wyeth 14,643, a selective peroxisomal proliferator-activated-α ligand,[54] upregulates renal Cyp2c44 expression, increases urine EET concentrations and normalizes the animals systemic blood pressures.[36] Thus, the hypertensive phenotype of *Cyp4a10*(−/−) mice could be rescued experimentally by inducing the Cyp2c44 AA epoxygenase and raising renal EET biosynthesis *in vivo*.[36] These associations between genetically controlled and/or experimentally induced changes in epoxygenase expression and activity, and systemic blood pressure support a role for EETs, as anti-hypertensive mediators[3–6,8,36] demonstrate that P450 participates in blood pressure regulation[36] and points to the EETs as endogenous regulators of ENaC activity.[36,48] Thirdly, amiloride is a powerful and selective inhibitor of ENaC activity and its administration reduces CD sodium reabsorption and potassium excretion.[55] Within a few days of administration, amiloride normalized the blood pressures of hypertensive *Cyp4a10*(−/−) mice to levels comparable with those of wild-type animals (Figure 4).[36] Furthermore, *Cyp4a10*(−/−) mice on amiloride remained normotensive even after the administration of 2% NaCl in their drinking water (Figure 4),[36] and the blood pressure effects of amiloride were reversible, since upon its removal, the hypertensive phenotype of the *Cyp4a10*(−/−) animals was restored (Figure 4).[36] In summary, these results identified ENaC as the molecular target of the hypertensive

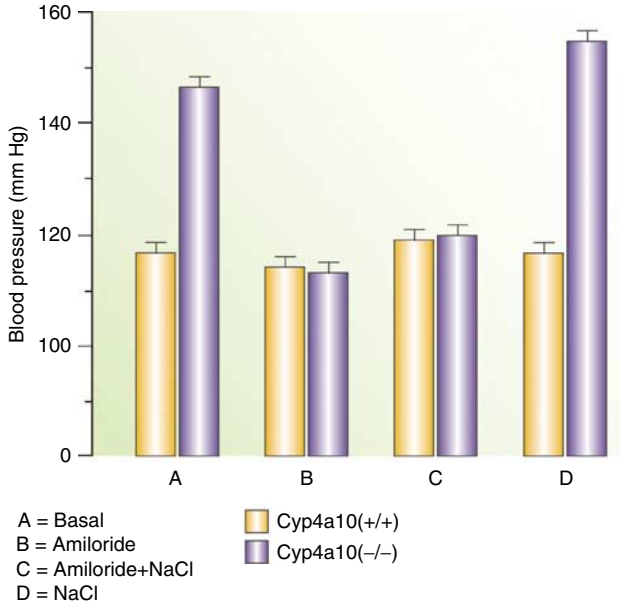

Figure 4 | Amiloride normalizes the systolic blood pressures of Cyp4a10(−/−) knockout mice. Groups of adult male wild-type and *Cyp4a10*(−/−) mice were fed standard mouse chow, and allowed free access to water (days 1–4), or to water containing either amiloride (50 μg/ml) (days 8–21), a mixture of amiloride (50 μg/ml) and NaCl (2% w/v) (days 22–28), or just NaCl (2% w/v) (days 29–34). The animal's systolic blood pressures were measured as in Nakagawa *et al*.[36] There were no significant changes in the blood pressure of wild-type mice during the different treatment regimes, nor were there differences between the wild-type and *Cyp4a10*(−/−) mice treated with amiloride, or amiloride in 2% NaCl. The blood pressures of the *Cyp4a10*(−/−) mice drinking water or a 2% solution of NaCl were significantly different to those of wild-type animals under similar treatment regimes ($P < 1 \times 10^{-3}$). The blood pressures of *Cyp4a10*(−/−) mice drinking a solution of amiloride were significantly different to that of *Cyp4a10*(−/−) mice drinking water ($P \leqslant \times 10^{-3}$). Shown are averages of daily blood pressure measurements carried out on at least five wild-type and five *Cyp4a10*(−/−) animals ± s.e.m. (Data from Nakagawa *et al*.[36])

phenotype brought about by the disruption of the *Cyp4a10* gene and demonstrated that altered tubular transport is the causative agent of the *Cyp4a10*(−/−) hypertensive phenotype.[36] Importantly, the documented ability of 11,12-EET to regulate ENaC gating in real time[36,48] indicates that this eicosanoid may function as an endogenous natriuretic agent.

Abundant functional data, and the characterization of *Cyp4a14*(−/−) and *Cyp4a10*(−/−) mice as hypertensive have identified a key physiological role for these P450s in the regulation of renal hemodynamics and tubular sodium transport, and in the pathophysiology of androgen and salt-sensitive hypertension, respectively.[35,36] Additionally, these studies identify members of the human *CYP4A* and *CYP2C* gene subfamilies, as candidate genes for studies of the molecular basis of human hypertension. The human genome contains two highly homologous *CYP4A* genes, that is, *CYP4A22* and *CYP4A11*,[8,10] and four *CYP2C* genes, that is, *CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*.[10] *CYP4A11* codes for an active kidney 20-HETE synthase,[56] *CYP4A22* codes for a non-functional protein, and is undetectable in kidney,[56] and CYP2C8 has been identified as a renal AA epoxygenase.[57] Importantly, two independent studies have associated a functional variant of the human *CYP4A11* gene with essential hypertension[58,59] and altered 20-HETE-associated natriuresis has been linked to human salt-sensitive hypertension.[60] Thus, genetic rat models of hypertension, genetic targeting of P450 in mice, and human studies point to the importance of AA P450 metabolites in water and electrolyte homeostasis and blood pressure regulation.

With few exceptions, the molecular bases of the most common forms of human hypertension are yet to be defined and thus, the early diagnosis and clinical management of this devastating disease remains challenging and mostly symptomatic. This situation reflects the complexity of a disease in which multiple environmental and genetic factors, as well as coexisting conditions may contribute to a multifaceted etiology. The studies summarized above identify a conceptually novel approach for studies of the molecular basis of human hypertension, and provide an experimental foundation for future clinical studies of the roles of these enzymes in human hypertension. Indeed, the efforts of several research groups are changing long-held views regarding the roles that P450s play in the metabolism of endogenous substrates and the regulation of cell, organ, and body physiology, *vis-a-vis*, their established roles in the metabolism and detoxification of foreign chemicals and drugs.

CONCLUSION AND FUTURE DIRECTIONS

There is now convincing evidence of a role for the microsomal AA monooxygenase in renal physiology and blood pressure regulation, as well as for them as potential contributors to the pathophysiology of subsets of human hypertension. Two additional properties of the P450-derived eicosanoids[5–7,9,30,61,62] are becoming of great interest. These include potential functional roles of: (a) EETs and 20-HETE as regulators of ion channel activity and, in particular, the physiological significance of their effects on vascular Ca^{++} activated K^+ channels,[5,30] and roles as endothelium-derived hyperpolarizing factor and anti-endothelium-derived hyperpolarizing factor mediators;[5,30] and (b) EETs as mitogenic and angiogenic mediators,[3,9,61,62] and their use as potential targets for the development of novel anti-angiogenic approaches for cancer therapy. Ongoing efforts in all these areas of interest hold the promise of providing new avenues for the understanding of basic cell, organ, and body physiology. Among the many issues that remain outstanding in this area of research, we include: (a) the characterization of the molecular mechanisms responsible for EET-mediated effects on ion membrane, ion permeability, and ion channel activity; (b) the study of signaling mechanisms for EETs and 20-HETE and the characterization of putative membrane bound receptors for these eicosanoids; (c) the biochemical, molecular, and functional characterization of organ/tissue/cell-specific AA monooxygenases, and of their human homologues; (d) the identification and molecular characterization of the mechanism by which Cyp4a isoforms regulate gene transcription; (e) the identification and molecular characterization of mechanisms that control the expression of AA monooxygenase in a organ/tissue/cell-specific manner; and (e) the analysis of correlations between human pathophysiological conditions and alterations in the genes coding for P450 AA epoxygenases and/or ω-hydroxylases.

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