Molecular Cloning and Functional Expression of a Phorbol Ester-inducible 8S-Lipoxygenase from Mouse Skin*

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One of the effects of topical application of phorbol ester to mouse skin is the induction of an 8S-lipoxygenase in association with the inflammatory response. Here we report the molecular cloning and characterization of this enzyme. The cDNA was isolated by polymerase chain reaction from mouse epidermis and subsequently from a mouse epidermal cDNA library. The cDNA encodes a protein of 677 amino acids with a calculated molecular mass of 76 kDa. The amino acid sequence has 78% identity to a 15S-lipoxygenase cloned recently from human skin and approximately 40% identity to other mammalian lipoxygenases. When expressed in vaccinia virus-infected Hela cells, the mouse enzyme converts arachidonic acid exclusively to 8S-hydroperoxyeicosatetraenoic acid while linoleic acid is converted to 9S-hydroperoxy-linoleic acid in lower efficiency. Phorbol ester treatment of mouse skin is associated with strong induction of 8S-lipoxygenase mRNA and protein. By Northern analysis, expression of 8S-lipoxygenase mRNA was also detected in brain. Immunohistochemical analysis of phorbol ester-treated mouse skin showed the strongest reaction to 8S-lipoxygenase in the differentiated epidermal layer, the stratum granulosum. The inducibility may be a characteristic feature of the mouse 8S-lipoxygenase and its human 15S-lipoxygenase homologue.

At least five distinct lipoxygenase enzymes are expressed in the mouse. Three of these enzymes are best known for their occurrence in different types of blood cells. In common with other mammals, a 5S-lipoxygenase is present in leukocytes and is responsible for synthesis of the pro-inflammatory mediators, the leukotrienes (1, 2). A 12S-lipoxygenase is found in platelets and several other tissues including skin (3–5). A second type of 12S-lipoxygenase, which is closely related in sequence to the human and rabbit “reticulocyte-type” of 15S-lipoxygenases, occurs in certain macrophages (5). The fourth mouse lipoxygenase to be characterized is another enzyme to have 12S-lipoxygenase activity; it was cloned recently from mouse skin and has been classified as an epidermal 12S-lipoxygenase (6, 7). All four of these murine lipoxygenases enzymes have been characterized at the cDNA and genomic levels.

The fifth known mouse lipoxygenase was described originally in 1986 by Fürstenberger, Marks, and coworkers (8) as an enzyme in skin forming 8-HETE1 and inducible by phorbol ester treatment. It was shown subsequently that this enzyme forms the 8S enantiomer (9), and isolation of the corresponding hydroperoxide confirmed identification of the enzyme as a lipoxygenase (10). Mouse skin is the only reported site of synthesis of 8S-HETE in animal tissues, and there is no indication from the literature pointing to a potential homologue of the mouse 8S-lipoxygenase in other mammals.

In the course of studies on HETE synthesis in skin, we recently cloned a second type of 15S-lipoxygenase from human skin (11). This enzyme is different from the well known reticuloocyte type of 15S-lipoxygenase in that it oxygenates arachidonic acid purely at C-15 and linoleic acid is a relatively poor substrate. For clarity, we will refer to the reticuloocyte-type of 15S-lipoxygenase as 15-Lox-1 and the more recently cloned enzyme as 15-Lox-2. It was not clear a priori what is the animal homologue of the new human lipoxygenase, 15-Lox-2. In searching for a potential murine homologue, we carried out a series of PCR reactions using mouse skin. This led to the detection of a new mouse cDNA that is characterized in this report.

EXPERIMENTAL PROCEDURES

Preparation of Mouse Epidermal Total RNA and cDNA Synthesis

Phorbol ester (PMA, 10 nmol) dissolved in 50 µl of acetone was applied topically onto dorsal skin of 6–7-day-old mice. At 21–24 h after PMA treatment, the mice were euthanized, and epidermis was prepared from the frozen dorsal skin as described previously (9). The frozen epidermis was dropped into guanidinium thiocyanate solution, the lysis buffer from the RNaseasy RNA extraction kit (QIAGEN). After a brief sonication using an ultrasonic probe (2 s, twice), total RNA was extracted according to the manufacturer instructions. Approximately 50 µg of total RNA was recovered in 50 µl of water. Twenty µl aliquots were used in 50 µl reactions for first strand cDNA synthesis using an oligo(dT)-adaptor primer (12). One µl aliquots of cDNA were used directly in PCR reactions.

PCR Cloning of Epidermal Lipoxygenase cDNA

Initial PCR Clone—Two upstream degenerate primers encoded the sequence DVWLLAK. The two primers differed only in using alternative codons for the 3′-lysine, AAA or AAG, and they are referred to below as WLLAK+(AAA) and WLLAK-(AAG) (11). For the first round PCR reaction, each upstream primer was used in separate reactions against a set of downstream primers encoding three amino acid se-

1 The abbreviations used are: HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HPODE, hydroperoxyoctadecadienoic acid; 15-Lox-1, reticuloocyte-type of 15S-lipoxygenase; 15-Lox-2, second type of human 15S-lipoxygenase; PMA, phorbol 12-myristate 13-acetate; HPLC, high pressure liquid chromatography; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; bp, base pair(s).
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quences beginning GQ that occur seven amino acids downstream of the most 3′-histidine ligand to the iron: the sequence GQLDV occurs in mammalian 12S- and 15S-lipoxygenases, GQYDW occurs in 5S-lipoxygenases, and GPQDS occurs in the new human 15S-lipoxygenase, 15-Lox-2 (11). The primer sequences were reported previously (11), except for the new degenerate downstream primer encoding GPQDS: 5′-CGA-GCA-GCA-RTC-AAA-ITT-GCC (where S, “strong,” encodes C or G). For the second round nested PCR reaction, the upstream primer was retained as before (WLLAK(AA) or WLLAK(AAG)) while the downstream primer was changed in all reactions to encode the sequence ELQXWWRR (11). After the second round PCR, only the reaction that originally used the WLLAK(AAG) and GPQDS primers yielded a visible PCR product. This product was 500 bp in size. The first round PCR reaction was primed with cDNA from phorbol ester-treated mouse epidermis, 1 μl per 50-μl PCR reaction (from a 50-μl cDNA synthesis using 20 μg total RNA), and using 10 mM Tris, pH 8.3, 50 mM KCl, 3 mM MgCl₂, with 0.2 μM of each dNTP and 0.25 μl (1.25 units) of AmpliTaq DNA polymerase (Perkin-Elmer) in a Perkin-Elmer 480 thermocycler. After the addition of the cDNA at 80 °C (hot start), the PCR was programmed as follows: 94 °C for 2 min, 1 cycle; 50 °C for 1 min, 72 °C for 1 min, 94 °C for 1 min, 30 cycles; 72 °C for 10 min, 1 cycle; and then the block temperature was held at 4 °C. The second round reaction was primed with the equivalent of 0.1 μl of the first round reaction products (added as a 10-fold dilution). The protocol was 94 °C for 2 min for 1 cycle and 58 °C for 1 min, 72 °C for 1 min, and 94 °C for 1 min for 30 cycles, and the protocol was completed with 1 cycle at 72 °C for 10 min and then the block temperature was held at 4 °C.

3′-RACE and 5′-RACE—The 3′-sequence was obtained using established upstream sequence 5′-G-AGC-CTT-GTC-TCT-GAA-ATA-GTC-AG-3′ against a downstream primer based on the adaptor-linked oligo(dT) primer used for cDNA synthesis (12). The 5′-RACE was accomplished using a kit from Life Technologies, Inc. according to the manufacturer instructions. The gene-specific downstream primers were 5′-GTG-AGG-AAT-CAA-TAG-CTT-GAA-GAG-3′, and 5′-AGT-GTG-GAC-GTC-CTC-GAT-GAT-G-3′.

Full-length Clones Obtained by PCR—The upstream primer encoded the N terminus with a HindIII site added at the 5′-end to facilitate subcloning. The upstream primer sequence was 5′-G-AGC-CTT-GTC-TCT-GAA-ATA-GTC-AG-3′, and the downstream primer encoded the C terminus of the protein with an added 5′ EcoRI site, 5′-G-AGG-GCT-ATC-TTT-GTT-GGC-GAC-ACT-GTT-3′. These two primers were purified by HPLC as the DMT (dimethoxytrityl) derivative (12). After deprotection, they were used in PCR reactions with a proof-reading mixture of Taq/Pfu/Bo DNA polymerase (Expand High Fidelity, Boehringer-Mannheim) according to the manufacturer instructions. The reaction conditions were 94 °C, 2 min for 1 cycle; 58 °C for 30 s, 72 °C for 1.5 min, and 96 °C for 15 s for 3 cycles; 68 °C for 2 min, and 96 °C for 15 s for 30 cycles; 72 °C for 10 min for 1 cycle; and the block temperature was held at 4 °C.

DNA Sequencing
cDNAs were sequenced using the Oncor Fidelity manual dideoxy chain termination method or by automated sequencing on an ABI Prism 310 Genetic analyzer and fluorescence-tagged dye terminator cycle sequencing (Perkin-Elmer).

HPLC Analysis of Lipoxygenase Metabolism

The lipoxygenase metabolism of [1-14C]linoleic acid was evaluated essentially as described previously (9). Following incubation with 100 μM substrate, products were extracted using the Bligh and Dyer procedure (13), and the extracts were analyzed by reversed phase-HPLC, normal phase-HPLC, and chiral column analysis (14). The hydroperoxide products were reduced with triphenylphosphine, methylated with diazomethane, purified by normal phase-HPLC, and then the stereochemistry was analyzed using a Chiralcel OD column.

Expression of Mouse 8S-Lipoxygenase Clones

The PCR products corresponding to the open reading frame of the cDNA were ligated directly into pCR3.1 (Invitrogen) and expressed by transient transfection in HeLa cells using VTF-7, a recombinant vaccinia virus containing the T7 RNA polymerase gene (15), or in human embryonic kidney (HEK) 293 cells using the adenovirus virus-associated RNA gene (7). In the former system, cells plated at 1 × 10⁶ cells/35-mm well 48 h earlier were transfected with 1 μg of plasmid DNA and 3 μg of Lipofectin and harvested after 12 h. In the HEK system, cells plated at 1 × 10⁶ cells/10-cm dish 24 h earlier were transfected with 10 μg of plasmid DNA by the calcium phosphate method and harvested after 2–3 days (7). The harvested cells were sonicated on ice, and the resulting homogenates were incubated with 100 μM [1-14C]arachidonic acid or [1-14C]linoleic acid for 45 min at room temperature. The metabolites were extracted and analyzed as described above.

Screening of cDNA Library

The library was a commercial α Unizap XR skin cDNA library prepared using poly(A)+ RNA isolated from whole skin of C57/Black female mice (Stratagene). It was screened with a 347-bp Bos MI fragment of the mouse 8S-lipoxygenase cDNA (PCR clone) as probe.

Northern Analysis

Poly(A)+ RNA was prepared from PMA- or acetone-treated frozen dorsal skin using Tri Reagent® (Molecular Research Center, Inc.) and Oligotex® (Qiagen) according to the manufacturer instructions. The poly(A)+ RNA was electroeluted and agarose-ethyldene glycol and then blotted to a Hybond-N+ nylon membrane (Amersham Corp.). The membrane was hybridized with 32P-labeled DNA probe (complementary with a 0.6-kilobase EcoRV/BamHI fragment of mouse epidermal 8S-lipoxygenase) prepared using the Multiprime DNA labeling kit (Amersham Corp.) and Rapid-hybridization buffer (Amersham Life Science, Inc.) and then washed according to the manufacturer specifications. Blots were exposed to Fuji x-ray film at −80 °C. Mouse cyclophilin cDNA was used as a house-keeping gene to access loading of RNA.

Western Analysis

After quantitation by Bradford assay (Bio-Rad), protein was separated by SDS-polyacrylamide gel electrophoresis and then transferred electrophoretically to Hybond ECL nitrocellulose membranes (Amersham Corp.). These were probed using a rabbit polyclonal antibody raised against the human 15-Lox-2. This antibody recognizes 15-Lox-2 and the mouse 8S-lipoxygenase, but not the human reticulocyte type of 15S-lipoxygenase (see “Results”). Donkey anti-rabbit Ig linked with horseradish peroxidase (Amersham Life Science, Inc.) was the secondary antibody. Specifically bound protein was detected by chemiluminescence using the ECL Western blotting detection reagents (Amersham Life Science, Inc.).

Immunohistochemical Analysis

The dorsal and tail skin of 6–7-day-old mice were treated with acetone or PMA (10 nmol for dorsal skin, 2 nmol for tail skin). After 24 h, the animals were euthanized, and the dorsal and tail skin were washed with soap and then rinsed thoroughly with water. Whole dorsal and tail skin was immersion-fixed for 24 h in 4% paraformaldehyde in phosphate-buffered saline (PBS), dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Skin sections were deparaffinized and rehydrated in graded alcohols. Endogenous peroxidase activity was blocked in 3% hydrogen peroxide/methanol for 20 min followed by incubation in 10% goat serum for 20 min. Sections were incubated at room temperature for 1 h in a 1/2500 dilution either of primary rabbit antisera that was used for Western analyses of 8S-lipoxygenase or of pre-immune serum. After rinsing in PBS, sections were incubated with the biotinylated secondary antibodies and peroxidase-labeled tertiary antisera supplied with an ABC Elite kit (Vector Corp., Burlingame, CA) followed by visualization of immunoprecipitate with 3,3-diaminobenzidine chromagen (Biogenex, San Ramon, CA).

RESULTS

Molecular Cloning by Reverse Transcriptase-PCR—A series of PCR reactions were carried out with cDNA prepared from phorbol ester-treated mouse skin as template and using degenerate primers based on well conserved sequences in mammalian lipoxygenases (11). The primers were identical to those used previously in the cloning of a novel 15S-lipoxygenase (15-Lox-2) from human skin, with the addition of an extra downstream primer that better represented the sequence of the new human enzyme. After running the protocol of nested PCR reactions, a strong band of the expected size of 500 bp was obtained one of the reactions that used the new downstream primer (see “Experimental Procedures” for details). The sequence of this PCR product showed a striking homology to the human lipoxygenase sequence. The remainder of the mouse cDNA was cloned by conventional 3′- and 5′-RACE (see “Experimental Procedures”). cDNA corresponding to the open read-
Characterization of Mouse 8S-Lipoxygenase

The sequence is from two identical cDNA library clones, except for the 5'-untranslated region, which was obtained by 5'-RACE. The consensus sequences used in PCR cloning are underlined. Seven PCR clones encoding the open reading frame and that expressed 8S-lipoxygenase activity were also fully sequenced. These contained multiple nucleotide substitutions that changed the encoded amino acid sequence yet had no apparent detrimental effect on their expressed 8S-lipoxygenase activity: clone G2, 112A (Leu to Met), 227-C (Val to Ala), 1607-A (Arg to Gln); clone G5, 227-C (Val to Ala), 1607-A (Arg to Gln); clone G11, 227-C (Val to Ala), 1607-A (Arg to Gln); clone K1, 227-C (Val to Ala); clone K2, same amino acid sequence as library clone; clone K7, 1237-G (Ile to Val); and clone K12, 95-G (Glu to Gly), 173-G (Pro to Arg). The cDNA sequence is available in GenBank with accession number U93277.

Fig. 1. Nucleotide and deduced amino acid sequences of the mouse 8S-lipoxygenase. The sequence is from two identical cDNA library clones, except for the 5'-untranslated region, which was obtained by 5'-RACE. The consensus sequences used in PCR cloning are underlined. Seven PCR clones encoding the open reading frame and that expressed 8S-lipoxygenase activity were also fully sequenced. These contained multiple nucleotide substitutions that changed the encoded amino acid sequence yet had no apparent detrimental effect on their expressed 8S-lipoxygenase activity.
each other and also exactly matched one of the PCR products in the open reading frame (Fig. 1). Fig. 2 shows an alignment with the human 15-Lox-2. The sequences are 78% identical at both the DNA and protein levels.

**Transient Expression in HEK and Hela cells**—The library clone was obtained relatively late in this study, and therefore much of the expression work described here was carried out using several of the PCR products. It became apparent very early on that there is some problem in expression of this mouse lipoxygenase. Using our standard transient expression system in HEK 293 cells, it was only very occasionally that we detected enzymatic activity in the expressed mouse lipoxygenase. Positive controls using the human reticulocyte-type of 15α-lipoxygenase (15-Lox-1) or the second type of human 15α-lipoxygenase (15-Lox-2) were run in every experiment, and these cDNAs always expressed with readily detectible activity. In the few instances when active mouse lipoxygenase was obtained in HEK cell expression, the enzyme converted arachidonic acid to 8S-hydroperoxyeicosatetraenoic acid (8S-HPETE).

Consistent expression of the mouse skin lipoxygenase was obtained using HeLa cells infected with vaccinia virus encoding the T7 RNA polymerase (15). In this system, using sonicated cells from a 35-mm well, typically 30–40% of added arachidonic acid (100 μM) was converted to 8S-HPETE as the sole enzymatic product (Fig. 3). The percent conversion of arachidonic acid in this system was always similar to that obtained using the human 15-Lox-2 as a positive control. Active enzyme was obtained using several of the PCR clones (that encode one, two, or three different amino acids from the library clone; see Fig. 1 legend) and the library clone itself.

Using the vaccinia expression system, linoleic acid was found to be a substrate for the mouse 8S-lipoxygenase although the conversion was 2–3-fold lower compared with arachidonic acid. The enzyme converted linoleic acid exclusively to 9S-HODE (Fig. 4).

**Effect of Phorbol Ester on Expression of 8S-Lipoxygenase in Mouse Skin**—The expression level of 8S-lipoxygenase in mouse skin is known to be strongly strain-dependent (16–18). Also, the highest activity is inducible in 6–10-day-old animals (8, 10). We examined several strains of mice and observed major differences in the level of constitutive expression (with no phorbol ester) and in the level after phorbol ester treatment. For example, using the Sencar strain, we observed high constitutive 8S-lipoxygenase activity in 6–10-day-old pups, with little extra induction by phorbol ester. The results shown here were obtained using a mixed breed of black Swiss animals that have low constitutive activity of 8S-lipoxygenase and exhibit strong induction with phorbol ester. Using 6–10-day-old pups, the inducing effect of phorbol ester clearly is related to induction of both mRNA and protein (Fig. 5).

**Cellular Localization of 8S-Lipoxygenase in Mouse Skin**—Expression of the 8S-lipoxygenase protein was examined in normal mouse skin following treatment with phorbol ester in acetone or acetone alone using the strain of black Swiss animals responsive to PMA. The histological analysis of skin from two differing body locations (thin dorsal skin and thick tail skin) revealed a marked hyperproliferative response to PMA (Figs. 6, A and D) and a diminished response to the acetone vehicle alone (Figs. 6, B and E). Most notable was an increase in the number of differentiated cells within the outer epidermal compartment, the stratum granulosum. The net result was more 8S-lipoxygenase positive cells in the PMA-treated samples as compared with the samples receiving acetone alone. No immunoreactivity was detected in any of the samples reacted with pre-immune serum. Hair follicles positioned within the underlying dermis also showed positive staining for 8S-lipoxygenase in differentiated cell layers (data not shown). Staining in these locations did not show a modulation in response to topical treatment with phorbol ester.

**Tissue Distribution of 8S-Lipoxygenase**—As the related hu-
man 15S-lipoxygenase, 15-Lox-2, is expressed in prostate (11), we used an activity assay (HPLC analysis of products formed from [1-14C]arachidonic acid) to examine for 8S-lipoxygenase activity in mouse prostate. Using young adult males of 8 weeks of age, high levels of cyclooxygenase and 12S-lipoxygenase activities were found in the prostate, but no 8S-lipoxygenase products were detected. Occurrence of the 8S-lipoxygenase transcript was examined in several different tissues by Northern analysis. This revealed expression of 8S-lipoxygenase transcript in mouse brain, with no detectible expression in heart, spleen, lung, liver, skeletal muscle, kidney, and testis (Fig. 7).

**DISCUSSION**

Mouse 8S-lipoxygenase cDNA was cloned by PCR using primers related to a recently characterized human 15S-lipoxygenase (15-Lox-2) (11). These two lipoxygenases have 78% amino acid identity, and the differences are mainly conservative substitutions. The two enzymes have only 30–45% identity to other mammalian lipoxygenases. The primary structure of the mouse 8S-lipoxygenase contains the absolutely conserved iron-binding histidines of lipoxygenases and the C-terminal isoleucine that is also an iron ligand (19, 20). A notable feature of the mouse 8S-lipoxygenase primary structure is the presence of a serine at amino acid position 558 as the putative 5th iron ligand (20). The equivalent residue in all other lipoxygenases is either a histidine or asparagine, with the exception of the human 15-Lox-2 in which a serine is also present (11). Based on the sequence similarity, our conclusion is that the 8S-lipoxygenase is the mouse homologue of the human 15-Lox-2. The extent to which the two enzymes are functionally homologous remains to be determined.

Initially we had difficulty studying the mouse enzyme as we could not obtain reliable expression of active lipoxygenase using our conventional HEK cell system (1, 4). The problem was solved by use of the recombinant vaccinia virus in a co-transfection system in Hela cells. In this procedure, the cells are co-transfected with the plasmid cDNA and vaccinia virus encoding the T7 RNA polymerase. The virus protein induces high level expression via the T7 promoter upstream of the lipoxygenase cDNA. The cells are harvested after 12 h. In this system, the mouse enzyme expressed with equivalent activity to either the 15-Lox-1 or 15-Lox-2 positive controls. Each of these lipoxygenases was expressed at a much higher level in the viral infected Hela cells than in the other procedure using the HEK cells.

Our Western results show clearly that HEK cells produce the 8S-lipoxygenase protein although at lower levels than the pos-
Panels B cellularity in the stratum granulosum compartment of the epidermis. Panels A and D show a thickened hyperproliferative epidermis after PMA treatment. An increase in 8S-lipoxygenase is due to an expansion in cellularity in the stratum granulosum compartment of the epidermis. Panels B and E show base-line staining of the stratum granulosum for 8S-lipoxygenase in skin receiving vehicle alone (acetone). Panels C and F show the absence of immunoreactivity after incubation of PMA-treated skin with pre-immune antiserum.

**Fig. 6. Localization of Mouse 8S-lipoxygenase protein in dorsal skin (A-C) and tail skin (D-F) by immunohistochemical analysis.** Panels A and D show a thickened hyperproliferative epidermis after PMA treatment. An increase in 8S-lipoxygenase is due to an expansion in cellularity in the stratum granulosum compartment of the epidermis. Panels B and E show base-line staining of the stratum granulosum for 8S-lipoxygenase in skin receiving vehicle alone (acetone). Panels C and F show the absence of immunoreactivity after incubation of PMA-treated skin with pre-immune antiserum.

**Fig. 7. Multiple tissue Northern analysis of mouse 8S-lipoxygenase.** A mouse tissue blot of mRNA (CLONTECH) was probed with a 618 bp EcoRV-BamHI fragment of 8S-lipoxygenase cDNA.

potentially, this substrate is available (21). Lehmann et al. (22) noted that the levels of 8-HETE and 9-HODE in mouse skin tend to change in parallel. Both are strikingly elevated in mouse skin papillomas and are lower than normal in skin carcinomas. The 8S chirality of the product from linoleic acid is one criterion that could be used to assess the contribution of the 8S-lipoxygenase to formation of 9-HODE in mouse skin. The main cyclooxygenase product from linoleic acid is the enantiomeric 9R-HODE (23), while non-enzymatic reactions would give racemic product.

Northern and Western analyses, as well as the activity assay, showed that PMA treatment strongly induced de novo synthesis of mouse 8S-lipoxygenase in the dorsal skin of the outbred mice used in this experiment. The histochemical analyses further defined the effect of PMA. Immunoreactive 8S-lipoxygenase protein was most prominent in a layer of differentiated epidermis, the stratum granulosum. The thickness of this cell layer increased markedly following 24 h of treatment with PMA. An increase in the number of cells that produce 8S-lipoxygenase, therefore, is one of the causes of the increased 8S-lipoxygenase activity induced by PMA.

In the Northern analysis using a multiple tissue blot, 8S-lipoxygenase mRNA was detected clearly in brain but not in the other seven tissues examined. Both the stratum granulosum of the epidermis and the neuronal tissues of the central nervous system were originally derived from the same ectodermal layer in early embryonic development, and both represent highly differentiated cell types. Occurrence of the 8S-lipoxygenase transcript in brain was unexpected as lipoxygenase-catalyzed formation of 8-HETE has not been reported in neuronal tissues. The negative reaction in liver is of interest in relation to the reported activity of 8S-HETE as a strong activator of the peroxisome proliferator-activated receptor, PPAR-α (24). In liver, there is the possibility of synthesis of 8-HETE via the microsomal cytochrome P-450 system, although in vitro this gives a nearly racemic 8-HETE product (25). The absence of 8S-lipoxygenase signal in the Northern analysis of liver, could, however, be related to the lack of induction in normal tissue. The same issue applies to the absence of detectable 8S-lipoxygenase activity in normal mouse...
prostate from young adult males (see “Results,” last section). Although the human homologue of the mouse 8S-lipoxygenase, 15-Lox-2, was readily detectible in human prostate (11), the pooled human sample would include tissue from older subjects, the majority of which are expected to exhibit benign prostatic hyperplasia (26). The induction of 8S-lipoxygenase in mouse skin by phorbol ester certainly is a striking feature of this enzyme. It remains to be seen whether inducibility is a general characteristic of this mouse enzyme and its human homologue, 15-Lox-2.

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