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Review

Arachidonic acid monooxygenase: Genetic and biochemical approaches to physiological/pathophysiological relevance

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ABSTRACT

Studies with rat genetic models of hypertension pointed to roles for the CYP2C and CYP4A arachidonic acid epoxygenases and ω-hydroxylases in tubular transport, hemodynamics, and blood pressure control. Further progress in defining their physiological functions and significance to human hypertension requires conclusive identifications of the relevant genes and proteins. Here we discuss unequivocal evidence of roles for the murine Cyp4a14, Cyp4a10, and Cyp2c44 genes in the pathophysiology of hypertension by showing that: (a) *Cyp4a14*(−/−) mice develop sexually dimorphic hypertension associated with renal vasoconstriction, and up-regulated expression of Cyp4a12a and pro-hypertensive 20-hydroxyeicosatetraenoic acid (20-HETE) levels, and b) *Cyp4a10*(−/−) and *Cyp2c44*(−/−) mice develop salt sensitive hypertension linked to downregulation or lack of the Cyp2c44 epoxygenase, reductions in anti-hypertensive epoxyeicosatrienoic acids (EETs), and increases in distal sodium reabsorption. Based on these studies, the human *CYP4A11* and *CYPs 2C8* and *2C9* genes and their products are identified as potential candidates for studies of the molecular basis of human hypertension.

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Cytochrome P450 (P450) hemeproteins belong to a complex gene superfamily expressed from bacteria to man, with approximately 57 genes identified in the human genome. The pharmacological and toxicological importance of mammalian P450s is well established, however, less is known regarding their physiological and/or pathophysiological relevance. The demonstration of roles for microsomal P450s in the metabolism of arachidonic acid (AA) and other polyunsaturated fatty acids suggested functional roles for the enzymes involved in these reactions. The subsequent characterization and chemical synthesis of most of the products generated from AA metabolism by the P450 enzymes led to the identification of the important biological activities associated

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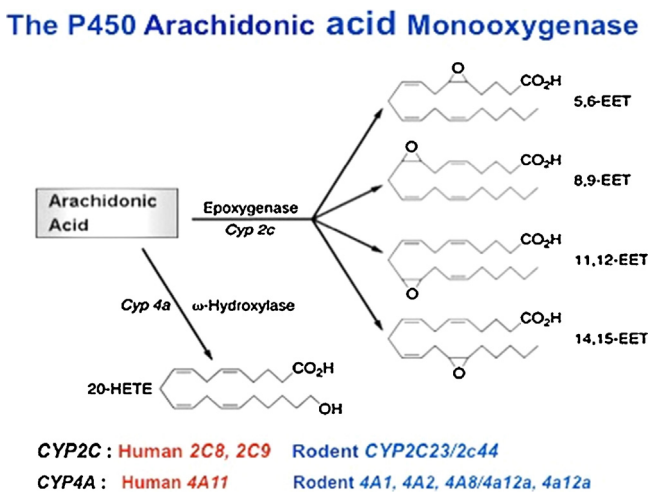

Fig. 1. The arachidonic acid monooxygenase and its epoxygenase and ω-hydroxylase branches. The scheme emphasizes members of the murine Cyp2c and Cyp4a gene subfamilies as functionally relevant kidney epoxygenases and ω-hydroxylases. Human and rodent CYP2C and CYP4A isoforms known to catalyze renal AA metabolism are indicated at the bottom.

with several of them [1–4]. These discoveries opened new avenues for studies of the physiological/pathophysiological relevance of P450 and roles in diseases such as hypertension, cancer, and inflammation [5–7].

The P450 monooxygenase metabolizes AA primarily to: (a) four regioisomeric epoxyeicosatrienoic acids (EETs) (*Epoxygenase*), and/or (b) 19- and 20-hydroxyeicosatetraenoic acids (19- and 20-HETE) (*ω/ω-1 hydroxylase*), with members of the *CYP2* and *CYP4* gene subfamilies identified as the predominant epoxygenases and ω-hydroxylases, respectively, in most rodent and human tissues [1] (Fig. 1).

The enzymatic hydration of 8,9-, 11,12-, and 14,15-EET to dihydroxyeicosatrienoic acids (8,9-, 11,12-, and 8,9-DHET) was shown to be predominantly catalyzed by soluble (cytosolic) epoxide hydrolase (sEH) in 1983 [8]. Subsequently, roles for sHE in the *in vivo* hydration of EET were proposed based on its steroselectivity for the EET enantiomers found endogenously in organ tissues [9]. Since then, extensive inhibitor studies characterized sHE as a key regulator of EET organ levels and functional responses, as well as target for drug development (reviewed in references [10,11]).

The identification of EETs and 20-HETE as components of human and rodent organs, urine, and plasma established the epoxygenase and ω-hydroxylase branches of the AA Monooxygenase as formal metabolic pathways (Fig. 1), and suggested that their metabolites were functionally relevant [1–7]. While the EETs have been characterized as vasodilator and pro-angiogenic lipids and as mediators of peptide hormonal release and signaling, nociception, and distal sodium excretion [3–6]; 20-HETE has been identified as inhibitor of Na/K-ATPase and proximal tubule transport, and as a potent vasoconstrictor [2,5,7]. Nonetheless, the identification of the epoxygenase and ω-hydroxylase P450 isoforms responsible for the *in vivo* biosynthesis of bioactive metabolites has been complicated by a multiplicity of P450 isoforms that share extensive amino acid sequence homology, metabolize AA to similar products, and often show common immunological determinants. The identification of the functionally significant enzymes is urgently needed to define their physiological contributions, mechanism(s) of action, regulatory control, and genetic properties. Several lines of evidence indicated that members of the *CYP2C* gene subfamily could be responsible for the biosynthesis of functionally important EETs in renal and vascular tissues, including: (a) the characterizations of rat CYP2C23 and its murine homologue, Cyp2c44, as stereo selective epoxygenases and as the predominant epoxygenases in rat and mouse kidney, (b) the identification of renal CYP2C23 and Cyp2c44 as dietary salt regulated epoxygenases, and (c) the demonstration of reduced CYP2C23 expression and EET biosynthesis in the kidneys of hypertensive Dahl salt sensitive rats [2–6,12–15]. Similarly, roles for rat CYP4A and mouse Cyp4a isoforms in the biosynthesis of functionally relevant 20-HETE were indicated by: (a) the documentation of up-regulated renal CYP4A expression and 20-HETE biosynthesis during the onset of hypertension in the SHR/WKY rat model of spontaneous hypertension, (b) differences in CYP4A2 expression and 20-HETE biosynthesis between salt resistant and sensitive Dahl rats (DR and DS genotypes, respectively), and (c) antisense nucleotide inhibition of renal CYP4A1/CYP4A2 expression and normalization of the blood pressures of hypertensive SHR rats [2,5–7,16,17]. Based on the above, as well as their tubular and vascular effects, anti- or pro-hypertensive properties were proposed for EETs and 20-HETE, and their corresponding CYP2C and CYP4A isoforms [16].

The availability of rat models of genetically determined hypertension opened the door to studies of gene-phenotype associations between products of the *CYP2C* and *CYP4A* genes and blood pressure control [2,5–7,16,17]. However, the multi-genic and complex nature of the SHR/WKY and Dahl genetic models of hypertension precluded an unequivocal identification of roles for distinct P450s genes in blood pressure control. The advent of gene targeting techniques and the development of mouse models of monogenic dysfunction allows now studies of the physiological and pathophysiological significance of specific P450 isoforms. To date, mouse lines carrying disrupted copies of the genes coding for *Cyp2c44, Cyp2j5, Cyp4a10, Cyp4a14*, and *Cyp4f4* have been generated and characterized to variable extents [18–22]. There is published evidence for the involvement of *Cyp2j5* in estrogen, but not AA metabolism [19], and for *Cyp4f4* in vitamin E catabolism [21]. We summarize herein studies performed in wild type (WT) and in mice carrying globally disrupted copies of the *Cyp4a10 (4a10 KO), Cyp4a14 (4a14 KO), or Cyp2c44 (2c44 KO)* genes (in isogenic *129SeV* backgrounds) that identify roles for these P450 genes on the control of blood pressures. Although not discussed, recent studies with *2c44 KO* mice also revealed that lack of the Cyp2c44 epoxygenase blunts tumor angiogenesis and growth [23].

1. Roles of the murine Cyp4a genes in blood pressure regulation

The mouse genome contains four *Cyp4a* genes: *Cyp4a10, Cyp4a12a, Cyp4a12b*, and *Cyp4a14* localized in chromosome 4. The *Cyp4a12a* and *Cyp4a12b* genes share extensive intron/exon sequence identity [24] and code for the only mouse Cyp4a proteins with significant 19- and 20-HETE synthase activity (Fig. 1) [18,24]. Cyp4a12a is expressed in male kidneys in an androgen sensitive manner [18,24]. Two highly homologous *CYP4A* genes, *CYP4A11* and *CYP4A22*, are present in chromosome 1 of the human genome (Fig. 1) [25]. While CYP4A11 metabolizes AA to mostly 20-HETE, an abnormal heme prosthetic group in CYP4A22 renders it catalytically inactive [26].

1.1. Cyp4a14*(−/−) mice, a model of androgen sensitive hypertension*

A link between the *Cyp4a14* gene and blood pressure regulation was established by the demonstration that its disruption causes a type of hypertension that is sexually dimorphic, male specific, and associated with: (a) increases in plasma androgens, (b) up-regulated kidney expression of Cyp4a12a, and (c) increased urinary excretion of 20-HETE [18]. Castration lowers renal Cyp4a12a