Drug Metab. Pharmacokinet. 20 (6): 478–484 (2005).

Regular Article

Localization of rat Cytochrome P450 in Various Tissues and Comparison of Arachidonic Acid Metabolism by Rat P450 with that by Human P450 Orthologs

Susumu IMAOKA[1]*, Takanori HASHIZUME[2] and Yoshihiko FUNAE[2]

[1]*Nanobiotechnology Research Center and Department of Bioscience, School of Science and Technology, Kwansei Gakuin University, Hyogo, Japan*
[2]*Department of Chemical Biology, Osaka City University Medical School, Osaka, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Metabolites of arachidonic acid produced by P450 are interesting substances with prominent physiological functions. To elucidate the physiological function of P450, it is necessary to identify a specific P450 in a particular tissue or organ and to characterize its catalytic activities. In this study, the expression of CYP2A1, 2B1, 2C23, 2J3, and 4F1 was investigated in liver, lung, kidney, spleen, heart, brain, and testis of rats by RT-PCR. Furthermore, arachidonic acid metabolism was investigated using the rat P450s described above and human CYP2A6, 2B6, 2C9, 2C18, 2C19, 2J2, and 4F2. Among the rat P450s, CYP2B1 and 2C23 efficiently produced EETs and CYP4F1 produced 19/20-HETE in abundace. CYP2B1 was specifically expressed in the lung. CYP2C23 was detected in all tissues used in this study. CYP4F1 was expressed in the kidney as well as in the liver. Among the human P450s, CYP2C9 and 2C19 efficiently produced EETs. CYP4F2 produced 19/20-HETE. The catalytic properties of rat CYP2C23 were similar to those of human CYP2C9 and 2C19. The catalytic properties of CYP4F isoforms were also similar between humans and rats. A systematic analysis of P450 expression in various tissues and of its catalytic property may provide valuable information on the physiological roles of P450s in each tissue.

Key words: Cytochrome P450; arachidonic acid; EET; RT-PCR; HETE

Introduction

Arachidonic acid is metabolized by cytochrome P450s (P450s) to many biologically active eicosanoids including epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs). This enzymatic pathway was first described in the liver,[1)] however, it is now clear that arachidonic acid can be metabolized by P450s in many tissues including brain, kidney, lung, heart, and blood vessels.[2)] Recent reports suggest that specific P450s localize in each tissue and contribute to the regulation of homeostasis in tissue function.[3,4)] Furthermore, Yamazaki and Shimada[5)] reported the possibility of interaction between drugs and endogenous substrates such as arachidonic acid. The P450s in the CYP2B, 2C, and 2J families are thought to be drug-metabolizing enzymes but are reported to produce EETs.[4,6,7)] The EETs have four isomers, 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET. In the brain, astrocytes produce 5,6-EET which is suggested to be a component of calcium influx factor.[8)] Cyp2j9 was isolated from mouse brain and is abundant in cerebellar Purkinje cells.[9)] Cyp2j9 produced 19-HETE which inhibited the activity of P/Q-type Ca^{2+} channels. These channels are expressed preferentially in cerebellar Purkinje cells and are involved in triggering neurotransmitter release. In the kidney, EETs promote salt excretion in proximal tubules and have potent effects on renal vascular tone. For example, 5,6-EET was found to dilate isolated blood vessels and to inhibit sodium reabsorption and

Received; August 15, 2005, Accepted; September 16, 2005
**To whom correspondence should be addressed*: Susumu IMAOKA, Ph.D., *Department of Bioscience, School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda 669-1337, Japan. Tel. & Fax. +81-79-565-7673, E-mail:imaoka@ksc.kwansei.ac.jp*
Abbreviations used are: P450 or CYP, cytochrome P450; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; RT-PCR, reverse transcription-polymerase chain reaction; SDS-PAGE, sodium dodecylsulphate-polyacrylamide gel electrophoresis; GAPDH, glyceraldehydes 3-phosphate dehydrogenase; DLPC, dilauroylphosphatidylcholine

Table 1. Oligonucleotide primers used in this study

Name	GenBank Accession No.	Sequence	Size
CYP2A1 sense	NM_012692	5′-GCTCAGTGAGCGCTATGGTC-3′	283
anti-sense		5′-GCCTGCCTCCTCCAGGATAC-3′	
CYP2B1 sense	J00719	5′-GGCCATATTGTGGAGAAGCA-3′	730
anti-sense		5′-AGAAGCAGATCTGGTACGTT-3′	
CYP2C23 sense	X55446	5′-GATGCTGTCTTCCGTCATGC-3′	252
anti-sense		5′-GTAATAGGCTTGATGTCAAG-3′	
CYP2J3 sense	U39943	5′-GCTCCTCACTTCAATATCAAC-3′	347
anti-sense		5′-CTTGTAGTAGTCTTGTCTGGG-3′	
CYP4F-1 sense	NM_019623	5′-AAACGGTTGATTTCAGAAGGCAGTT-3′	205
anti-sense		5′-ACAGCAGGTCCATGAACAGCAAAGG-3′	
GAPDH sense	BC059110	5′-TCGTCTCATAGACAAGATGG-3′	136
anti-sense		5′-GTAGTTGAGGTCAATGAAGGG-3′	

GAPDH, Glyceraldehyde-3-phosphate dehydrogenase

potassium secretion in isolated perfused collecting tubules.[10] P450s in the CYP4A and 4F families are also reported to produce 19- and 20-HETEs.[11] 19-HETE is a stimulator of renal Na^+-K^+-ATPase[12] and 20-HETE is a potent vasoconstrictor of isolated rat aorta.[13] In the lung, 11,12-EET causes significant changes in rat airway electrical parameters, suggesting that it is involved in the control of lung fluid and electrolyte transport.[14] 5,6-EET and 14,15-EET increase endothelial permeability by depletion of endoplasmic reticulum Ca^{2+} stores in rat lung.[15] CYP2J3 was isolated from a rat heart cDNA library.[16] Recombinant CYP2J3 produced 8,9-EET, 11,12-EET, and 14,15-EET. 11,12-EET improved the recovery of cardiac contractility with the addition of perfusate prior to global ischemia of the heart, suggesting that 11,12-EET plays an important functional role in the response of the heart to ischemia.[16] These findings indicate that P450s have much more specific and complex physiological functions than are currently recognized. To clarify the physiological function of P450, it is necessary to identify a specific P450 in a particular tissue or organ and to characterize its catalytic activities. As described above, metabolites of arachidonic acid are one of the most interesting substances which have prominent physiological functions. The aim of the present study was to compare the arachidonic acid metabolism of rat P450s with those of human P450s in the same gene family.

In this study, we investigated the expression of five P450 isoforms (CYP2A1, 2B1, 2C23, 2J3, and 4F1) in liver, lung, kidney, spleen, heart, brain, and testis of rats. As described above, these P450s seem to play an important role in physiology in extrahepatic tissues. The metabolism of arachidonic acid by these rat P450s were compared with those of human P450s, CYP2A6, 2B6, 2C9, 2C18, 2C19, 2J2, and 4F2, expressed in insect cells using a baculovirus system.

Materials and Methods

Chemicals and P450s: Dilauroylphosphatidylcholine (DLPC) and arachidonic acid were obtained from Sigma Chemical Co. (St. Louis, MO). [1-^{14}C]Arachidonic acid was purchased from New England Nuclear (Boston, MA). Human CYP2A6, 2B6, 2C9, 2C18, 2C19, 2J2, and 4F2 expressed by the Baculovirus system were obtained from Gentest (Wobum, MA). NADPH was from Oriental Yeast Co. (Tokyo, Japan). All other chemicals and solvents were obtained from Wako Pure Chemical Industries (Osaka, Japan). CYP2A1, 2B1, 2J3, and 4F1 were purified from hepatic microsomes of Sprague-Dawley rats (Nippon Clea) as described previously.[17,18] CYP2C23 was purified from rat kidney microsomes.[19] Cytochrome b_5 and NADPH-P450 reductase were purified from rat liver microsomes as described previously.[17]

Detection of P450 expression by RT-PCR: Total RNA was isolated from the frozen liver, lung, kidney, spleen, heart, brain, and testis of rats using Isogen (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. After treatment with DNase, total RNA was converted to cDNA by reverse-transcription as described previously.[20] The cDNA produced was amplified using the primers listed in **Table 1**. PCR was done in a reaction mixture (50 μL) containing cDNA (0.1 μg), each primer (10 pmol), dNTP (0.2 mM), $MgCl_2$ (2.5 mM), and Ampli Taq Gold (Perkin-Elmer, Branchburg, NJ). PCR amplification was done for 30–40 cycles (94°C for 1 min, 56°C for 1 min, and 72°C for 2.5 min). We chose the maximum number of PCR cycles that can maintain the linearity of the amplification. The products were analyzed on a 2% agarose gel. The gels were stained with ethidium bromide, and visualized with UV.

Arachidonic acid metabolism: The metabolism of arachidonic acid by rat and human P450s was assayed

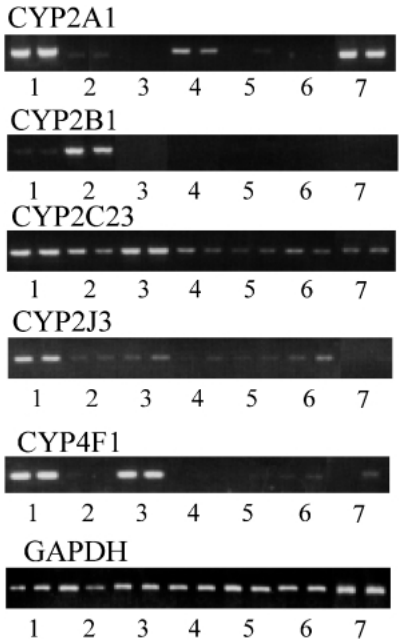

Fig. 1. Detection of P450 mRNAs by RT-PCR

Total RNA was isolated from each tissue and RT-PCR was done with primer sets indicated in **Table 1**. Tissues used in duplicate experiments were isolated from different rats. Amplified DNA fragments were analyzed by electrophoresis with 2% agarose. Lane 1, liver; lane 2, lung; lane 3, kidney; lane 4, spleen; lane 5 heart; lane 6, brain; and lane 7, testis

with [^{14}C]arachidonic acid by separation using HPLC with a reversed-phase column as described previously.[21,22] The reaction mixture containing recombinant human P450 (50 pmol) expressed by the baculovirus system or purified rat P450 (50 pmol) with cytochrome b_5 (50 pmol), NADPH-P450 reductase (0.1 units), and DLPC (5 μg) was incubated with arachidonic acid (100 μM) and NADPH for 30 min (for recombinant human P450) or 15 min (for purified rat P450) at 37°C. Metabolites were extracted and analyzed by HPLC. Activity of P450s was measured under conditions in which the metabolism was proportional to the P450 concentration and incubation time.

Results

Expression of P450 isoform mRNAs in various tissues: The expression of CYP2A1, 2B1, 2C23, 2J3 and 4F1 was investigated in liver, lung, kidney, spleen, heart, brain, and testis of rats by RT-PCR (**Fig. 1**). CYP2A1 was expressed in liver, spleen, and testis. The amount of P450 in spleen is reported to be small.[23] It is interesting that CYP2A1, which may have a specific function in the spleen, was detected. CYP2B1 was abundant in lung. The result is consistent with a previous report.[24] CYP2C23 was originally purified from rat kidney[19] and detected in all tissues examined in this study. Its levels were high in the liver and the kidney. CYP2J3 was first purified from the livers of starved rats.[25] In humans, its homologous P450 cDNA was isolated from heart.[26] In the present study, CYP2J3 was detected in abundance in the liver and in small amounts in the kidney and brain. In mice, a brain-dominant isoform, Cyp2j9, was reported.[9] CYP4F1 was first purified from rat liver and leukotriene ω-hydroxylase.[17,18] Its expression was detected in liver and kidney.

Metabolism of arachidonic acid by purified rat P450s: Production of 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, and 19/20-HETE from arachidonic acid was investigated in a reconstituted system including purified rat P450s (**Fig. 2**). CYP2A1 exhibited no activity toward arachidonic acid. CYP2B1 produced all metabolites detected in this study and produced 14,15-EET most efficiently. CYP2C23 efficiently produced EETs. 11,12-EET was produced most abundantly and CYP2C23 did not produce 5,6-EET. The results were consistent with a previous study.[22] CYP2J3 produced EETs but its levels of activity was low. In humans, CYP2J is reported to be an arachidonic acid epoxygenase.[26] CYP4F1 specifically produced 19/20-HETE.

Metabolism of arachidonic acid by human P450s and comparison of their activities with those of rats P450s: The metabolic activity of recombinant human P450s toward arachidonic acid are shown in **Fig. 3**. CYP2A6 revealed almost no activity similar to rat CYP2A1. CYP2B6 produced small amounts of EETs although rat CYP2B1 had moderate activity. CYP2C9, 2C18, and CYP2C19 are typical drug-metabolizing isoforms in human liver. CYP2C18 had weak activity but CYP2C9 and 2C19 efficiently metabolized arachidonic acid to HETEs and EETs. CYP2C19 revealed the highest levels of activity of any of the human P450 isoforms used in this study. CYP2J2 is reported as an arachidonic acid epoxygenase and also produced EETs in this study.[26] Its activity was stronger than that of rat CYP2J2 but much weaker than that of CYP2C9 or CYP2C19. Like rat P450, CYP4F2 produced 19/20-HETE specifically and efficiently.

Discussion

There are many reports dealing with the metabolism of arachidonic acid by P450s (reviewed in ref. 27). The physiological functions of EETs and 19- or 20-HETE among metabolites of arachidonic acid have been focused on. Choudhary *et al.*[23,28] reported a systematic investigation of the expression of many P450 mRNAs in mouse and human tissues. In the present study, we investigated the tissue distribution of several P450s in rats and compared the activity of rat P450s to metabolize arachidonic acid with that of human P450s. Rat CYP2A1 and human CYP2A6 had little activity

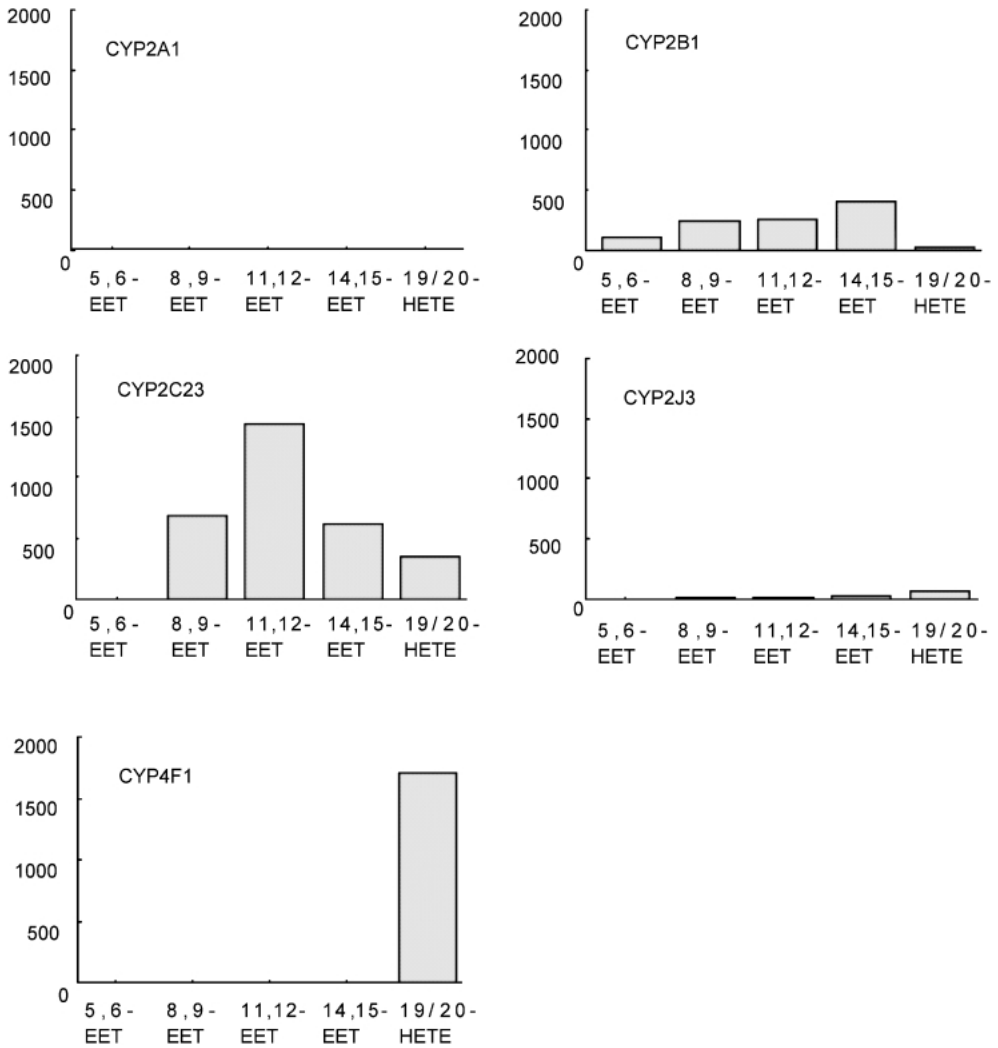

Fig. 2. Metabolism of arachidonic acid by purified rat cytochrome P450s

The y-axis indicates catalytic activity expressed as pmol of product/min/nmol of P450. EET, epoxyeicosatrienoic acid and HETE, hydroxyeicosatetraenoic acid

toward arachidonic acid. CYP2A1 mRNA was expressed in liver, spleen, and testis. Rat CYP2B1 metabolized arachidonic acid to produce four EETs efficiently but human CYP2B6 did not. CYP2B1 mRNA is expressed specifically in rat lung and reported to be present in airway epithelial cells including Clara cells.[29] CYP2B1 efficiently produced EETs which may play a role in regulating the volume and composition of airway surface liquids by modulating airway transepithelial transport.[30] CYP2C23 is highly expressed in rat kidney, where it is involved in regulating the biosynthesis of EETs. EETs contribute to integrated kidney function by directly affecting tubular transport processes and vascular tone.[3] In the present study, CYP2C23 mRNA was detected in abundance in rat kidney and was detected in all tissues tested. In humans, CYP2C9 was detected in the proximal tubules of the kidney[4] and distributed in many tissues although expression of CYP2C19 was detected in a limited tissue.[31] CYP2C9 produced 14,15-EET most efficiently and little 5,6-EET. CYP2C23 produced 11,12-EET most efficiently and did not 5,6-EET. 11,12-EET and 14,15-EET are found to have several physiological functions such as regulation of ion-transport and blood pressure.[32] Both CYP2C9 and CYP2C23 are major CYP2C forms in human and rat kidney, respectively.[4,33] In hypertensive rats, expression of CYP2C23 in renal tubules was decreased, concomitant with the decrease in EET production, suggesting that these P450s play important role in kidney functions.[34] CYP2C18 and CYP2C19 had different catalytic properties from CYP2C9. CYP2C18 is expressed in human kidney but CYP2C19 is not.[31] CYP2C18 had little activity toward arachidonic acid. CYP2C19 exhibited strong activity and produced

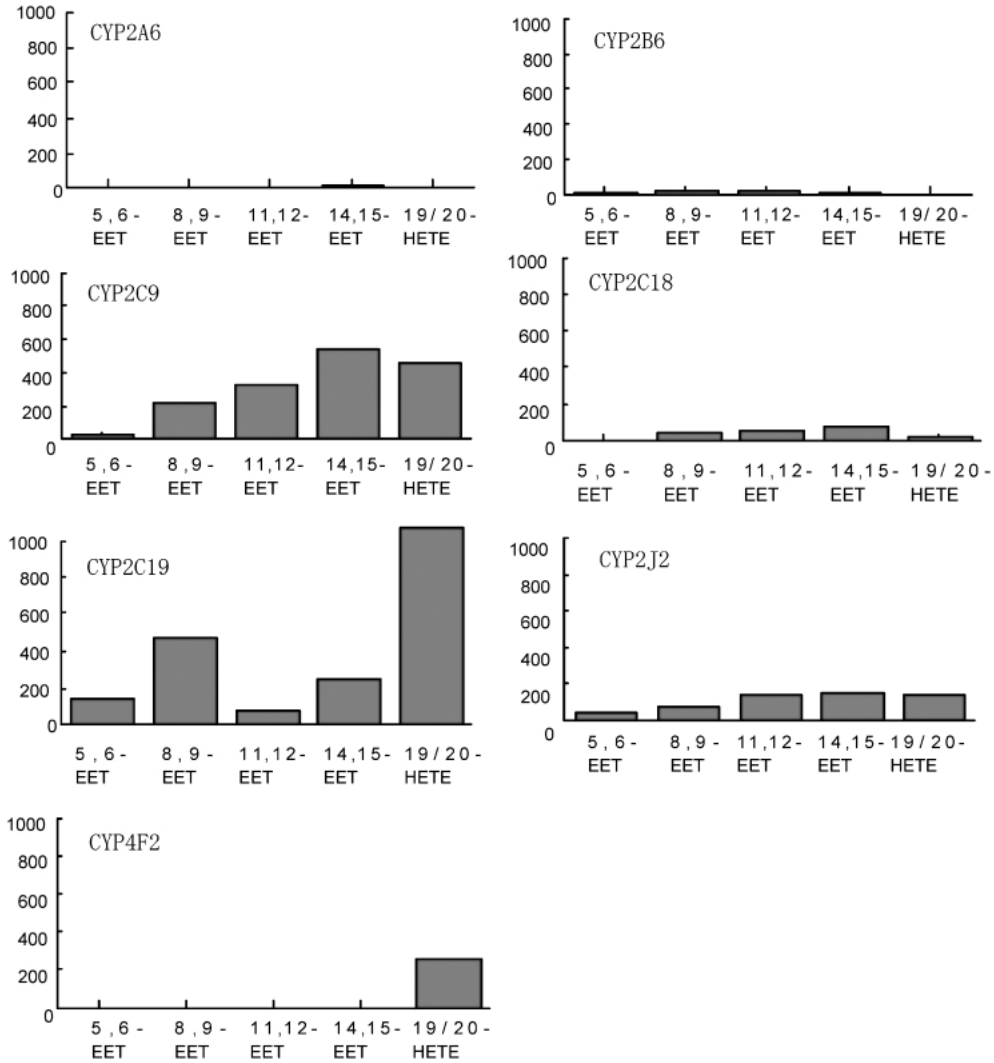

Fig. 3. Metabolism of arachidonic acid by human cytochrome P450s expressed in the baculovirus system

The y-axis indicates catalytic activity expressed as pmol of product/min/nmol of P450. EET, epoxyeicosatrienoic acid and HETE, hydroxyeicosatetraenoic acid

19/20-HETE in abundance. CYP2C19 also produced EETs, especially 8,9-EET. We first isolated CYP2J3 from rat liver.[25] CYP2J3 was thought to be an epoxygenase of arachidonic acid like CYP2C23 but produced little EET. In humans, CYP2J2 cDNA was first isolated from a heart library. In the present study, CYP2J3 was expressed in abundance in rat liver, followed by kidney and brain and not detected in heart. Recently, a brain-specific CYP2J form was isolated from mice.[9] It may have an important physiological role in the brain. CYP4F1 was dominantly expressed in liver and kidney, especially kidney. CYP4F1 specifically and efficiently produced 19/20-HETE. Human CYP4F2 had similar catalytic properties. Lasker *et al.*[11] found that CYP4F2 was present in proximal tubules of human kidney, and suggested that CYP4F2 partakes in pivotal renal functions, including the regulation of the salt and water balance.

A systematic analysis of the expression of P450 in various tissues may provide valuable information on the physiological roles of P450s in each tissue. Furthermore, a systematic comparison of human P450s with rat P450s may prove helpful in predicting the physiological functions of human P450s in each tissue.

Acknowledgments: This study was partially supported by a Grant-in-aid for Scientific Research (c) and a special Grant-in-aid of the Advanced Program of High Profile Research for Academia-Industry Cooperation, sponsored by the Ministry of Education, Science, Culture, Sports and Technology of Japan. This study was also partially supported by a Grant-in-aid from Kwansei Gakuin University.

References

1) Capdevila, J., Chacos, N., Werringloer, J., Prough, R. A. and Estabrook, R. W.: Liver microsomal cytochrome P-450 and the oxidative metabolism of arachidonic acid. *Proc. Natl. Acad. Sci. U S A*, **78**: 5362–5366 (1981).
2) Zeldin, D. C.: Epoxygenase pathways of arachidonic acid metabolism. *J. Biol. Chem.*, **276**: 36059–36062 (2001).
3) Fleming, I.: Cytochrome p450 and vascular homeostasis. *Circ. Res.*, **89**: 753–762 (2001).
4) Enayetallah, A. E., French, R. A., Thibodeau, M. S. and Grant, D. F.: Distribution of soluble epoxide hydrolase and of cytochrome P450 2C8, 2C9, and 2J2 in human tissues. *J. Histochem. Cytochem.*, **52**: 447–454 (2004).
5) Yamazaki, H. and Shimada, T.: Effects of arachidonic acid, prostaglandins, retinol, retinoic acid and cholecalciferol on xenobiotic oxidations catalysed by human cytochrome P450 enzymes. *Xenobiotica*, **29**: 231–241 (1999).
6) Daikh, B. E., Lasker, J. M., Raucy, J. L. and Koop, D. R.: Regio- and stereoselective epoxidation of arachidonic acid by human cytochromes P450 2C8 and 2C9. *J. Pharmacol. Exp. Ther.*, **271**: 1427–1433 (1994).
7) Zeldin, D. C., Plitman, J. D., Kobayashi, J., Miller, R. F., Snapper, J. R., Falck, J. R., Szarek, J. L., Philpot, R. M. and Capdevila, J. H.: The rabbit pulmonary cytochrome P450 arachidonic acid metabolic pathway: characterization and significance. *J. Clin. Invest.*, **95**: 2150–2160 (1995).
8) Rzigalinski, B. A., Willoughby, K. A., Hoffman, S. W., Falck, J. R. and Ellis, E. F.: Calcium influx factor, further evidence it is 5, 6-epoxyeicosatrienoic acid. *J. Biol. Chem.*, **274**: 175–182 (1999).
9) Qu, W., Bradbury, J. A., Tsao, C. C., Maronpot, R., Harry, G. J., Parker, C. E., Davis, L. S., Breyer, M. D., Waalkes, M. P., Falck, J. R., Chen, J., Rosenberg, R. L. and Zeldin, D. C.: Cytochrome P450 CYP2J9, a new mouse arachidonic acid omega-1 hydroxylase predominantly expressed in brain. *J. Biol. Chem.*, **276**: 25467–25479 (2001).
10) Escalante, B., Erlij, D., Falck, J. R. and McGiff, J. C.: Effect of cytochrome P450 arachidonate metabolites on ion transport in rabbit kidney loop of Henle. *Science*, **251**: 799–802 (1991).
11) Lasker, J. M., Chen, W. B., Wolf, I., Bloswick, B. P., Wilson, P. D. and Powell, P. K.: Formation of 20-hydroxyeicosatetraenoic acid, a vasoactive and natriuretic eicosanoid, in human kidney. Role of Cyp4F2 and Cyp4A11. *J. Biol. Chem.*, **275**: 4118–4126 (2000).
12) Escalante, B., Falck, J. R., Yadagiri, P., Sun, L. M. and Laniado-Schwartzman, M.: 19 (S)-hydroxyeicosatetraenoic acid is a potent stimulator of renal Na^+-K^+-ATPase. *Biochem. Biophys. Res. Commun.*, **152**: 1269–1274 (1988).
13) Schwartzman, M. L., Falck, J. R., Yadagiri, P. and Escalante, B.: Metabolism of 20-hydroxyeicosatetraenoic acid by cyclooxygenase. Formation and identification of novel endothelium-dependent vasoconstrictor metabolites. *J. Biol. Chem.*, **264**: 11658–11662 (1989).
14) Pascual, J. M., McKenzie, A., Yankaskas, J. R., Falck, J. R. and Zeldin, D. C.: Epoxygenase metabolites of arachidonic acid affect electrophysiologic properties of rat tracheal epithelial cells1. *J. Pharmacol. Exp. Ther.*, **286**: 772–779 (1998).
15) Alvarez, D. F., Gjerde, E. A. and Townsley, M. I.: Role of EETs in regulation of endothelial permeability in rat lung. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **286**: L445–451 (2004).
16) Wu, S., Chen, W., Murphy, E., Gabel, S., Tomer, K. B., Foley, J., Steenbergen, C., Falck, J. R., Moomaw, C. R. and Zeldin, D. C.: Molecular cloning, expression, and functional significance of a cytochrome P450 highly expressed in rat heart myocytes. *J. Biol. Chem.*, **272**: 12551–12559 (1997).
17) Funae, Y. and Imaoka, S.: Purification and characterization of liver microsomal cytochrome P-450 from untreated male rats. *Biochim. Biophys. Acta*, **926**: 349–358 (1987).
18) Hashizume, T., Imaoka, S., Mise, M., Terauchi, Y., Fujii, T., Miyazaki, H., Kamataki, T. and Funae, Y.: Involvement of CYP2J2 and CYP4F12 in the metabolism of ebastine in human intestinal microsomes. *J. Pharmacol. Exp. Ther.*, **300**: 298–304 (2002).
19) Imaoka, S., Nagashima, K. and Funae, Y.: Characterization of three cytochrome P450s purified from renal microsomes of untreated male rats and comparison with human renal cytochrome P450. *Arch. Biochem. Biophys.*, **276**: 473–480 (1990).
20) Hiroi, T., Imaoka, S., Chow, T. and Funae, Y.: Tissue distributions of CYP2D1, 2D2, 2D3 and 2D4 mRNA in rats detected by RT-PCR. *Biochim. Biophys. Acta*, **1380**: 305–312 (1998).
21) Tanaka, S., Imaoka, S., Kusunose, E., Kusunose, M., Maekawa, M. and Funae, Y.: Omega- and (omega-1)-hydroxylation of arachidonic acid, lauric acid and prostaglandin A1 by multiple forms of cytochrome P-450 purified from rat hepatic microsomes. *Biochim. Biophys. Acta*, **1043**: 177–181 (1990).
22) Imaoka, S., Wedlund, P. J., Ogawa, H., Kimura, S., Gonzalez, F. J. and Kim, H. Y.: Identification of CYP2C23 expressed in rat kidney as an arachidonic acid epoxygenase. *J. Pharmacol. Exp. Ther.*, **267**: 1012–1016 (1993).
23) Choudhary, D., Jansson, I., Schenkman, J. B., Sarfarazi, M. and Stoilov, I.: Comparative expression profiling of 40 mouse cytochrome P450 genes in embryonic and adult tissues. *Arch. Biochem. Biophys.*, **414**: 91–100 (2003).
24) Imaoka, S., Terano, Y. and Funae, Y.: Expression of four phenobarbital-inducible cytochrome P-450s in liver, kidney, and lung of rats. *J. Biochem. (Tokyo)*, **105**: 939–945 (1989).
25) Imaoka, S., Terano, Y. and Funae, Y.: Changes in the amount of cytochrome P450s in rat hepatic microsomes with starvation. *Arch. Biochem. Biophys.*, **278**: 168–178 (1990).
26) Wu, S., Moomaw, C. R., Tomer, K. B., Falck, J. R. and Zeldin, D. C.: Molecular cloning and expression of

CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. *J. Biol. Chem.*, **271**: 3460–3468 (1996).
27) Kroetz, D. L. and Zeldin, D. C.: Cytochrome P450 pathways of arachidonic acid metabolism. *Curr. Opin. Lipidol.*, **13**: 273–283 (2002).
28) Choudhary, D., Jansson, I., Stoilov, I., Sarfarazi, M. and Schenkman, J. B.: Expression patterns of mouse and human CYP orthologs (families 1–4) during development and in different adult tissues. *Arch. Biochem. Biophys.*, **436**: 50–61 (2005).
29) Keith, I. M., Olson, E. B., Jr., Wilson, N. M. and Jefcoate, C. R.: Immunological identification and effects of 3-methylcholanthrene and phenobarbital on rat pulmonary cytochrome P-450. *Cancer Res.*, **47**: 1878–1882 (1987).
30) Tsao, C. C., Coulter, S. J., Chien, A., Luo, G., Clayton, N. P., Maronpot, R., Goldstein, J. A. and Zeldin, D. C.: Identification and localization of five CYP2Cs in murine extrahepatic tissues and their metabolism of arachidonic acid to regio- and stereoselective products. *J. Pharmacol. Exp. Ther.*, **299**: 39–47 (2001).
31) Klose, T. S., Blaisdell, J. A. and Goldstein, J. A.: Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. *J. Biochem. Mol. Toxicol.*, **13**: 289–295 (1999).
32) Capdevila, J. H., Falck, J. R. and Harris, R. C.: Cytochrome P450 and arachidonic acid bioactivation. Molecular and functional properties of the arachidonate monooxygenase. *J. Lipid Res.*, **41**: 163–181 (2000).
33) Muller, D. N., Theuer, J., Shagdarsuren, E., Kaergel, E., Honeck, H., Park, J. K., Markovic, M., Barbosa-Sicard, E., Dechend, R., Wellner, M., Kirsch, T., Fiebeler, A., Rothe, M., Haller, H., Luft, F. C. and Schunck, W. H.: A peroxisome proliferator-activated receptor-alpha activator induces renal CYP2C23 activity and protects from angiotensin II-induced renal injury. *Am. J. Pathol.*, **164**: 521–532 (2004).
34) Wang, M. H., Smith, A., Zhou, Y., Chang, H. H., Lin, S., Zhao, X., Imig, J. D. and Dorrance, A. M.: Downregulation of renal CYP-derived eicosanoid synthesis in rats with diet-induced hypertension. *Hypertension*, **42**: 594–599 (2003).