Cytochrome P450 CYP2J9, a New Mouse Arachidonic Acid ω-1 Hydroxylase Predominantly Expressed in Brain*

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A cDNA encoding a new cytochrome P450 was isolated from a mouse brain library. Sequence analysis reveals that this 1,958-base pair cDNA encodes a 57–59-kDa polypeptide that is 70–91% identical to CYP2J subfamily P450s and is designated CYP2J9. Recombinant CYP2J9 was co-expressed with NADPH-cytochrome P450 oxidoreductase (CYPOR) in S9 cells using a baculovirus system. Microsomes of CYP2J9/CYPOR-transfected cells metabolize arachidonic acid to 19-hydroxyeicosatetraenoic acid (HETE) thus CYP2J9 is enzymologically distinct from other P450s. Northern analysis reveals that CYP2J9 transcripts are present at high levels in mouse brain. Mouse brain microsomes biosynthesize 19-HETE. RNA polymerase chain reaction analysis demonstrates that CYP2J9 mRNAs are widely distributed in brain and most abundant in the cerebellum. Immunoblotting using an antibody raised against human CYP2J2 that cross-reacts with CYP2J9 detects a 56-kDa protein band that is expressed in cerebellum and other brain segments and is regulated during postnatal development. In situ hybridization of mouse brain sections with a CYP2J9-specific riboprobe and immunohistochemical staining with the anti-human CYP2J2 IgG reveals abundant CYP2J9 mRNA and protein in cerebellar Purkinje cells. Importantly, 19-HETE inhibits the activity of recombinant P/Q-type Ca2+-channels that are known to be expressed preferentially in cerebellar Purkinje cells and are involved in triggering neurotransmitter release. Based on these data, we conclude that CYP2J9 is a developmentally regulated P450 that is abundant in brain, localized to cerebellar Purkinje cells, and active in the biosynthesis of 19-HETE, an eicosanoid that inhibits activity of P/Q-type Ca2+-channels. We postulate that CYP2J9 arachidonic acid products play important functional roles in the brain.

Cytochromes P450 catalyze the NADPH-dependent oxidation of arachidonic acid to cis-epoxyeicosatrienoic acids (5,6-, 8,9-, 11,12-, and 14,15-EET),1 mid-chain cis-trans-conjugated dienoals (5-, 8-, 9-, 11-, 12-, and 15-HETE), and/or ω-terminal alcohols of arachidonic acid (16-, 17-, 18-, 19-, and 20-HETE) (1–3). These eicosanoids are biosynthesized in numerous tissues where they possess a myriad of potent biological activities. For example, the EETs have been shown to control peptide hormone secretion in the pancreas (4), regulate vascular tone in the intestine, kidney, heart, and lung (5–9), affect ion transport in the kidney (6, 10, 11), and have anti-inflammatory properties (12). 20-HETE constricts renal and aortic vessels by inhibiting smooth muscle cell large conductance Ca2+-activated K+ channels (13, 14) and affects renal tubular ion transport (15). 19-HETE has also been reported to have effects on vascular tone and ion transport in the kidney (16, 17).

Remarkably little is known about cytochrome P450-dependent arachidonic acid metabolism in the central nervous system. Multiple different P450 isoforms capable of arachidonic acid metabolism are known to be expressed constitutively in brain tissue including members of the CYP1A, CYP2B, CYP2C, CYP2D, CYP2E, CYP4A, and CYP4F subfamilies (18–25). In addition, CYP1A1, CYP1B2, CYP2C, CYP2E, and CYP4A sub-family members have been shown to be inducible by xenobiotics in the brain (18, 26, 27). EETs are biosynthesized in the pituitary and hypothalamus where they have been shown to stimulate the release of various neuropeptides including somatostatin, arginine vasopressin, oxytocin, and luteinizing hormone-releasing hormone (28–30). EETs are also produced by a cytochrome P450 (most likely a CYP2F2 isoform) in brain astrocytes, cells that are anatomically and functionally associated with cerebral microvessels (31–33). Harder and colleagues (32, 33) have proposed that astrocyte-derived EETs participate in functional hyperemia and the local regulation of cerebral blood flow by dilating adjacent cerebral arterioles through a mechanism that involves activation of vascular smooth muscle K+ channels. One of the EETs has also been proposed as calcium influx factor, the elusive link between release of Ca2+ from intracellular stores and capacitative Ca2+ influx, in astrocytes (34). In contrast, 20-HETE, the primary arachidonic acid metabolite produced in cerebral arteries by a CYP4A iso-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF336850.

The sequence reported in this paper was submitted to the Committee on Standardized Cytochrome P450 Nomenclature and has been designated CYP2J9.

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1 The abbreviations used are: EET, cis-epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HPLC, high performance liquid chromatography; P450, cytochrome P450; CYPOR, NADPH-cytochrome P450 oxidoreductase; PFB, pentafluorobenzyl; kb, kilobase; PCR, polymerase chain reaction; GC/MS, gas chromatography/mass spectroscopy.

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form, activates vascular smooth muscle L-type Ca$^{2+}$ channels and promotes cerebral vasodilation (35).

Herein, we report the cDNA cloning and heterologous expression of CYP2J9, a new mouse P450 that is primarily expressed in the brain, regulated during postnatal brain development, and active in the $\omega$-1 hydroxylation of arachidonic acid to 19-HETE. We further demonstrate, using in situ hybridization and immunohistochemical techniques, that CYP2J9 mRNA and protein are localized to Purkinje cells in the cerebellum. Importantly, we show that 19-HETE significantly inhibits the activity of recombinant P/Q-type Ca$^{2+}$ channels, voltage-gated channels which are known to be preferentially expressed in Purkinje cells and are involved in triggering the release of neurotransmitters. Based on these data, we postulate that CYP2J9 products play important functional roles in the brain.

**EXPERIMENTAL PROCEDURES**

**Materials**—[125I]dATP, [1-14C]arachidonic acid, and [1-14C]linoleic acid were purchased from PerkinElmer Life Sciences. Restriction enzymes were purchased from New England Biolabs (Beverly, MA). PCR reagents including AmpliTaq® DNA polymerase were purchased from PerkinElmer Life Sciences. Oligonucleotides were purchased from Life Technologies, Inc. All other chemicals and reagents were purchased from Sigma unless otherwise specified.

**Cloning of the CYP2J9 cDNA**—An oligo(dT)-primed Agt10 C57Bl/6 mouse brain cDNA library (a gift from Dr. David Burt, University of Maryland) was screened with a radiolabeled 1.6-kb CYP2J8 cDNA fragment (GenBankTM accession number AF218857). Nucleic acid hybridizations were done at 57 °C in 0.9 M NaCl containing 0.05 M NaHPO$_4$/NaH$_2$PO$_4$ (pH 7.0), 0.5% SDS, 0.01 μM EDTA, 5X Denhardt’s solution, and 0.1 mg of heat-denatured salmon sperm DNA/ml. Fourteen duplicate positive clones were identified, of which 10 clones, selected at random, were plaque-purified and rescued into pBluescript SK(−) (Stratagene). Plasmid DNAs were isolated using a Qiagen Plasmid Purification Kit (Qiagen Inc., Chatsworth, CA), and insert sizes were determined by agarose gel electrophoresis after EcoRI digestion. The pBluescript cDNA inserts were partially sequenced on an ABI model 377 automated DNA sequencing using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase (PerkinElmer Life Sciences) and T3/T7 universal oligo-nucleotide primers. Nucleotide sequence chromatograms were edited using the software GCG (Genetics Computer Group, Inc., Madison, WI). One of the duplicate positive clones (clone WQ24-1, 1.8-kb) contained a novel sequence that shared homology with several human and rodent CYP2 family P450s (36). Based on alignment with other CYP2J subfamily members, it was determined that this clone was missing ∼120 nucleotides at the 5’-end of the coding region, and the 3’-end contained frameshift mutational events. A 2255-bp insert obtained by 5’-rapid amplification of cDNA ends using Mouse Brain Marathon-Ready™ cDNA (CLONTECH Laboratories, Palo Alto, CA) and the following gene-specific primer, 5’-GTCTCATGAGCCAACCTC- TGGTCTACC-3’. The resulting 2.0-kb cDNA was gel-purified, cloned into the pCR3.0TM-2.1 vector using the Original TA Cloning kit (Invitrogen Corp., Carlsbad, CA), and replicated in DH5α-competent Escherichia coli. Ten of the resulting clones contained identical sequences, one of which (clone WQ23J9-7) was completely sequenced utilizing a total of 14 oligonucleotide primers (20–25 nucleotides, each) that spanned the entire length of the sense and antisense cDNA strands.

**Heterologous Expression of Recombinant CYP2J9**—Co-expression of the protein encoded by the cloned 1.858-kb WQ23J9-7 cDNA insert (CYP2J9) with CYPOR in S9 insect cells was accomplished with the pAcUW51-CYPOR shuttle vector (kindly provided by Dr. Cosette Sera-bjit-Singh, GlaxoSmithKline, Research Triangle Park, NC) and the BaculoGold Baculovirus Expression System (PharMingen, San Diego, CA) using methods similar to those described previously (37–39). The CYP2J9 cDNA (nucleotides 130–1649) including the entire coding region was amplified by PCR and ligated into a slightly modified pAcUW51-CYPOR vector at the NheI and KgnI sites. The orientation and identity of the resulting expression vector (pAcUW51-CYPOR-CYP2J9) was confirmed by sequence analysis. In this construct, the expression of CYPOR is controlled by the p10 promoter, whereas the expression of CYP2J9 is independently controlled by the polyhedrin promoter. Cultured S9 cells were co-transfected with the pAcUW51-CYPOR-CYP2J9 vector and linear wild-type BaculoGold viral DNA, and recombinant viruses were purified as described (37, 38). Cultured S9 cells grown in spinner flasks at a density of 1.5–2 × 10$^6$ cells/ml were then infected with high titer CYP2J9-CYPOR recombinant baculovirus stock in the presence of δ-aminolevulinic acid and iron citrate (100 μM each). Cells co-expressing recombinant CYP2J9 and CYPOR were harvested 72 h after infection, washed twice with buffered saline, and used to prepare microsomal fractions by differential centrifugation at 4 °C. P450 content was determined spectrophotically according to the method of Omura and Sato (40) using a Shimadzu UV-3000 dual-wavelength/double-beam spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). S9 cells expressing only recombinant CYPOR (no P450s) were prepared as described (38).

**Incubations of Recombinant CYP2J9 with Arachidonic Acid**—**Product Characterization**—Reaction mixtures containing 0.05 μM Tris-Cl buffer (pH 7.5), 0.15 μM KCl, 0.01 μM MgCl$_2$, 8 mM sodium iodate, 0.5 IU/ml isocitrate dehydrogenase, CYP2J9-CYPOR-transfected S9 cell microsomes (an amount corresponding to 0.1–0.2 nmol P450/ml, final concentration), and [1-14C]arachidonic acid (55–56 μCi/μmol, 100 μM, final concentration) were constantly stirred at 37 °C. After temperature equilibration, NADPH (1 μM, final concentration) was added to initiate the reaction. At different time points (5–20 min, selected to ensure that the quantitative assessment of the rates of product formation reflect initial rates), aliquots were withdrawn, and the reaction products were extracted and analyzed by reverse-phase HPLC as described (37, 38). An authentic standard was identified by comparing their retention times with those of authentic EET phase HPLC properties with tetakis(acetoxyethyl) EET and HETE standards. In addition, the CYP2J9-derived 19-HETE was analyzed by negative ion chemical ionization GC/MS using methane as the reagent gas as described (41). Briefly, the 19-HETE was derivatized to the corresponding pentfluorobenzyl (PFB) ester, trimethylsilyl ether, and injected on a ThermoQuest Trace MS equipped with a 30-m Spheros SPB-5 capillary column (0.32 mm inner diameter, 0.25-μm film thickness). The gas chromatograph was programmed from 150 to 300 °C at a rate of 10 °C/min with an initial hold time of 1 min. Both full scan and selected ion monitoring data were collected. For chiral analysis, the EET and 19-HETE were collected from the HPLC eluant, derivatized to the corresponding PFB or methyl esters, and purified by normal-phase HPLC. The 11,12-EET-PFB, 9,10-EET-PFB, and 14,15-EET-methyl esters were resolved into corresponding antipodes by chiral-phase HPLC and quantified by liquid scintillation as described previously (42, 43). Chiral analysis of 19-HETE-methyl ester was performed on naphthylated derivatives using a Pirkle covalent n-phenyl glycine column (5 μm, 4.6 × 250 mm, Regis Chemical Co., Morton Grove, IL) equilibrated with 99.75% hexane, 0.25% isopropyl alcohol at 1 ml/min as described (44). Using the start conditions and by increasing the retention times for authentic (19S)- and (19R)-HETEs (76 and 80 min, respectively, with a resolution factor of 0.93). Control studies were performed by incubating uninfected S9 cells and microsomes and baculovirus-infected S9 cell microsomes expressing recombinant CYPOR but containing no spectrally evident P450 with arachidonic acid under identical conditions. In some experiments, [1-14C]arachidonic acid (55 μCi/μmol, 100 μM, final concentration) was substituted for [1-14C]linoleic acid, and the products were identified by co-chromatography with authentic hydroxyeicosatetraenoic acid and epoxyeicosatetraenoic acid standards on reverse-phase HPLC.

**Incubations of Mouse Brain Microsomes with Arachidonic Acid**—**Microsomal fractions were prepared from freshly isolated mouse brains by differential centrifugation and resuspended in 50 mM Tris-Cl buffer (pH 7.4), 1 mM dithiothreitol, 1 mM EDTA, and 20% glycerol. Reaction mixtures containing 0.05 mM Tris-Cl buffer (pH 7.5), 0.15 μM KCl, 0.01 μM MgCl$_2$, 8 mM sodium iodate, 0.5 IU/ml isocitrate dehydrogenase, 1 mM NADPH, 5 μM of brain microsomal protein/ml, and 100 μM [1-14C]arachidonic acid were incubated at 37 °C for 30–90 min. Reaction products were extracted and analyzed by HPLC as described (37, 38). All products were identified by comparing their reverse- and normal-phase HPLC properties with those of authentic EET and HETE standards.

**Northern Blot Hybridization and RNA PCR Analysis**—Normal mouse tissues (lung, brain, liver, kidney, spleen, heart, stomach, small intestine, colon, and testes) were obtained from adult male and female C57BL/6J mice fed NIH 31 rodent chow (Agway, St. Mary, OH) ad libitum and sacrificed by lethal CO$_2$ inhalation. For some experiments, the brain was subdivided into the following anatomic sections using conventional morphologic criteria as follows: cerebellum, cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, brainstem, and pituitary. RNA was prepared using TRIreagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s instructions. For Northern analysis, total RNA (20 μg) was denatured and electrophoresed in 1.2% agarose gels containing 2.2 M formaldehyde as de-
scribed (45). After capillary-pressure transfer to Hybond-N+ membrane (Amersham Pharmacia Biotech), the blots were hybridized with the cloned 1.958-kb CYP2J9 cDNA insert. Hybridizations were performed at 42 °C in 50% formamide containing 0.7 M NaCl, 70 mM sodium citrate, 5 × Denhardt’s solution, 0.5% SDS, and 0.1 mg of heat-denatured salmon sperm DNA/ml. Deformalized DNA was denatured at 0.25% (v/v) formamide, 0.5 M NaCl for 10 min. Sense or antisense RNA probes were hybridized to the sections at 55 °C for 18 h. After hybridization, the sections were washed once in 5 × SSC plus 10 mM β-mercaptoethanol for 30 min and once more in 50% formamide, 2 × SSC, and 100 mM β-mercaptoethanol for 60 min at 65 °C. After two additional washes in 10 mM Tris-Cl, 50 mM EDTA, 500 mM NaCl, sections were treated with RNAase A (10 μg/ml) at 37 °C for 30 min, followed by another wash in TNE at 37 °C. Sections were then washed twice in 2 × SSC and two more times in 0.1 × SSC at 65 °C. Slides were dehydrated with graded ethanol containing 300 mM ammonium acetate, dipped in photoemulsion (Ilford K5, Knutsford, Cheshire, UK) diluted 1:1 with 2% glycerol, and exposed for 4–5 days at 4 °C. After developing in Kodak D-19, slides were counterstained with hematoxylin.

For immunohistochemistry, adult mouse brain was fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Localization of CYP2J9 protein expression was investigated on serial sections (5–6 μm) with the anti-CYP2J9 IgG (1:100 dilution) using methods described previously (12, 37, 38). Preimmune rabbit IgG was used as the negative control and placed in the primary antibody.

Exposure of Mice to Mercury Vapor—Female mice (7 weeks old) were randomly divided into mercury-exposed and control groups (n = 5 each). Mice were exposed to mercury vapor (4.0 mg/m3) by nose only for 2 h/day for 3 consecutive days. Control mice where placed in holding tubes in a nose only exposure system and exposed to conditioned air at the same flow rates as the animals receiving mercury. After the final exposure, all animals were immediately euthanized by CO2 asphyxiation. Brains were quickly removed, frozen in liquid nitrogen, and used to prepare RNA as described above. All animal studies were conducted in accordance with principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals and approved by the NIEHS Animal Care and Use Committee.

Expression of Recombinant Ca2+ Channel Subunits in Xenopus Oocytes—cDNAs for the α1, α1B, α1C, and β1a subunits of voltage-gated Ca2+ channels, subcloned into pBluescript SK+ (α1A and β1a), pBlsta (α1B), and pGEM-3 (α1C), were generous gifts from Drs. William Horne (North Carolina State University; α1A and β1a), Diane Lipscombe (Brown University; α1B), and Lucie Parent (University of Montreal; α1C). Linearized cDNA templates were obtained by digestion with HindIII or EcoRI, and β1a, β1b, and β1c cDNA's were transcribed in vitro with T7 RNA polymerase using the Messame Machine kit (Ambion, Austin, TX) according to the manufacturer’s instructions and resuspended in water. Oocytes were surgically removed from female African clawed frogs, Xenopus laevis (Nasco, Atkinson, WI), and treated with collagenase (Sigma) to remove the follicular cell layer. Oocytes were injected with 10–15 ng of α1A, α1B, or α1C and 25 ng β1, β1a, β1b, or β1c cRNA (molar ratio 1:2:1) and incubated at 18°C for 2–5 days in ND96 media (96 mM NaCl, 2 mM KCl, 1 mM MgCl2, 1.8 mM CaCl2, and 5 mM Na-Hepes (pH 7.5)) supplemented with 50 μM gentamicin and 0.55 mM mg sodium pyruvate.

Two-electrode Voltage Clamp—Two-microelectrode voltage clamp was performed with a GeneClamp 500 amplifier driven by pCLAMP6 software (Axon Instruments, Foster City, CA). Microelectrodes were filled with 3 M KCl and had resistances of 0.5–2.1 megohms. To minimize the contribution of endogenous Ca2+-activated chloride channels to the electrical recordings, oocytes were injected with 46 nl of 50 mM 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (final intracellular concentration ~5 mM) ~15 min before recording (49). After two-electrode voltage clamp was established, oocytes were superfused with a chloride-free Ba2+ recording solution (5 mM Ba(OH)2, 102.5 mM NaOH, 1 mM KOH, 10 mM Na-Hepes, methanesulfonic acid (pH 7.2). The holding potentials were ~80 mV (α1A or α1B) or ~60 mV (α1C).

Test pulses of 650-ms duration were applied every 30 s. Test potentials were ~10 mV (α1A or α1B) or ~10 mV (α1C); these test potentials were at the peak of the current-voltage curve for the respective channel type. Currents were filtered at 1 kHz with an 8-pole Bessel low-pass filter and were sampled at 2 kHz (Axon Instruments). Ba2+ currents were recorded for 6–8 min to obtain a stable baseline and then the solution was switched to Ba2+ recording solution containing either 100 mM racemic 19-HETE, (19S)-HETE, or (19R)-HETE. Because addition of 0.01% ethanol (vehicle for 19-HETE) caused a small, transient inhibition of channels in some oocytes, we recorded currents in a solution containing 0.01% ethanol prior to
switching to the solution containing 19-HETE. In this way, the concentration of ethanol was the same in control and 19-HETE-containing solutions.

**Synthetic Procedures**—The [1-14C]EET internal standards were synthesized from [1-14C]arachidonic acid (56–57 Ci/mmol) by nonselective epoxidation as described previously (50). EET, mid-chain HETE, and 20-HETE standards were prepared by total chemical synthesis according to published procedures (51–53). 19-HETE enantiomers were synthesized as described (17). All synthetic compounds were purified by reverse-phase HPLC prior to use (37, 38, 54).

**Statistical Analysis**—All values are expressed as means ± S.E. Data were analyzed by analysis of variance using SYSTAT software. When F values indicated that a significant difference was present, Tukey’s HSD test for multiple comparisons was used. Values were considered significantly different if P < 0.05.

**RESULTS**

**Cloning of Mouse CYP2J9 cDNA**—Screening of a mouse brain cDNA library with the CYP2J9 cDNA probe yielded a novel clone (clone WQ24-1) that shared some sequence homology with several different human and rodent P450s; however, this clone did not contain an open reading frame and, based on alignment with other P450s, was missing the 5'-end including the start codon. The full-length cDNA (clone WQ2J9-7) was obtained by 5'-rapid amplification of cDNA ends. Complete nucleic acid sequence analysis of clone WQ2J9-7 revealed that the cDNA was 1958 nucleotides long, contained an open reading frame between nucleotides 141 and 1649 flanked by initiation (ATG) and termination (TGA) codons, had a 140 nucleotide 5'-untranslated region, and had a 308 nucleotide 3'-untranslated region with a polyadenylation tail (Fig. 1). The cDNA encoded a 502-amino acid polypeptide that had a derived molecular mass of 57,935 Da. The deduced amino acid sequence for WQ2J9-7 contained a putative heme-binding peptide (FSMGKRACLGEQLA) with the underlined conserved residues and the invariant cysteine at position 448. The polypeptide encoded by WQ2J9-7 also contained other structural features associated with CYP2 family P450s including an N-terminal hydrophobic peptide and a proline cluster between residues 40 and 51 (Fig. 1). A comparison of the deduced WQ2J9-7 amino acid sequence with that of other P450s indicated that it was (a) <31% identical to members of the CYP1, CYP3, CYP4, CYP5, CYP6, CYP7, and CYP11 families; (b) 40–43% identical with members of the CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, CYP2F, and CYP2G subfamilies; and (c) 72–91% identical to human, rabbit, rat, and mouse CYP2J subfamily P450s (37, 38, 46, 55–57). Based on the amino acid sequence homology with other CYP2Js, the new mouse hemoprotein encoded by clone WQ2J9-7 was designated CYP2J9 by the Committee on Standardized Cytochrome P450 Nomenclature.

**Heterologous Expression and Enzymatic Characterization of CYP2J9**—The protein encoded by clone WQ2J9-7 (CYP2J9) was co-expressed with CYPOR in recombinant P450—Heterologous Expression and Enzymatic Characterization of CYP2J9—

**Cloning of Mouse CYP2J9 cDNA**—Screening of a mouse brain cDNA library with the CYP2J9 cDNA probe yielded a novel clone (clone WQ24-1) that shared some sequence homology with several different human and rodent P450s; however, this clone did not contain an open reading frame and, based on alignment with other P450s, was missing the 5'-end including the start codon. The full-length cDNA (clone WQ2J9-7) was obtained by 5'-rapid amplification of cDNA ends. Complete nucleic acid sequence analysis of clone WQ2J9-7 revealed that the cDNA was 1958 nucleotides long, contained an open reading frame between nucleotides 141 and 1649 flanked by initiation (ATG) and termination (TGA) codons, had a 140 nucleotide 5'-untranslated region, and had a 308 nucleotide 3'-untranslated region with a polyadenylation tail (Fig. 1). The cDNA encoded a 502-amino acid polypeptide that had a derived molecular mass of 57,935 Da. The deduced amino acid sequence for WQ2J9-7 contained a putative heme-binding peptide (FSMGKRACLGEQLA) with the underlined conserved residues and the invariant cysteine at position 448. The polypeptide encoded by WQ2J9-7 also contained other structural features associated with CYP2 family P450s including an N-terminal hydrophobic peptide and a proline cluster between residues 40 and 51 (Fig. 1). A comparison of the deduced WQ2J9-7 amino acid sequence with that of other P450s indicated that it was (a) <31% identical to members of the CYP1, CYP3, CYP4, CYP5, CYP6, CYP7, and CYP11 families; (b) 40–43% identical with members of the CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, CYP2F, and CYP2G subfamilies; and (c) 72–91% identical to human, rabbit, rat, and mouse CYP2J subfamily P450s (37, 38, 46, 55–57). Based on the amino acid sequence homology with other CYP2Js, the new mouse hemoprotein encoded by clone WQ2J9-7 was designated CYP2J9 by the Committee on Standardized Cytochrome P450 Nomenclature.

**Heterologous Expression and Enzymatic Characterization of CYP2J9**—The protein encoded by clone WQ2J9-7 (CYP2J9) was co-expressed with CYPOR in Sf9 insect cells using the Sf9 baculovirus-infected insect cell microsomes. To reconstitute CYP2J9 activity and to ascertain the catalytic mechanism, we incubated mouse brain microsomes with radiolabeled arachidonic acid. The principal product formed was 19-HETE (retention time = 16–17 min) which was 66% of the total (Fig. 2A). This compound was identified based upon the following: (a) co-elution with authentic 19-HETE on reverse-phase HPLC (Fig. 2A); (b) co-elution with authentic 19-HETE on normal-phase HPLC (Fig. 2B); and (c) analysis of its PFB ester, trimethylsilyl ether derivative, by negative ion chemical ionization GC/MS in both full scan (data not shown) and single ion monitoring modes (Fig. 2C). EETs and other HETEs were formed in lower amounts (22 and 12% of the total, respectively). None of the metabolites were formed when NADPH was omitted from the reaction, when uninfected Sf9 cell microsomes were used, or when baculovirus-infected Sf9 cell microsomes expressing only CYPOR were used (data not shown).

Stereochemical analysis of the CYP2J9-derived 19-HETE revealed a preference for the (19R)-HETE enantiomer (optical purity 78%). Regiochemical analysis of the CYP2J9-derived EETs revealed a slight preference for epoxidation at the 14,15-olefin (42% of total EETs), whereas epoxidation at the 11,12- and 8,9-olefins occurred less often (28 and 30% of total EETs, respectively). Epoxidation at the 5,6-olefin occurred only rarely (<1% of the total EETs). Stereochemical analysis of the EETs revealed a slight preference for (14S,15R)-, (11R,12S)-, and (8R,9S)-EETs (optical purities, 60, 60, and 54%, respectively). Based on the above data, we conclude the following: (a) CYP2J9 is predominantly an arachidonic acid ω-1 hydroxylase, and (b) CYP2J9 is enzymologically distinct from other known P450s.

To determine if arachidonic acid was a preferred endogenous substrate for CYP2J9, we looked at other possible substrates. Recombinant CYP2J9 metabolized [1-14C]linoleic acid to mixtures of hydroxyoctadecenoic acids and epoxyoctadecenoic acids. Interestingly, the rate of linoleic acid metabolism by CYP2J9 was nearly 5-fold lower (0.08 nmol product/nmol P450/ min) than the rate of arachidonic acid metabolism by CYP2J9. We also examined several xenobiotic substrates, but without exception, the rates of metabolism were low-undetectable. Based on these data, we conclude that arachidonic acid is a preferred substrate for CYP2J9.

**Metabolism of Arachidonic Acid by Mouse Brain Microsomes**—To examine if 19-HETE is formed in mouse brain, we incubated mouse brain microsomes with radiolabeled arachidonic acid in the presence of NADPH. Reverse-phase HPLC analysis of the resulting products revealed that the major brain metabolite (retention time = 16–17 min) co-elutes with authentic 19- and 20-HETE (Fig. 2D) and the major 19-HETE peak produced by recombinant CYP2J9 (Fig. 2A). Indeed, the similarities between mouse brain and recombinant CYP2J9 chromatograms are striking. Since the HETEs are not well resolved under the reverse-phase HPLC conditions used, we collected the radioactive material generated by mouse brain microsomes which eluted between 15 and 20 min and re-injected it on a normal-phase HPLC system that resolves individual HETE regioisomers. The results show that this fraction contains mainly 20-HETE (45%) and 19-HETE (19%), with other mid-chain HETEs formed in lower amounts (Fig. 2E). Thus, the major CYP2J9 product 19-HETE is produced by mouse brain microsomes.

We also examined whether the anti-CYP2J9pep2 could inhibit brain microsomal arachidonic acid metabolism. Unfortunately, the anti-CYP2J9pep2 did not inhibit arachidonic acid metabolism by recombinant CYP2J9, even at concentrations as high as 5 mg of IgG/nmol of P450. Thus, although the anti-CYP2J9pep2 is specific for CYP2J9 on Western blots, it is not inhibitory. Moreover, the anti-CYP2J9 IgG is not inhibitory. Therefore, with currently available reagents, we are unable to
determine the exact contribution of CYP2J9 to 19-HETE biosynthesis in the brain. Other P450s may also contribute to brain 19-HETE production.

**Tissue Distribution of CYP2J9 mRNA—**To determine the relative organ abundance of CYP2J9 transcripts, total RNA extracted from adult male mouse tissues was blot-hybridized.
FIG. 2. Metabolism of arachidonic acid by recombinant CYP2J9 and mouse brain microsomes. A, reverse-phase HPLC chromatogram of the metabolites generated during an incubation of Sf9 insect cell microsomes containing recombinant CYP2J9 and CYPOR with [1-14C]arachidonic acid (AA). Under the HPLC conditions used, authentic 19-HETE elutes at 16–17 min. Chromatogram is representative of 14 incubations with four independent preparations of enzyme. B, normal-phase HPLC chromatogram of the 16–17-min peak produced during incubation of CYP2J9 with [1-14C]arachidonic acid. The retention times of authentic 19- and 20-HETE are 16 and 20 min, respectively, under the normal-phase conditions employed. Chromatogram is representative of three independent experiments. C, negative ion chemical ionization GC/MS analysis of the PFB ester, trimethylsilyl ether derivative of the 16–17-min peak produced during incubation of CYP2J9 with [1-14C]arachidonic acid. The top chromatogram shows the retention times of derivatized 19-HETE and 20-HETE standards (selected ion monitoring at $m/z$ 391), and the bottom chromatogram shows co-elution of the derivatized 14C-labeled 16–17-min peak with 19-HETE (selected ion monitoring at $m/z$ 393). D, reverse-phase HPLC chromatogram of the metabolites generated during an incubation of mouse brain microsomes with [1-14C]arachidonic acid. Chromatogram is representative of nine incubations with three different preparations of microsomes, each prepared from brains of six mice. E, normal-phase HPLC chromatogram of the the 15–20-min fraction produced during incubation of mouse brain microsomes with [1-14C]arachidonic acid. The retention times of authentic 12-, 15-, 11-, 19- and 20-HETE standards are 7, 8, 12, 16, and 20 min, respectively. Chromatogram is representative of three independent experiments.
under high stringency conditions with the full-length CYP2J9 cDNA probe. As shown in Fig. 3A, the CYP2J9 cDNA hybridized strongly with brain RNA to produce two transcripts as follows: (a) an abundant 2.0-kb transcript consistent with the size of the CYP2J9 mRNA; and (b) a larger 3.5-kb transcript of lower intensity. The identity of the 3.5-kb transcript is unknown but may represent another mouse P450 that shares nucleic acid sequence homology with CYP2J9 or an alternate splice variant of CYP2J9. The 2.0- and 3.5-kb CYP2J9 transcripts were also present, albeit at much lower levels, in mouse kidney RNA but were absent from RNA prepared from other mouse tissues including lung, liver, spleen, heart, stomach, small intestine, colon, and testis (Fig. 3A). The differences in mRNA abundance were not due to differences in the amount of RNA applied to each lane as assessed by ethidium bromide staining of the gel prior to transfer (Fig. 3A) and the membrane following transfer (data not shown). To determine if there were interanimal or gender differences in the abundance of CYP2J9 transcripts in brain or kidney, we performed Northern analysis on tissues collected from several adult male and female mice. As shown in Fig. 3B, whereas there was some interanimal variability in expression of the 2.0-kb CYP2J9 transcript in mouse brain, there were no consistent differences between the two sexes. In contrast, the 2.0-kb CYP2J9 transcript was more abundant in male versus female kidney RNA (Fig. 3C).

To confirm independently the tissue distribution of CYP2J9 mRNAs, we used a sensitive RNA PCR method that was highly specific for CYP2J9. We were able to amplify a 451-bp DNA fragment (predicted size) from reverse-transcribed mouse brain RNA and to a lesser extent from mouse kidney and colon RNA (Fig. 4A). We were unable to amplify a fragment from lung, liver, spleen, heart, stomach, small intestine, or testis RNA indicating that CYP2J5 mRNA expression was below the limit of detection of the RNA PCR assay in these tissues. RNA-PCR analysis using β-actin sequence-specific primers confirmed that the observations were not due to differences in the quality or amount of RNA used (Fig. 4A).

To examine the relative distribution of CYP2J9 transcripts within the central nervous system, we applied the above RNA PCR method to different portions of the mouse brain. As shown in Fig. 4B, we amplified a 451-bp DNA fragment from all brain sections including the cerebellum, cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, brainstem, and pituitary. Densitometric analysis based on data from 12 mice and normalized to the corresponding β-actin signals demonstrated that CYP2J9 transcripts were most abundant in the cerebellum (p < 0.0001) (Fig. 4C). Thus, based upon the Northern analysis and RNA PCR, we conclude that CYP2J9 mRNA is primarily present in mouse brain, expressed at lower levels in mouse kidney and colon, and absent from other mouse tissues. Moreover, within the brain, CYP2J9 transcripts are widely distributed but particularly abundant in the cerebellum. 

Expression of CYP2J9 Protein in Mouse Brain—Previous work (12, 37, 38, 46) has shown that the anti-CYP2J2 IgG is immunospecific for the CYP2J subfamily (i.e. it reacts with known CYP2J subfamily P450s in human, rat, and mouse but does not cross-react with non-CYP2Js). Immunoblotting of microsomal fractions prepared from S29 insect cells infected with recombinant CYP2J9 baculovirus using this anti-CYP2J2 IgG produced a band at ~57–58 kDa indicating that this antibody also cross-reacted with mouse CYP2J9 (Fig. 5A). In order to examine the distribution of CYP2J9 protein within the mouse brain, we performed immunoblotting of lysates prepared from different brain sections. The anti-CYP2J2 IgG detected a single protein at ~56 kDa in hippocampus, hypothalamus, frontal lobe, cerebral cortex, brainstem, cerebellum, and striatum (Fig.
This suggests that CYP2J immunoreactivity is widely distributed in the mouse brain and is consistent with the RNA PCR data (Fig. 4, B and C) showing the presence of CYP2J9 mRNAs in all brain sections. Immunoblotting of lysates prepared from the hippocampus, hypothalamus, frontal lobe, cerebral cortex, brainstem, and cerebellum of mice ages 3 days to 7 months showed that brain CYP2J immunoreactivity was present shortly after birth, gradually increased during the first 3 weeks of life, and achieved maximal levels during adulthood (Fig. 5C). These data indicate that brain CYP2J expression is regulated during postnatal development and that the developmental expression patterns are similar in different portions of the brain.

The electrophoretic mobility of the recombinant CYP2J9 protein was slightly lower (i.e. slightly higher molecular mass) than that of the CYP2J9 immunoreactive protein band detected in mouse brain tissues by the anti-CYP2J2 IgG (Fig. 5A). These differences, although minor, suggest that (a) the endogenous brain CYP2J9 protein is produced in a truncated form or post-translationally modified, or (b) the endogenous protein de-
are representative of five independent experiments. Lysates prepared from mouse cerebral cortex (30 μglane) were electrophoresed on Tris glycine gels, and the resolved proteins were transferred to nitrocellulose membranes. Membranes were cut and immunoblotted with either anti-CYP2J2 IgG (left side) or anti-CYP2J9pep2 IgG (right side). Lysates prepared from mouse hippocampus, hypothalamus, frontal lobe, cerebral cortex, brainstem, cerebellum, and striatum (30 μglane) were electrophoresed, transferred to nitrocellulose, and immunoblotted with the anti-CYP2J2 IgG. Results are representative of five independent experiments. C, Lysates prepared from 3-, 9-, 12-, and 21-day-old and 7-month-old mouse brain sections (30 μglane) were electrophoresed, transferred to nitrocellulose, and immunoblotted with the anti-CYP2J2 IgG. Results are representative of three independent experiments. D, Microsomal fractions prepared from Sf9 cells infected with recombinant CYP2J2, CYP2J3, CYP2J5, CYP2J6, CYP2J8, and CYP2J9 baculovirus (0.5 pmol of P450/lane) were electrophoresed, transferred to nitrocellulose, and immunoblotted with the anti-CYP2J9pep2 IgG. E, Lysates prepared from mouse cerebral cortex, striatum, cerebellum, brainstem, frontal lobe, hypothalamus, and hippocampus (30 μglane) were transferred to nitrocellulose, and immunoblotted with an antibody to recombinant CYP2J9 (0.5 pmol P450/lane) were electrophoresed, transferred to nitrocellulose, and immunoblotted with the anti-CYP2J9pep2 IgG. Results are representative of four independent experiments.

**Fig. 5. Expression of CYP2J9 protein in mouse brain by immunoblotting.** A, Microsomal fractions prepared from Sf9 cells infected with recombinant CYP2J9 were electrophoresed on Tris glycine gels, and the resolved proteins were transferred to nitrocellulose membranes. Membranes were cut and immunoblotted with either anti-CYP2J2 IgG (left side) or anti-CYP2J9pep2 IgG (right side). B, Lysates prepared from mouse hippocampus, hypothalamus, frontal lobe, cerebral cortex, brainstem, cerebellum, and striatum (30 μglane) were electrophoresed, transferred to nitrocellulose, and immunoblotted with the anti-CYP2J2 IgG. Results are representative of five independent experiments. C, Lysates prepared from 3-, 9-, 12-, and 21-day-old and 7-month-old mouse brain sections (30 μglane) were electrophoresed, transferred to nitrocellulose, and immunoblotted with the anti-CYP2J2 IgG. Results are representative of three independent experiments. D, Microsomal fractions prepared from Sf9 cells infected with recombinant CYP2J2, CYP2J3, CYP2J5, CYP2J6, CYP2J8, and CYP2J9 baculovirus (0.5 pmol of P450/lane) were electrophoresed, transferred to nitrocellulose, and immunoblotted with the anti-CYP2J9pep2 IgG. E, Lysates prepared from mouse cerebral cortex, striatum, cerebellum, brainstem, frontal lobe, hypothalamus, and hippocampus (30 μglane) were transferred to nitrocellulose, and immunoblotted with an antibody to recombinant CYP2J9 (0.5 pmol P450/lane) were electrophoresed, transferred to nitrocellulose, and immunoblotted with the anti-CYP2J9pep2 IgG. Results are representative of four independent experiments.

Inhibition of Recombinant Ca<sup>2+</sup> Channels by 19-HETE—We have shown previously that 11,12- and 14,15-EET inhibit cardiac L-type Ca<sup>2+</sup> channels, probably via a direct mechanism (58). In order to determine if 19-HETE, the major metabolite of CYP2J9, could also inhibit voltage-gated Ca<sup>2+</sup> channels, we tested its effects on various Ca<sup>2+</sup> channels expressed in Xenopus oocytes. Fig. 8 shows Ba<sup>2+</sup> currents through P/Q-type Ca<sup>2+</sup> channels (expressed as a combination of α1A/β1a sub-


FIG. 6. Localization of CYP2J9 mRNA and CYP2J9 protein in mouse brain. A, dark-field photomicrograph of a mouse cerebellar section stained with an 35S-labeled sense CYP2J9 RNA probe. There is intense silver grain deposition at the interface of the granular and molecular layer of the cerebellum. B, dark-field photomicrograph of an adjacent mouse cerebellar section stained with an 35S-labeled sense CYP2J9 RNA probe showing only low level background silver grain deposition (negative control). C, light-field illumination of the section in A at higher magnification showing localization of silver grain deposition to Purkinje cells. D, adjacent mouse cerebellar section stained with anti-CYP2J2 IgG showing strong immunostaining in Purkinje cells. Magnifications, × 92 (A and B) and 185 (C and D).

FIG. 7. Effect of mercury exposure on CYP2J9 mRNA expression. A, total RNA (20 μg) isolated from control and mercury-exposed mouse brains was blot-hybridized with the CYP2J9 cDNA probe as described in Fig. 3. After autoradiography, the radiolabeled CYP2J9 probe was removed by boiling, and the blot was rehybridized with a mouse β-actin probe. Results are representative of three independent experiments. B, autoradiographs were scanned, and relative CYP2J9 mRNA levels were determined by normalization to the β-actin signal. Values are the mean ± S.E. (n = 5). The * indicates p < 0.05 for comparison between control and mercury-treated groups by Student t test.

muscle, and neurons (60) were inhibited by 100 nM racemic 19-HETE (maximal inhibition, 11.4 ± 2.2%, n = 3, p < 0.05) (Fig. 8C). The kinetics of voltage-dependent activation and inactivation of each channel type were not altered by 19-HETE. For each channel type, inhibition by racemic 19-HETE was apparent within 30 s of application and reached maximal levels in 1–2 min (Fig. 8D, shown only for the P/Q-type Ca2+ channel). During sustained application of racemic 19-HETE, there was a small, variable recovery of the current magnitude. After removal of the 19-HETE, current amplitudes recovered partially to values that were approximately halfway between the pretreatment level and the level of maximal inhibition (Fig. 8D). The extent of inhibition by 100 nM racemic 19-HETE was approximately the same as that of 100 nM 11,12-EET on these channels (data not shown). Together, these data demonstrate that racemic 19-HETE, like the EETs, inhibits voltage-gated Ca2+ channels, including P/Q-types that are preferentially expressed in cerebellar Purkinje neurons. To our knowledge, this is the first demonstration of a physiologically relevant effect of a P450 ω-1 hydroxylase metabolite in the brain.

To determine if the effect of 19-HETE was stereoselective, we added synthetic (19R)- and (19S)-HETE to Xenopus oocytes expressing recombinant P/Q-type Ca2+ channels. Interestingly, we found that whereas both (19R)- and (19S)-HETE significantly inhibited these channels, the effect of (19R)-HETE was much more pronounced (Fig. 9). Thus, the maximal inhibition observed with 100 nM (19R)-HETE (11.0 ± 1.2%, n = 4) was comparable to that observed with racemic 19-HETE, whereas the maximal inhibition observed with 100 nM (19S)-HETE was only 5.0 ± 0.7% (n = 3, p < 0.05 versus, (19R)-HETE). Based on these data, we conclude that the major CYP2J9 enantiomer, (19R)-HETE, was also the most active in inhibiting P/Q-type Ca2+ channels.

DISCUSSION

Cytochromes P450 have been the subject of intense investigation by toxicologists and pharmacologists in the past because they catalyze the metabolism of a wide variety of drugs, industrial chemicals, environmental pollutants, and carcinogens (36, 61). The highest concentrations of P450 enzymes are found in the liver, and as a result, most studies have focused on the molecular and biochemical characterization of hepatic hemeprotein involved in xenobiotic metabolism and on their regulatory properties. Some P450s can also catalyze the NADPH-dependent oxidation of arachidonic acid to biologically active eicosanoids (EETs and HETEs) that have been shown to play critical roles in renal, pulmonary, intestinal, and cardio-
vascular function (1–17, 37, 57, 58). Recently, there has been growing interest in studying P450 metabolism of arachidonic acid in the brain in light of well documented effects of P450-derived eicosanoids in the release of neuropeptides and in the control of cerebral blood flow (28–35). In this report, we describe the cDNA cloning of CYP2J9, a new mouse P450 that is primarily expressed in the brain, regulated during postnatal brain development and active in the biosynthesis of 19-HETE. We further demonstrate that CYP2J9 is particularly abundant in cerebellar Purkinje cells and that 19-HETE inhibits P/Q-type Ca²⁺ channels, voltage-gated channels that are known to influence neurotransmitter release. CYP2J9 expression in Purkinje cells is also more abundant than in other cortical neurons (59, 60).

We found that CYP2J9 expression is particularly abundant in the cerebellum and highly localized to Purkinje cells, neurons that integrate information from within and outside the cerebellum, and communicate with motor nuclei. Tirumalai and co-workers (22) have shown that brain P450 content varies depending on the region, with the brainstem and cerebellum showing the highest levels. P450RAI-2 appears to be highly localized to the cerebellum and highly localized to Purkinje cells, neurones showing the highest levels. P450RAI-2, a protein that is predominantly expressed in the brain and is responsible for the metabolism of all-trans-retinoic acid to more polar, inactive metabolites. Among these enzymes, only CYP2D18 has been reported to metabolize arachidonic acid (64).

We found that CYP2J9 expression is particularly abundant in the cerebellum and highly localized to Purkinje cells, neurones that integrate information from within and outside the cerebellum, and communicate with motor nuclei. Tirumalai and co-workers (22) have shown that brain P450 content varies depending on the region, with the brainstem and cerebellum showing the highest levels. P450RAI-2, a protein that is predominantly expressed in the brain and is responsible for the metabolism of all-trans-retinoic acid to more polar, inactive metabolites. Among these enzymes, only CYP2D18 has been reported to metabolize arachidonic acid (64).

The recombinant CYP2J9 protein catalyzes the NADPH-dependent metabolism of arachidonic acid to 19-HETE as the principal reaction product; other HETEs and EETs are formed in much lower amounts. Hence, CYP2J9 is mainly an arachidonic acid ω-1 hydroxylase. The catalytic turnover of CYP2J9 is comparable to that of other P450s that are known to metabolize arachidonic acid including members of the CYP1A, CYP2B, CYP2C, CYP2E, CYP2G, and CYP4A subfamilies, other CYP2J isoforms, and CYP2D18 (37, 46, 64, 69–71). Importantly, the CYP2J9 product profile is distinct from that previously reported for other CYP2J enzymes. Thus, CYP2J1, CYP2J6, and

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2 D. C. Zeldin, unpublished observations.

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**Fig. 8.** Inhibition by 19-HETE of voltage-activated Ca²⁺ channels expressed in *Xenopus* oocytes. Inward Ba²⁺ currents (shown as downward deflections) of P/Q-type Ca²⁺ channels (A), N-type Ca²⁺ channels (B), and L-type Ca²⁺ channels (C) were evoked by depolarization from a holding potential (HP) of −80 mV (A and B) or −60 mV (C) to a test potential of −10 mV (A and B) or +10 mV (C) during the interval shown by the bar at the top. In each panel, the larger inward current amplitude (lower trace) was obtained before application of the racemic 19-HETE, and the smaller current amplitude (upper trace) was obtained 1–2 min after changing the bathing solution to one containing 100 nM 19-HETE. The time/amplitude scale is the same for A–C. Results shown are representative of 3–5 independent experiments. D shows a typical time course of racemic 19-HETE inhibition of P/Q-type Ca²⁺ channels (α1A/β1a). Normalized peak inward current amplitude during the depolarization is plotted versus time, with the duration of application of 100 nM 19-HETE shown by the bar. For this experiment, the maximal current amplitude was −2.25 μA.

**Fig. 9.** Inhibition of P/Q-type Ca²⁺ channels by (19R)- and (19S)-HETE. A, inward Ba²⁺ currents of P/Q-type Ca²⁺ channels, evoked by depolarizations from −80 to −10 mV, were recorded before (lower trace) and 3 min after (upper trace) addition of 100 nM (19R)-HETE. The inhibition was ~13% in this experiment. B, P/Q-type Ca²⁺ channels in a different oocyte before and 3 min after exposure to 100 nM (19S)-HETE. The inhibition was ~5% in this experiment.
CYP2J9 do not significantly metabolize arachidonic acid (55); CYP2J2 is predominantly an arachidonic acid epoxygenase (46); CYP2J3 and CYP2J4 are arachidonic acid epoxygenases and ω-1 hydroxylases (37, 56); and CYP2J5 is an arachidonic acid epoxygenase and mid-chain hydroxylase (38). Moreover, the CYP2J9 product profile is different from non-CYP2J P450s that metabolize arachidonic acid (64, 69–73). Among these, CYP2D18 biosynthesizes mainly EETs and forms only low amounts of 19-HETE (64); CYP4A1, CYP4A2, and CYP4A3 make primarily 20-HETE (71); and CYP2E1 forms mainly (19S)-HETE and (18R)-HETE (73). Therefore, we conclude that CYP2J9 possesses unique enzymological properties.

Little is known about the functional role of P450-derived eicosanoids in the brain. In the pituitary and hypothalamus, EETs are known to stimulate the release of neuropeptides, most likely through mechanisms that involve release of cAMP and increased intracellular calcium (28–30, 74). EETs are also potent vasodilators in the brain where they are believed to regulate local cerebral blood flow by enhancing K+ outward current, hyperpolarizing the resting membrane potential, and inhibiting voltage-gated Ca2+ channels in vascular smooth muscle cells (31–34). Indeed, astrocyte-derived EETs have been proposed to be the elusive link between neuronal metabolic activation and increased nutritive blood flow (i.e. mediators of functional hyperemia) (75). In contrast, 20-HETE is a known cerebral vasoconstrictor and is thought to act by enhancing L-type Ca2+ channel currents (35). We are unaware of previous studies evaluating the physiological role of 19-HETE in the brain.

The localization of CYP2J9 expression to Purkinje cells in the cerebellum suggested that CYP2J9 products may be essential for Purkinje cell function. An important finding of this study is that the major CYP2J9 product (19-HETE) significantly inhibits voltage-gated Ca2+ channels. It is now well established that Ca2+ influx through voltage-gated channels is important in triggering release of neurotransmitters, stimulating contraction of smooth and cardiac muscle, initiating the transcription of numerous genes, and controlling other critical cellular processes (60). In the central nervous system, Ca2+ influx through L-type Ca2+ channels may initiate specific gene expression, whereas Ca2+ influx through P/Q- and N-type Ca2+ channels is more important for triggering neurotransmitter release (60). The effect of 19-HETE was to inhibit all three types of voltage-gated Ca2+ channels. The P/Q-, N-, and L-type Ca2+ channels were each significantly inhibited by 19-HETE. The largest effect was observed in P/Q-type channels that are preferentially expressed in cerebellar Purkinje cells. Importantly, these effects occurred at nanomolar concentrations, levels of 19-HETE that are likely to be achievable in vivo (16). Although the level of inhibition that we observed (10–20%) is not very large, there is a steep power relationship between the rise in intracellular Ca2+ mediated by the influx through the voltage-gated channels and the rate of neurotransmitter release (76). Thus, even modest inhibition of Ca2+ channel activity by 19-HETE is likely to cause a profound inhibition of downstream events like neurotransmitter release and gene expression. Previous work has documented effects of 19-HETE on renal vascular tone and Na+/K+-ATPase activity (16, 17).

Mercy compounds are common pollutants and are considered to be some of the most toxic substances in the environment (77). Following mercury vapor exposure, the central nervous system is affected, and significant neurologic abnormalities can result (77). In fact, the cerebellum is a primary target for mercury-induced lesions, and Purkinje cells show pronounced mercury accumulation after mercury vapor exposure (77). The present study demonstrates that CYP2J9 expression is particularly abundant in the cerebellum and highly localized in Purkinje cells. Interestingly, mercury vapor exposure in vivo caused enhanced expression of CYP2J9 mRNA in mouse brain. Thus, an important environmental toxicant can regulate CYP2J9 expression at a pretranslational level. Further work will be necessary to define the molecular mechanisms underlying mercury-induced alteration in CYP2J9 expression and its potential toxicological and functional consequences, but the possibility exists that CYP2J9 up-regulation could be a sensitive biochemical marker of exposure to this toxic transition metal.

In summary, we report the CDA cloning and heterologous expression of CYP2J9, a new mouse P450 that is primarily expressed in the brain, regulated during postnatal brain development, and active in the ω-1 hydroxylation of arachidonic acid to 19-HETE. We further show, using in situ hybridization and immunohistochemical techniques, that CYP2J9 mRNA and protein are localized to Purkinje cells in the cerebellum. Importantly, we demonstrate that 19-HETE significantly inhibits the activity of recombinant P/Q-type Ca2+ channels, voltage-gated channels that are known to be expressed preferentially in Purkinje cells and are involved in triggering the release of neurotransmitters. Based on these data, we conclude that CYP2J9 is an enzymologically distinct heme-thiolate protein that contributes to the ω-1 hydroxylation of arachidonic acid in cerebellar Purkinje cells. In light of the effects of 19-HETE on voltage-gated Ca2+ channel activity, and the documented role of intracellular Ca2+ mediated by the influx through these channels on rates of neurotransmitter release, we postulate that CYP2J9 products play important functional roles in the brain.

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