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P450 SUBFAMILY CYP2J AND THEIR ROLE IN THE BIOACTIVATION OF ARACHIDONIC ACID IN EXTRAHEPATIC TISSUES*

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ABSTRACT

Historically, there has been intense interest in P450 metabolic oxidation, peroxidation, and reduction of xenobiotics. More recently, there has been a growing appreciation for the role of P450s in the oxidation of lipophilic endobiotics, such as bile acids, fat-soluble vitamins, and eicosanoids. This review details the emerging CYP2J subfamily of P450s and their role as catalysts of arachidonic acid metabolism.

I. INTRODUCTION

The P450 superfamily is comprised of heme-thiolate proteins that act as the terminal oxidases in the mixed-function oxidase system [1,2]. Ubiquitous in living organisms, over 750 P450s have been identified so far in bacteria, yeast, plants, and animals [3]. All mammalian P450 activities require NADPH, as well as NADPH:P450 reductase (CPR), which transports electrons from NADPH to the heme-thiolate protein. Historically, toxicologists and pharmacologists have directed intensive investigation of P450 metabolic oxidation, peroxidation, and

reduction of a wide variety of drugs, industrial chemicals, environmental pollutants, and carcinogens [1,4,5]. P450 metabolism of these xenobiotics can result in altered drug efficacy or production of toxic metabolites, some of which have been implicated in the pathogenesis of human diseases, including birth defects and cancer [1,4,5]. More recently, there has been growing appreciation for the role of P450s in the oxidation of lipophilic endobiotics, such as bile acids, fat-soluble vitamins, and eicosanoids [6–9]. Remarkably diverse in substrate preference and catalytic role, as many as 50 different P450 enzymes may exist in a given species, with the complement of the P450 pool varying interindividually, resulting in dramatic variability in pharmacologic metabolism and response and in toxin susceptibility [10]. As information accumulates about the regulation and function of individual P450s and interindividual variation due to genetic polymorphism, the P450 complement should serve as a biomarker for assessing pharmacologic efficacy, potential drug interactions, environmental chemical risk, and tissue/body homeostasis.

According to the recommended systematic nomenclature for the P450 superfamily [1,11], based on primary sequence identity there are currently 14 gene families represented in all mammals, subdivided into 29 subfamilies. Each subfamily is comprised of one or more individual P450 constituents. This review will focus on our expanding knowledge of the CYP2J subfamily. Initial studies have focused on characterizing the different CYP2J enzymes with respect to their structural, catalytic, and regulatory properties, their organ and cellular distribution, and the biological activities of their eicosanoid metabolites. To date, sequence data for six members of the CYP2J subfamily have been entered into respective databanks [1,11,12], with at least four additional members currently being characterized.

A. Regulation and Expression of CYP2J Subfamily

Most previously described mammalian P450s are expressed primarily in liver, and at lower or undetectable levels in extrahepatic tissues [5]. As a result, much of what is known about P450 biochemistry, molecular biology, and toxicology is based on work with the liver enzymes. Some P450s are expressed constitutively at high levels (e.g., CYP2A, CYP2C, and CYP3A subfamily members). Others are inducible by xenobiotics, including polycyclic aromatic hydrocarbons (e.g., CYP1A), barbiturates (e.g., CYP2B), hypolipidemic drugs (e.g., CYP4A), and simple hydrocarbons (e.g., CYP2E) [1,4,5]. Xenochemicals can affect P450 activity by altering transcription, mRNA degradation, translation, and protein turnover, or by causing suicide inhibition [13].

Conversely, the CYP2J enzymes have been detected predominantly in extrahepatic tissues, including the heart, intestine, kidney, and pancreas [14–18], and,

within these tissues, localized to specific cell types [16,18–21]. In most cases, specific monoclonal antibodies have not yet been developed for this growing novel subfamily of enzymes; thus, current reports on tissue and cell distributions are subject to the limitations of using cross-reactive antibodies to characterize proteins in different species. Exhaustive data suggest that CYP2J members are constitutive hemoproteins that are not readily induced with known P450 inducers such as phenobarbital, β-naphthoflavone, clofibrate, or acetone [15,22]. Moreover, CYP2J protein levels do not appear to change in selected animal models of disease, including salt-sensitive hypertension, oxygen-induced lung injury, and cardiac ischemia/reperfusion [22]. Emerging data do, however, suggest that CYP2J isoforms may be nutritionally and/or age regulated [18,23]. Preliminary data also suggest that CYP2Js may be alternatively spliced, which may serve as a mechanism to regulate endogenous P450 function.

B. Catalytic Activity of the CYP2J Enzymes

1. Xenobiotics

The P450 superfamily of enzymes, although remarkably diverse in substrate recognition, catalyze a limited number of reactions, including epoxidation, dealkylation, and hydroxylation [24]. Inhibition of P450 activities [6] may be accomplished by mechanism-based (e.g., gestodene, disulfiram) or competitive inhibitors (e.g., quinidine, sulfaphenazole), competing substrates, compounds which ligand or oxidize the heme moiety (e.g., cimetidine, troleandomycin) or adduct the protein (e.g., chloramphenicol) [25].

Currently, more is known about the xenochemicals CYP2J enzymes that do not oxidize than about their substrates. Native and recombinant CYP2J1 and recombinant CYP2J3 oxidize benzphetamine at comparable, appreciable rates [14,16,26]. Similarly, aminopyrine and *N*,*N*-dimethylaniline are both oxidized by CYP2J1 [26]. In contrast, CYP2J2 demonstrates significantly lower activity toward benzphetamine [15]. Both CYP2J2 and CYP2J5 catalyze the hydroxylation of diclofenac and bifuralol (C. Crespi, J. Ma, and D. C. Zeldin, unpublished data).

The CYP2J enzymes have low or undetectable activities toward other xenochemicals: CYP2J1 toward 7-ethoxycoumarin, benzo[*a*]pyrene, 7-ethoxyresorufin, myristate, or aniline [26]; CYP2J3 toward 7-methoxyresorufin, 7-ethoxyresorufin, 7-pentoxyresorufin, or 7-benzoxyresorufin (M. F. Oleksiak, J. J. Stegeman, and D. C. Zeldin, unpublished data); and CYP2J4 toward (*R*)- and (*S*)-warfarin [17].

2. Endobiotics

Several members of the CYP1, CYP2, and CYP4 families oxidize arachidonic acid (5,8,11,14-eicosatetraenoic acid). This polyunsaturated fatty acid is present,

esterified to cellular glycerophospholipids, in most cell membranes, and is a participant in a variety of receptor/agonist-mediated signaling cascades [9,27,28]. Under basal conditions, the cellular levels of free arachidonic acid are very low. In response to stimuli, phospholipases mobilize arachidonic acid from the *sn*-2 position of glycerophospholipids, making the free arachidonate available for oxidative metabolism by several enzyme systems, including prostaglandin H_2 synthases, lipoxygenases, and P450 monooxygenases [9,27,28].

The P450 monooxygenases, including members of the CYP2J subfamily, bioactivate arachidonic acid to three eicosanoid products (Fig. 1). Olefin epoxidation forms four epoxyeicosatrienoic acids (5,6-, 8,9-, 11,12-, and 14,15-EETs). The EETs are hydrated by epoxide hydrolases to dihydroxyeicosatrienoic acids (DHETs) [29–31]. Allylic oxidation forms six midchain conjugated dienols (5-, 8-, 9-, 11-, 12-, and 15-hydroxyeicosatetraenoic acids or HETEs). ω-Terminal hydroxylation forms C_{16}–C_{20} alcohols of arachidonic acid (16-, 17-, 18-, 19-, and 20-HETEs) [9,27,28]. Regiospecific and stereospecific products of CYP2J arachidonate oxidation will be further detailed for the individual enzymes.

Recent preliminary studies demonstrated that both CYP2J2 and CYP2J5 oxidized testosterone (C. Crespi, J. Ma, and D. C. Zeldin, unpublished data); whereas CYP2J4 showed low activity toward testosterone and progesterone [17]. Metabolism studies have revealed only very low CYP2J activities toward other endogenous substrates, such as prostaglandins [26] and lauric acid (Y. Kikuta, personal communication). Little is known about CYP2J activity toward other endobiotics, including estrogens, other fatty acids, or bile acids.

C. Biological Effects of P450-Derived Arachidonate Metabolites

The eicosanoid products of prostaglandin H_2 (PGH_2) synthases and lipoxygenases have been studied extensively, and their functional roles in a variety of fundamental processes including inflammation and cellular proliferation have been documented [32]. Current studies are focused on elucidating the roles that P450-derived eicosanoids may play in cell and organ physiology, and whether aberrant P450 expression due to environmental and/or endogenous factors leads to cell and organ dysfunction and disease.

Different from the prostanoids, epoxyeicosatrienoic acids (EETs) can be esterified to phospholipids, where they may regulate membrane physical and/or chemical properties (e.g., permeability), and, upon release, may serve as mediators of transmembrane signaling [33,34]. These P450-derived eicosanoids exhibit a multitude of potent biological activities in vitro or in situ, serving as peptide hormone secretagogues in the pancreas, pituitary, and hypothalamus [35–38], regulators of body volume and fluid composition by affecting ion and water transport in the kidney [9,27,28,39,40], and modulators of vascular tone and cellular proliferation in multiple tissues [40–44]. P450-derived arachidonate metabolites

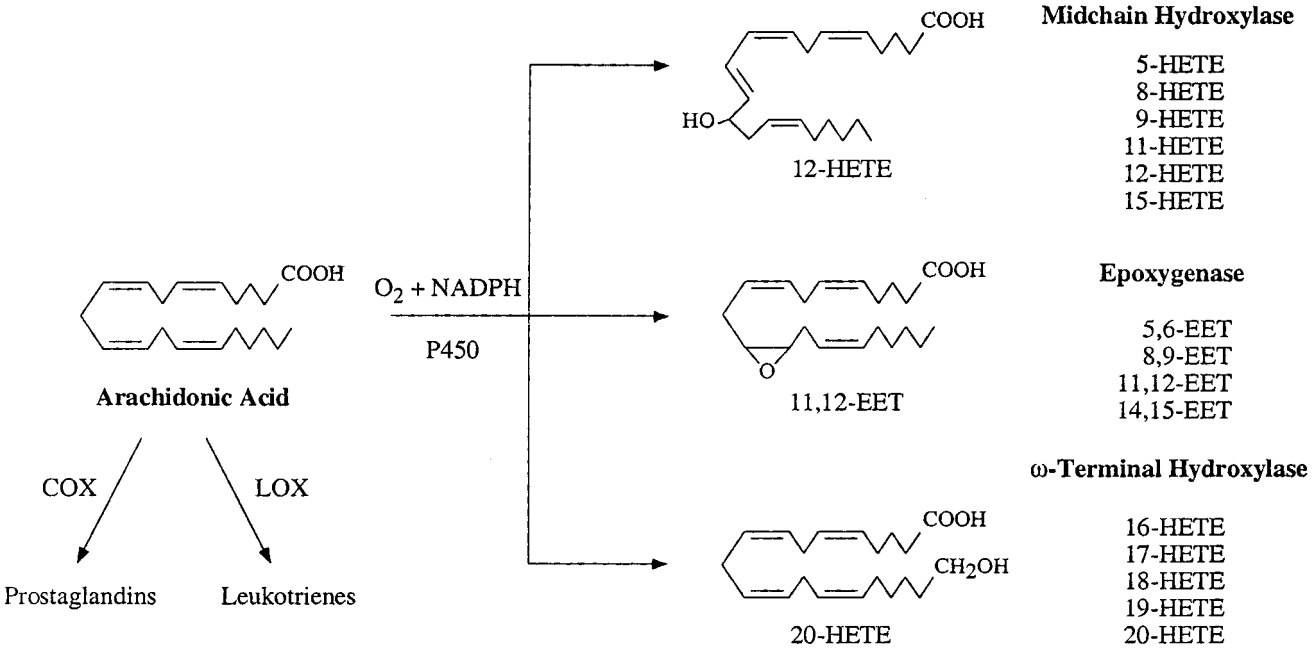


FIG. 1. Reactions catalyzed by microsomal P450s during the metabolism of arachidonic acid.

are postulated to be involved in the pathogenesis of hypertension in rodents and humans [40,42,45–47]. Changes in P450 expression that occur in response to environmental and/or endogenous factors (e.g., exposure to xenobiotics, nutritional alterations, or developmental factors) may result in altered P450-mediated metabolism of arachidonic acid, which may lead to physiological abnormalities and disease [23,46–50]. A more comprehensive understanding of the biological importance of CYP2J metabolites will come as experimental animal models are developed which exhibit altered CYP2J expression.

D. Structural Characteristics of the CYP2J Enzymes

1. Primary Sequence

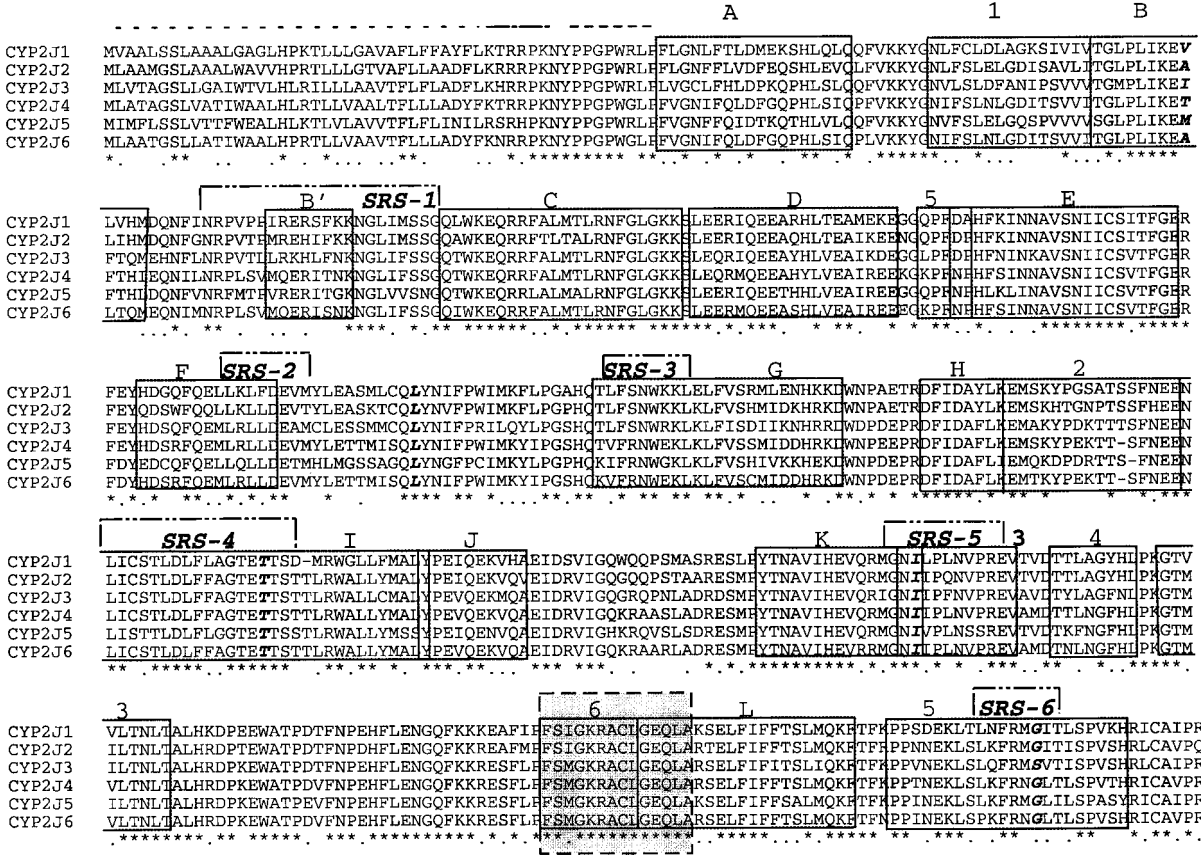
Sequence identity among the six described CYP2Js is between 69% and 80%, the exceptions being CYP2J4 and CYP2J6, which are 94% identical and are likely orthologous (Table 1). Functional studies should yield more information on the likelihood that the six CYP2J subfamily members are representatives of several distinct P450 genes with orthologues among species.

Primary sequence alignment (Fig. 2) [12] reveals that, overall, 53% of the amino acids are identical and 78% are either identical or conservatively replaced among the six described CYP2J subfamily members. Roughly 10 regions of clustered homology are distributed among the 6 putative substrate recognition sites (SRSs), based on those proposed by Gotoh [51] for the CYP2 family, the most notable being the region between SRS-5 and SRS-6. Of these ~100 residues, all but 6 positions are either identical or conservatively replaced. The SRSs display variable homology, ranging from 25% to 74% identity, with SRS-1 exhibiting the greatest variability and SRS-4 being the most conserved. The six CYP2Js also contain all three structural features associated with other members of the

TABLE 1

Primary Sequence Identity Among the CYP2Js

	Rabbit CYP2J1	Human CYP2J2	Rat CYP2J3	Rat CYP2J4	Mouse CYP2J5
Mouse CYP2J6	72%	76%	78%	94%	73%
Mouse CYP2J5	69%	70%	71%	74%	
Rat CYP2J4	73%	76%	79%		
Rat CYP2J3	72%	73%			
Human CYP2J2	80%				



CYP2 family: (1) an N-terminal hydrophobic peptide, (2) a proline-rich segment between residues 40 and 51, and (3) an invariant position 447 or 448 cysteine in a putative heme-binding peptide which lies within the highly conserved stretch between SRS-5 and SRS-6 (Fig. 2).

2. Secondary Structure

Three-dimensional structures have been reported for four bacterial P450 enzymes: $P450_{cam}$, a camphor hydroxylase from *Pseudomonas putida* [52,53]; $P450_{BM-3}$, a fatty acid monooxygenase from *Bacillus megaterium* [54]; $P450_{terp}$, an ω-terpinol monooxygenase also from *Pseudomonas* [55]; and $P450_{eryF}$, a 6-deoxyerythronolide β-hydroxylase from *Saccharopolyspora erythrea* [56]. Primary sequence alignment based on Gotoh's report [51] and structural alignment by Hasemann et al. [57] has allowed rational analysis and identification of putative secondary structural features in P450s from other species. Figure 2 illustrates the putative α-helices and β-structures within the CYP2J subfamily based on alignment with other CYP2 family members and with $P450_{cam}$.

3. Tertiary Structure

Molecular replacement and rule-based modeling, derived from the four solved bacterial P450 structures, indicate that topology of all P450s should be ''quite similar,'' particularly within the heme-binding core [57]. The accumulating three-dimensional data along with analyses of naturally occurring CYP2 family variants have facilitated studies of structure–activity relationships and identification of key residues involved in substrate recognition for several CYP2 family members [58]. Negishi et al. reported that polarity was secondary to bulk and flexibility

FIG. 2. Alignment and putative structural features of CYP2Js. Primary sequence alignment, based on those by Gotoh [51] and Haseman [57], illustrates the N-terminal hydrophobic peptide (···), followed by the polycationic segment (–·–·), and proline-rich region (–––). Boxed are the putative α-helices (lettered), β-structures (numbered), and heme-binding region (shaded). Putative substrate recognition sites (SRSs) are indicated (–··–··). Key residues involved in substrate recognition for the CYP2 family, as identified by Negishi et al. [58], are in bold italics within α-helix B, between SRS-2 and SRS-3, and within SRS-4, SRS-5, and SRS-6.

of particular amino acid side chains in determining substrate specificity in the CYP2 family. Based on Hasemann's alignment [57], these critical residues identified by Negishi et al. align three-dimensionally with the $P450_{cam}$ residues P100-T101, M184-T185, T252, L294, and V396. The analogous positions within the CYP2Js are illustrated in Fig. 2. Such elegant studies which utilize the available structural data illustrate a need to establish a standardized P450 numbering system to allow clarity in comparison studies and analysis of postulated mechanisms for catalysis, inhibition and stimulation, and functional changes observed in substitution experiments.

4. Quaternary Structure

Membrane topology studies using immunochemistry, chemical modification, proteolysis, and genetic engineering indicate that microsomal P450s are anchored to subcellular membranes by one or two transmembrane peptides near the NH_2-terminus. These hydrophobic stretches, approximately 20 amino acids in length, are generally followed by a short polycationic segment which serves as a signal to halt transfer into the membrane [59]. Both such features are found among the CYP2J members (Fig. 2).

II. THE CYP2J SUBFAMILY: IDENTIFICATION, DISTRIBUTION, REGULATION, AND METABOLISM

The discussion which follows details the characterization studies of the individual CYP2J subfamily members described to date. Tables 2 and 3 summarize

TABLE 2

Physical Properties of the CYP2J Enzymes

Enzyme	cDNA Length (bp)	Open reading frame	Molecular mass (Da)	Amino acid number
Rabbit CYP2J1	1829	26–1528	57,193	501
Human CYP2J2	1876	6–1511	57,653	502
Rat CYP2J3	1778	10–1515	57,969	502
Rat CYP2J4	1843	48–1550	57,878	501
Mouse CYP2J5	1886	42–1544	57,784	501
Mouse CYP2J6	2046	248–1750	57,819	501

TABLE 3

Catalytic Properties of the CYP2J Enzymes

Enzymes	Known substrates	Catalytic turnover (min^{-1})
CYP2J1	Benzphetamine	3.90
	Aminopyrine	1.50
	N,*N*-Dimethylaniline	0.90
CYP2J2	Arachidonic acid	0.10
	Testosterone	0.30
	Benzphetamine	0.08
	Diclofenac	0.60
	Bufuralol	0.17
CYP2J3	Arachidonic acid	0.20
	Benzphetamine	3.00
CYP2J4	Arachidonic acid	0.15
	Testosterone	0.01
	Progesterone	0.02
CYP2J5	Arachidonic acid	7.20
	Testosterone	0.10
	Diclofenac	0.01
	Bufuralol	0.04
CYP2J6	Unknown	—

physical and catalytic properties for the individual enzymes. Turnover numbers in Table 3 are comparable to other P450s (e.g., CYP2C8 and CYP2B4) which metabolize arachidonic acid [60,61].

A. Rabbit CYP2J1

1. Identification

The first enzyme of this new subfamily was isolated in 1985 by Ichihara et al. [26]. A ~56-kDa protein (then designated P-450ib) purified from rabbit small intestine microsomes demonstrated a typical b-type cytochrome spectrum and was distinct from other known rabbit P450s as assessed by peptide mapping and double-diffusion methods. Subsequently, the P-450ib cDNA was identified from a rabbit small intestine cDNA library [14]. A 1.8-kb (kilobase) clone contained an open reading frame encoding a 501 amino acid 57,193-Da polypeptide with a 35–41% primary sequence identity to known CYP2 family members and less than a 30% identity to members of the other P450 families. The first member

of the novel subfamily was designated CYP2J1 according to the recommended nomenclature. On Western blots of microsomal preparations from COS-7 cells expressing recombinant CYP2J1, a protein that was recognized by polyclonal antibodies against P-450ib exhibited a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) migration identical to the native protein in rabbit small intestine microsomes.

2. Distribution

Northern blots of total RNA prepared from various rabbit tissues suggested that CYP2J1 transcripts are expressed primarily in the small intestine [14], but are also detected in the liver, lung, kidney, and heart [22]. The expression of CYP2J1 protein in nonintestinal rabbit tissues has not been investigated, nor has CYP2J1 regulation been documented.

3. Metabolism

The CYP2J1 oxidation of benzphetamine and related compounds has been reported, as well as negligible activity toward PGA_1 [26]. Kikuta and co-workers did not observe appreciable lauric acid or arachidonate oxidation by CYP2J1 (Y. Kikuta, personal communication).

B. Human CYP2J2

1. Identification

In an attempt to identify the human kidney P450(s) involved in arachidonate metabolism, a human kidney cDNA library was screened with a CYP2C9 cDNA probe, and a new P450 sequence identified [15]. The novel cDNA had an open reading frame that encoded a 57-kDa polypeptide that was 80% identical to rabbit CYP2J1 and 40–43% identical to other CYP2 family members. Identical sequences were also obtained by screening human heart, liver, and intestinal cDNA libraries [15,20; J. Ma and D. C. Zeldin, unpublished data]. Based on the deduced amino acid sequence, the new human enzyme was designated CYP2J2. A baculovirus expression system was used to express CYP2J2 with or without CPR in insect cells. The recombinant CYP2J2 protein, purified to near electrophoretic homogeneity, displayed typical b-type cytochrome spectral characteristics and migrated as a discrete band on SDS–PAGE with an apparent molecular mass of 57 kDa [15].

2. Distribution

Northern analysis of human RNA demonstrated that CYP2J2 transcripts, unlike other previously described P450s, were most abundant in the heart and were present at lower but significant levels in the liver, intestine, lung, and kidney [15,19,20]. More sensitive reverse transcriptase–polymerase chain reaction (RT–PCR) methods detected CYP2J2 transcripts in RNA preparations from the human brain, heart, vasculature, lung, intestine, spleen, liver, kidney, ovary, uterus, and testes (P. E. Scarborough, P. Adler, J. Ma, and D. C. Zeldin, unpublished data). Immunoblot analysis with polyclonal antibodies raised against purified recombinant CYP2J2 confirmed the unusual extrahepatic tissue distribution of CYP2J2 protein. The polyclonal anti-CYP2J2, which did not cross-react with other known human P450s, detected a prominent ~56-kDa band in microsomal fractions prepared from human heart, lung, hypothalamus, pituitary, intestine, pancreas, liver, kidney, skeletal muscle, and vascular tissues [15,19–21; D. C. Zeldin, unpublished data]. Immunoreactive protein was most abundant in heart microsomes. CYP2J2 is the only human P450 known to be expressed at high levels in the heart. Also detected were two additional bands in liver microsomes: one of slightly lower mobility and intensity, and the other of slightly higher mobility and of least intensity [15]. Presently unidentified, these may represent alternative splice variants of CYP2J2, other uncharacterized human liver CYP2Js, or other P450s.

The tissue distribution of CYP2J immunoreactivity and CYP2J2 mRNA was similar; however, there was a discordance between mRNA and protein levels in several human tissues. Human stomach, duodenum, and kidney CYP2J protein levels were high despite low CYP2J2 mRNA levels [15,20]. Other investigators have noted a lack of correlation between protein and mRNA levels for human P450s and have implicated the balance between translation rate and protein turnover in determining human P450 hemoprotein levels [62]. The results also suggest the possibility that more than one human CYP2J may be present in some tissues.

3. Regulation

Preliminary evidence suggests that CYP2J2 RNA may be alternatively spliced and that the putative alternative splice variants are expressed in a tissue-specific fashion. Alternative splicing may be one mechanism regulating the endogenous function of the CYP2Js. Two putative CYP2J2 alternative splice variants, identified as CYP2J2-H2 and CYP-H5, were isolated from a human heart cDNA library (J. Ma and D. C. Zeldin, unpublished data). Both were identical to CYP2J2, except in the region upstream from the putative exon 1/intron 1 splice site. In

this region, CYP2J2 contained a 214-bp (base pair) sequence, whereas CYP2J2-H2 and CYP2J2-H5 contained 67-bp and 124-bp sequences, respectively. Based on the deduced amino acid sequence, both the CYP2J2-H2 and CYP-H5 cDNAs encode polypeptides that contain a putative heme-binding peptide but do not have an N-terminal proline cluster that is thought to be important for proper folding and incorporation of the heme moiety [63]. CYP2J2-H2 mRNAs were amplified by RT–PCR methods from human heart, lung, intestine, pancreas, kidney, liver, spleen, and brain. CYP2J2-H2 mRNAs were not detected in the ovary, uterus, and testes despite the presence of CYP2J2 transcripts (P. E. Scarborough, P. Adler, J. Ma, and D. C. Zeldin, unpublished data). In contrast, CYP2J2-H5 transcripts were only detected in the heart, spleen, and uterus. Currently, efforts are focused on the following: (1) determining the relevant genomic sequence of the CYP2J2 to confirm whether CYP2J2-H2 and CYP-H5 indeed arise by alternative splicing; (2) evaluating catalytic function of these putative splice variants; and (3) developing specific, peptide-based antibodies to determine the tissue distribution and cellular localization of the CYP2J2, CYP2J2-H2, and CYP2J2-H5 components.

4. Metabolism

Recombinant CYP2J2, when incubated with arachidonic acid, CPR (or coexpressed with CPR), and NADPH, catalyzed the formation of all four regioisomeric EETs as the major products, with limited production of 19-HETE, indicating that CYP2J2 is primarily an arachidonic acid epoxygenase [15]. Epoxidation occurred preferentially at the 14,15-olefin and, to a lesser degree, at the 5,6-, 8,9-, and 11,12-olefins. Furthermore, epoxidation at the 14,15-olefin was highly stereoselective for 14(*R*),15(*S*)-EET, whereas epoxidation at the 8,9- and 11,12-olefins was non-enantioselective. Importantly, the regiochemical and stereochemical product profile of CYP2J2 was different from that of other known arachidonate epoxygenases, including several different members of the CYP1A, CYP2B, and CYP2C subfamilies [48,60,61,64,65]. CYP2J2 did not significantly oxidize benzphetamine [15], but catalyzed the hydroxylation of testosterone and several xenobiotic substrates, including diclofenac and bufuralol (C. Crespi, J. Ma, and D. C. Zeldin, unpublished data).

C. Rat CYP2J3

1. Identification

To identify rodent orthologue(s) of CYP2J2 and to facilitate the development of animal models to study CYP2J function and regulation in vivo, homology

screening of a rat liver cDNA library identified a full-length cDNA, designated CYP2J3 [16]. The deduced 501 amino acid sequence shared 72–73% identity to CYP2J1 and CYP2J2 and less than 42% identity with other CYP2 family members. Recombinant CYP2J3 protein, expressed in insect cells, displayed typical P450 spectral characteristics, migrated as a discrete band on SDS–PAGE with an apparent molecular mass of ~58 kDa and was immunoreactive with anti-CYP2J2 polyclonal antibodies.

2. Distribution

Northern analysis of rat RNA, using either the CYP2J3 cDNA or a sequence-specific oligonucleotide, detected a prominent band in the liver and less intense bands in the heart, lung, intestine, and kidney, demonstrating the wide tissue distribution of CYP2J3 transcripts [16,19,20]. Specific RT–PCR methods amplified CYP2J3 mRNAs in the heart, lung, brain, intestine, liver, and kidney (W. Qu and D. C. Zeldin, unpublished data). Western blots of microsomal fractions prepared from rat tissues or host cells expressing recombinant CYP2J3 demonstrated a prominent anti-CYP2J2 antibody cross-reactive band in the heart and liver, with less intense bands in the lung, kidney, intestine, pancreas, pituitary, hypothalamus, and vascular tissues, further supporting a wide tissue distribution of rat CYP2J immunoreactive protein(s) [19–21; D. C. Zeldin, unpublished data].

3. Regulation

Developmental regulation of CYP2J protein expression has been observed in the rat. Immunoblots of kidney microsomal fractions from rats (ages newborn to adult) showed that CYP2J protein was expressed at very low levels until shortly after birth, increased gradually during the first 3–4 weeks of life, and reached adult levels by 8 weeks of age (W. Qu, G. Engelmann, and D. C. Zeldin, unpublished data). A similar pattern of developmental expression was observed on Western blots of microsomes prepared from rat hearts. Subsequent investigations will explore whether exposure of the animal to endogenous or environmental agents during the early postnatal period contributes to the age-related changes in CYP2J expression.

Dietary factors also appear to alter the expression of CYP2J P450s. Intestine, liver, and kidney microsomes of rats that underwent fasting and refeeding were examined for CYP2J expression using polyclonal anti-CYP2J2 antibodies. After 48 h of fasting, rat intestinal CYP2J levels were markedly reduced, returning to prefasting levels within 6 h of refeeding (W. Qu and D. C. Zeldin, unpublished data). In contrast, there were no significant differences in liver or kidney CYP2J

with fasting or refeeding [23], suggesting that nutritional status regulates CYP2J protein expression in an organ-specific fashion.

4. Metabolism

Microsomal fractions, prepared from host cells co-expressing recombinant CYP2J3 and CPR, oxidized arachidonic acid to EETs and 19-HETE as the major products, demonstrating that CYP2J3 acts as both arachidonate epoxygenase and ω-1 hydroxylase [16]. Analysis of the EETs, revealed regiospecific epoxidation at the 14,15- $>$ 8,9- and 11,12- $\gg$ 5,6-olefins with a minor stereochemical prevalence of 14(*S*),15(*R*)-, 11(*R*),12(*S*)-, and 8(*R*),9(*S*)-EETs. CYP2J3-derived 19-HETE was recovered primarily as the (*R*)-stereoisomer [66]. In contrast to observations with CYP2J2, rat CYP2J3 readily oxidized benzphetamine [16]. Primary sequence comparison and arachidonate metabolism data suggest that CYP2J2 and CYP2J3 are distinct nonorthologous enzymes. Similar to human CYP2J2, CYP2J3 is the only known rat P450 arachidonic acid epoxygenase expressed at high levels in the heart.

D. Rat CYP2J4

1. Identification

Concurrent with the identification of rat CYP2J3 by Wu et al., Zhang and coworkers identified a second rat enzyme, designated CYP2J4, from rat small intestine [17]. The 501-residue polypeptide shared 73–79% identity with CYP2J1, CYP2J2, and CYP2J3. Recombinant CYP2J4, expressed using a baculovirus system, displayed typical b-type cytochrome spectra and migrated as a discrete ~55-kDa band as detected on Western blots by polyclonal antibodies against a synthetic peptide common to both CYP2J3 and CYP2J4 (anti-CYP2J3/4 peptide antibody).

2. Distribution

Zhang et al. examined the tissue distribution of rat CYP2J4 mRNA by Northern analysis using a 0.9-kb cDNA fragment of the coding region [17]. A 2.0-kb transcript was abundant in the liver, small intestine, and olfactory mucosa and was present at much lower levels in the kidney, heart, and lung. A larger 2.4-kb transcript was also detected in olfactory mucosa and, to a lesser extent, in the small intestine. This larger mRNA may represent alternative splicing of the

CYP2J4 gene product or another homologous rat P450. Recently, RT–PCR methods have detected CYP2J4 transcripts in the rat brain, heart, lung, intestine, kidney, liver, and muscle (W. Qu and D. C. Zeldin, unpublished data).

Immunoblot analysis, with the anti-CYP2J3/4 peptide antibody, of microsomal fractions from various rat tissues detected the following: (1) a protein with the same electrophoretic mobility as recombinant CYP2J4 in small intestine and, at lower levels, in liver and olfactory mucosa; (2) a second, less abundant protein of slightly lower apparent molecular weight in the kidney, liver, small intestine, lung, and heart; (3) a protein of intermediate mobility but strong intensity in olfactory mucosa; and (4) a $\sim$ 39-kDa protein in the kidney and small intestine [17]. The rat proteins recognized by the anti-CYP2J3/4 peptide antibody remain to be identified. As with human CYP2J2, there was discordance between rat CYP2J mRNA and protein levels in certain tissues. For example, in the rat heart, CYP2J immunoreactive protein was abundant despite relatively low CYP2J3 and CYP2J4 mRNA levels. Nothing is known about regulation of CYP2J4.

3. Metabolism

Similar to rat CYP2J3, recombinant CYP2J4 exhibited both arachidonic acid epoxygenase and ω-1 hydroxylase activities [17]. Interestingly, the CYP2J3 and CYP2J4 profiles are remarkably similar to that of the recently described fish CYP2P3, from *Fundulus heteroclitus*. This P450 is phylogenetically most closely related to mammalian CYP2J subfamily members [66]. The stereochemical composition of CYP2J4 arachidonate metabolites are not yet known. Recombinant CYP2J4 also has low activities toward testosterone and progesterone [17].

E. Mouse CYP2J5

1. Identification

A full-length mouse CYP2J cDNA was recently identified from a liver cDNA library and designated CYP2J5 [18]. CYP2J5 encoded a polypeptide with 69–74% identity to the other CYP2Js, and when expressed with or without CPR in insect cells, displayed typical P450 spectral characteristics.

2. Distribution

Northern and RT–PCR analyses of mouse RNAs, using the CYP2J5 cDNA or sequence-specific oligonucleotides, detected transcripts primarily in the kid-

ney, at lower levels in the liver, and in no other mouse tissues [18]. Polyclonal antibodies against a CYP2J5-specific peptide (anti-CYP2J5pep) detected a protein, with the same electrophoretic mobility as recombinant CYP2J5, in kidney microsomes and, at lower levels, in liver microsomes.

3. Regulation

There is increasing evidence that CYP2J5 may be regulated in an age-related fashion in the liver and kidney. Immunoblotting of microsomal fractions, prepared from kidneys of mice ages 18-day fetal to adult, with the anti-CYP2J5pep detected CYP2J5 protein at very low levels before birth, increasing gradually from the newborn period until 1 week of age, reaching maximal levels between 2 and 4 weeks, and then remaining relatively constant [18]. In contrast, mouse liver microsomal CYP2J5 protein was undetectable during the fetal, newborn, and early postnatal ages, first appeared at 1 week, and then remained relatively constant from 1 to 10 weeks of age. Current efforts are focused on elucidating the biochemical and molecular mechanisms involved in the regulation of CYP2J5 expression.

4. Metabolism

Microsomal fractions of host cells, expressing recombinant CYP2J5, exhibited both arachidonate epoxygenase and midchain hydroxylase activities, with EETs and midchain HETEs as the principal products [18]. Similar to rat CYP2J3, regiochemical and stereochemical analysis revealed a predominance of 14(*S*),15(*R*)- $>$ 11(*R*),12(*S*)- and 8(*R*),9(*S*) $\gg$ 5,6-EET. However, distinct from the other CYP2J enzymes, CYP2J5 catalyzed arachidonate midchain hydroxylation at the 11- and 15-positions. Recombinant CYP2J5 oxidation of testosterone and several xenobiotic substrates, including diclofenac and bufuralol, also have been observed (C. Crespi, J. Ma, and D. C. Zeldin, unpublished data).

F. Mouse CYP2J6

1. Identification

Another mouse cDNA, isolated from a liver cDNA library and designated CYP2J6, encoded a polypeptide that was 94% identical to rat CYP2J4 and 72–78% identical to the other CYP2J members (J. Ma and D. C. Zeldin, unpublished data). Recombinant CYP2J6 was expressed using the baculovirus system and

displayed typical P450 spectral characteristics; however, the CYP2J6 hemoprotein was unstable in the absence of detergent as evidenced by a rapid depletion of the sodium hydrosulfite-reduced, carbon monoxide-bound spectral absorbance at 450 nm and the appearance of a peak at 420 nm consistent with loss of the heme moiety. Interestingly, SDS–PAGE of recombinant CYP2J6 host cell microsomes showed that the ~57-kDa component was not degraded to lower-molecular-weight bands with repeated freezing and thawing.

2. Distribution

Northern and RT–PCR analyses of mouse tissue RNA, using either the CYP2J6 cDNA or sequence-specific oligonucleotides, showed CYP2J6 mRNAs to be abundant in the intestine and present at lower levels in the heart, lung, brain, kidney, and liver (J. Ma and D. C. Zeldin, unpublished data). A CYP2J6-specific peptide antibody (anti-CYP2J6pep) failed to detect an ~57-kDa protein in mouse tissue microsomal fractions; however, it did detect an ~25-kDa component with a tissue distribution similar to that of CYP2J6 mRNAs in accordance with the presumed instability of the recombinant protein (J. Ma and D. C. Zeldin, unpublished data). The apparent instability of CYP2J6 suggests the possibility that posttranslational regulation and/or protein turnover may be important in controlling steady-state levels of this hemoprotein. Ways to stabilize the recombinant CYP2J6 hemoprotein are currently under investigation in order that the catalytic properties can be characterized.

G. Other CYP2J Enzymes

Three additional mouse CYP2Js recently have been identified, with designations CYP2J7, CYP2J8, and CYP2J9 by the nomenclature committee (W. Qu, J. Ma, and D. C. Zeldin, unpublished data). These newly described P450s share approximately 70–75% identity with each other, and with CYP2J5 and CYP2J6. In addition, a new CYP2J cDNA fragment has been identified recently from rat sebaceous gland RNA using RT–PCR (D. Kinney, personal communication). This cDNA, tentatively named CYP2J10, shares approximately 80% identity with rat CYP2J3 and CYP2J4. Although tissue distributions, regulation, and catalytic activities of these new CYP2J isoforms have not been characterized, it appears that the CYP2J subfamily, like other CYP2 subfamilies, contains multiple genes. Further work will be necessary to determine the functional significance of these newly described CYP2J isoforms.

H. Possible Early Vertebrate Forms of the Mammalian CYP2J Enzymes

Recently, two novel P450 gene subfamilies (CYP2N and CYP2P) have been described in the primitive vertebrate *Fundulus heteroclitus* ''killifish'' [66,67]. Members of these P450 subfamilies are phylogenetically most closely related to the mammalian CYP2Js. In addition, the tissue distribution of CYP2N and CYP2P transcripts is similar to that of the mammalian CYP2Js (i.e., high levels in the heart, intestine, and kidney). Functional similarities may exist among members of these subfamilies.

Recombinant CYP2N1, CYP2N2, and CYP2P3 were individually co-expressed with CPR in insect cells, and their substrate specificities and arachidonic acid metabolic profiles were compared to the mammalian CYP2Js. Both CYP2N1 and CYP2N2 catalyzed the NADPH-dependent oxidation of arachidonic acid to EETs and midchain HETEs with a product profile remarkably similar to that of mouse CYP2J5 [18,67]. Recombinant CYP2P3 oxidized arachidonic acid to EETs and 19-HETE, remarkably similar to rat CYP2J3 [16,66]. Incubations with xenobiotic substrates confirmed the similarities in the substrate specificities among the three P450 subfamilies. Therefore, based on the structural and metabolic similarities among CYP2J, CYP2N, and CYP2P isoforms, investigators postulate that members of these three P450 subfamilies may have originated from a common ancestral gene and that the newly described fish P450s may represent early vertebrate forms of the mammalian CYP2J hemoproteins.

III. GENE ORGANIZATION OF *Cyp2j5*

In order to facilitate studies on the biochemical and molecular mechanisms responsible for the tissue- and cell-specific expression and developmental regulation of CYP2Js, a mouse strain 129 embryonic stem cell genomic λ phage library was screened with radiolabeled CYP2J5 cDNA fragments. Three distinct overlapping clones were isolated that together comprise >27 kb of *Cyp2j5*, including >4 kb of the 5′ flanking region (J. Ma, R. Langenbach, and D. C. Zeldin, unpublished data). *Cyp2j5*, like all previously described CYP2 family genes, contains nine exons and eight introns, with intron–exon junctions consistent with the consensus for known splice sites within eukaryotic genes. The exons vary in length from 139 to 210 nucleotides with the eighth and ninth exon containing the nucleotides encoding the putative heme-binding peptide. The nucleotide sequences of the exons were identical to those obtained for the CYP2J5 cDNA [18]. The introns vary in length from 525 nucleotides (intron 1) to 5.3 kb (intron 5). *Cyp2j5* intron–exon boundaries are in similar positions to those of other CYP2 family

genes, confirming that no changes in intron–exon patterns have occurred since the CYP2J and other CYP2 family genes diverged from a common ancestral gene over 100 million years ago [1].

IV. CHROMOSOMAL MAPPING OF THE *CYP2J* CLUSTER

A PCR-based, allele-specific, oligonucleotide hybridization mapped *Cyp2j5* and *Cyp2j6* to the same subchromosomal interval distal to *jun* and proximal to *misty* on mouse chromosome 4 [68]. Human *CYP2J2* was assigned to chromosome 1 on a panel of somatic hybrid cell lines, and to 1p31.3–p31.2 by fluorescence in situ hybridization analysis. The results with *Cyp2j5* and *Cyp2j6* extend earlier studies which found that genes within a given P450 subfamily are closely linked [1]. Although P450 genes are spread throughout the genome, comparative gene mapping showed that considerable synteny exists between mouse and human. The assignment of the *CYP2J* cluster to mouse chromosome 4 and the *CYP2J2* locus to human chromosome 1p, provided further evidence to support the synteny between these mouse and human chromosomal regions [68]. Interestingly, *Cyp2j5* and *Cyp2j6* map to a region of chromosome 4 that is in close proximity to mouse *Cyp4a10* and *Cyp4a12*. Similarly, the human *CYP2J2* and *CYP4B1* appear to be closely linked on chromosome 1, to which the human orthologue of mouse *Cyp4a10* (i.e., *CYP4A11*) has also been mapped [1]. Thus, it appears as though genes of the CYP2J, CYP4A, and CYP4B subfamilies are closely linked on homologous regions of mouse chromosome 4 and human chromosome 1. The CYP4A subfamily are known to be active in the ω-hydroxylation of arachidonic acid [69]. The close proximity of the *CYP2J* cluster to the *CYP4A* cluster suggest that these genes may be part of a cassette of P450 genes involved in the bioactivation of fatty acids [68].

V. POTENTIAL PHYSIOLOGIC IMPLICATIONS OF CYP2J ENZYMES

In contrast to most other P450s, CYP2J subfamily protein levels are considerable in extrahepatic tissues, including the heart and kidney. Several studies have focused on establishing immunohistochemical localization of CYP2J expression within these tissues, and the presence and biological effects of the principle CYP2J metabolites in tissues and cells where CYP2J proteins are abundant.

A. Heart

Immunohistochemistry of human and rat heart tissue sections found CYP2J immunoreactive staining in both atrial and ventricular myocytes and less intense staining in endothelial cells lining the endocardium, whereas subendocardial connective tissue did not stain [16]. EETs, the major CYP2J arachidonate metabolites, were present in human heart tissue [15], with stereochemistry that matched the chirality of CYP2J2 products. This was the first documentation of EETs as endogenous constituents of heart tissue, data which provided direct evidence that the P450 monooxygenase pathway participates in cardiac arachidonic acid metabolism.

The localization of CYP2J protein to cardiac myocytes and EET components in heart tissue prompted an effort to elucidate the biological role of these eicosanoids in the heart. In an isolated perfused rat heart model, 11,12-EET, at concentrations similar to those estimated to be present endogenously in rat heart tissue, significantly improved recovery of heart contractile function following prolonged, global ischemia [16]. The effects were dose dependent and measurable at concentrations as low as 1 μM. Other CYP2J products failed to improve functional recovery following ischemia [16], whereas arachidonic acid and prostaglandins have detrimental effects in the heart following an ischemic event [70,71].

To further elucidate the mechanisms involved in the cardioprotective effects of 11,12-EET, cardiac action potentials were measured in isolated-perfused rabbit papillary muscle preparations, under basal conditions and during ischemia [70]. Perfusion with 11,12-EET caused hyperpolarization of the resting membrane potential and shortening of the action potential duration. After infusion and washout of 11,12-EET, the time to onset of cell-to-cell electrical uncoupling during ischemia was lengthened. Thus, exposure to 11,12-EET ''preconditioned'' a delay of cell-to-cell electrical uncoupling during ischemia.

The electrophysiologic effects on the cardiac action potential (i.e., hyperpolarization and shortening of the action potential duration) suggested that 11,12-EET may modulate Ca^{2+} and/or K^+ channel activity in cardiac myocytes. Therefore, subsequent experiments measured effects of 11,12-EET on single cardiac L-type Ca^{2+} channels reconstituted into planar lipid bilayers [72]. 11,12-EET, whether added to the extracellular or the intracellular side, inhibited cardiac L-type Ca^{2+} channels, with a decrease in open probability that was partially due to an acceleration of channel inactivation during depolarizations. In addition, single-channel conductance was decreased. Persistence of the effect in the presence of phosphatase inhibitors exonerated channel dephosphorylation for the observed effects [72].

Other groups have also documented biological effects of P450 epoxygenase

products within the heart. EETs relax coronary arteries [73,74], activate Ca^{2+}-dependent K^+ channels in coronary artery smooth-muscle cells [73,75,76], and the hydration product of 11,12-EET (i.e., 11,12-DHET) inhibits cardiac Na^+/K^+-ATPase [77]. In addition, 19-HETE is vasoactive [78,79].

Figure 3 illustrates a working model for the potential biological roles of CYP2Js in the heart. The CYP2Js, present in both cardiac myocytes and endothelial cells, catalyze the formation of EETs and smaller amounts of 19-HETE. The 11,12-EET has inhibitory effects on cardiac myocyte L-type Ca^{2+} channels resulting in secondary effects on cardiac contractility, action potential, cellular coupling, and/or calcium-dependent enzymes (e.g., calpain) through mechanisms that remain unknown. Together, these effects result in cardioprotection during ischemia. The eicosanoids also have effects on cardiac Na^+/K^+-ATPase and vascular tone, and they may affect cardiac K^+ channels through mechanisms similar to their effects on K^+ channels in vascular smooth muscle. Current efforts are underway to develop a transgenic animal model to study the effects of increased cardiac CYP2J expression on heart function with hope that the information gleaned will lead to the development of novel therapeutic and/or preventative strategies for ischemic heart disease.

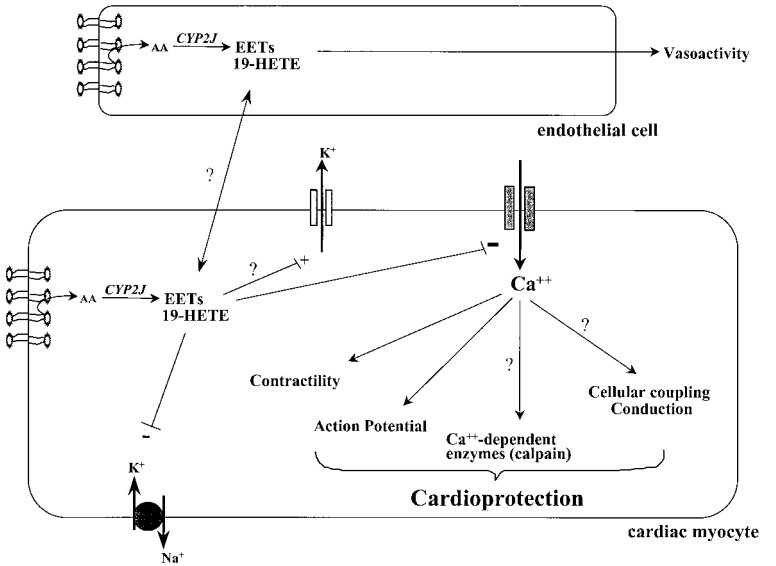


FIG. 3. A working model of potential biological roles for CYP2Js in the heart.

B. Kidney

The EETs are endogenous constituents of human and rat kidney, with regiochemistry similar to that of recombinant CYP2J2 and CYP2J3 products [15,16,43,80]. In both species, the major EET regioisomer present in the kidney is 14,15-EET.

A number of P450s are present in rat and human kidney, including CYP2C and CYP4A members [27,28,65,81,82]. The nephron segment with the highest concentration of P450 arachidonic acid metabolic activity is the proximal tubule, although some activity has also been observed in the medullary thick ascending limb and the collecting ducts [40,42,81]. Immunohistochemical staining, with anti-CYP2J2 polyclonal antibodies, of rat, mouse, and human kidney sections revealed that CYP2J protein was primarily in the proximal tubule and collecting ducts [18]. CYP2J immunoreactivity was generally intense throughout the proximal tubules; however, the straight part of the proximal tubules (corresponding to the P2/P3 segments) was generally more intense than the convoluted part (corresponding to the P1 segment). Within the collecting duct, immunostaining was most intense in the papillary collecting ducts and was present at lower levels in the inner and outer medullary collecting ducts. Immunoreactivity was lower in distal tubules (including the thick ascending limb and distal convoluted tubule) and generally absent from vascular structures, glomeruli, the thin limbs of Henle's loop, and intervening stroma.

The CYP2Js were localized to regions of the nephron where energy-dependent absorption or secretion take place, and thus could contribute in these functions. For example, the EETs inhibit Na^+ transport, mediate angiotensin II-induced rise in cytosolic Ca^{2+}, and inhibit Na^+/K^+-ATPase in the proximal tubule [83–85]. In the collecting duct, the EETs inhibit Na^+ reabsorption and K^+ secretion, vasopressin-stimulated water reabsorption, and stimulate prostaglandin synthesis [86–88]. In addition, the EETs have potent effects on renal vascular tone [43], mesangial cell proliferation, and Na^+/H^+ exchange [44]. Thus, EETs are biologically active in regions of the nephron where CYP2J proteins are abundant.

Recent data suggest that the renal P450 arachidonate metabolic pathway may be involved in the pathogenesis of hypertension [9,27,28,40,45–47]. Increased dietary salt intake results in increased urinary excretion of P450 arachidonic acid epoxygenase metabolites in rats [45,46]. Treatment of rats on a high-salt diet with the P450 inhibitor clotrimazole results in reduced urinary excretion of epoxygenase metabolites and increased mean arterial blood pressure. Genetically controlled alterations of the renal P450 arachidonic acid epoxygenase also induce hypertension in rats fed a high-salt diet [46]. Taken together, these findings suggest that renal epoxygenases may protect against the development of salt-sensitive hypertension in experimental animals. Moreover, urinary excretion of epoxygenase metabolites is increased in patients with pregnancy-induced hypertension,

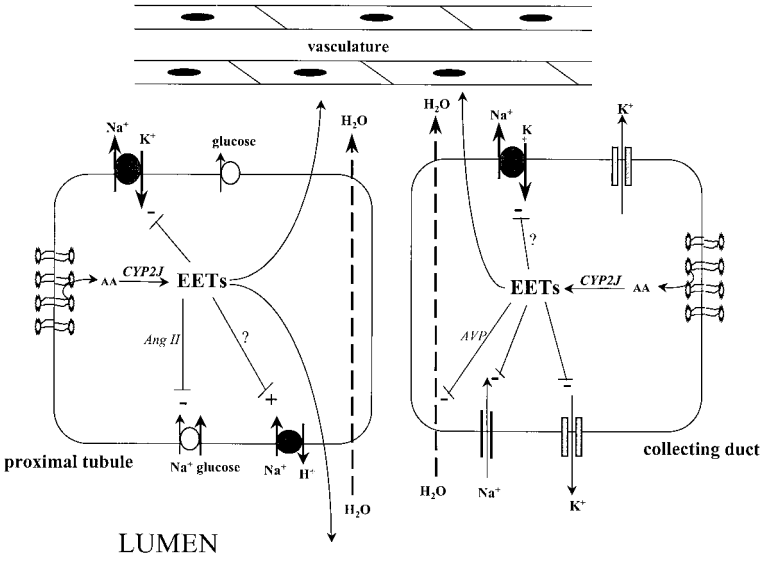


FIG. 4. A working model of potential biological roles for CYP2Js in the kidney.

thus implicating renal P450-derived arachidonate metabolites in the pathogenesis of human hypertension [89].

Figure 4 illustrates a working model for the potential biological roles of CYP2J products in the kidney. CYP2J enzymes, present in both proximal tubules and collecting ducts, catalyze the formation of EETs. These eicosanoids have a variety of biological effects on renal tubular transport processes and renal vascular tone. Proximally formed EETs may be secreted into the tubular lumen and affect distal tubular transport processes. The EETs may also diffuse through the interstitium, where they may enter the circulation and affect renal vascular tone. Current efforts focus on developing a transgenic animal model to study the effects of reduced CYP2J products on kidney function and blood pressure.

VI. SUMMARY

Several members of the CYP2J subfamily have been identified in rodents, although only a single gene has been identified in humans. The CYP2J cDNAs encode heme-thiolate proteins that bioactivate arachidonic acid and can also cata-

lyze the metabolism of several xenobiotic substrates including benzphetamine, diclofenac, and bufuralol. The CYP2Js are abundant in extrahepatic tissues, including the heart and kidney, and within these organs, CYP2J expression is localized to specific cells. EETs, the major eicosanoid metabolites of the CYP2J enzymes, are endogenous constituents of tissues where CYP2J proteins are abundant and exhibit potent biological activities therein. The CYP2J isoforms appear to be regulated during development and in a tissue-specific fashion. Future studies should evaluate the effects of altered CYP2J protein expression on cell and organ function and elucidate the biochemical and molecular mechanisms involved in the regulation of the CYP2J enzymes.

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