Differentiating Keratinocytes Express a Novel Cytochrome P450 Enzyme, CYP2B19, Having Arachidonate Monoxygenase Activity*

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Diane S. Keeney‡,‡, Colin Skinner‡,‡, Jeffrey B. Travers, Jorge H. Capdevila‡,‡, Lillian B. Nannya‡,‡, Lloyd E. King, Jr.§ §, and Michael R. Waterman‡

From the Departments of ‡Biochemistry, Medicine (‡Dermatology and **Nephrology), and ‡‡Plastic Surgery, Vanderbilt University School of Medicine, and §§Department of Veterans Affairs Medical Center, Nashville, Tennessee 37232 and the ‡Department of Dermatology and H. B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana 46202

The novel cytochrome P450, CYP2B19, is a specific cellular marker of late differentiation in skin keratinocytes. CYP2B19 was discovered in fetal mouse skin where its onset of expression coincides spatially (upper cell layer) and temporally (day 15.5) with the appearance of loricin-expressing keratinocytes during the stratification stage of fetal epidermis. CYP2B19 is also present postnatally in the differentiated keratinocytes of the epidermis, sebaceous glands, and hair follicles. CYP2B19 mRNA is tightly coupled to the differentiated (granular cell) keratinocyte phenotype in vivo and in vitro. In primary mouse epidermal keratinocytes, it is specifically up-regulated and correlated temporally with calcium-induced differentiation and expression of the late differentiation genes loricin and profilaggrin. Recombinant CYP2B19 metabolizes arachidonic acid and generates 14,15- and 11,12-epoxyeicosatrienoic (EET) acids, and 11-, 12-, and 15-hydroxyeicosatetraenoic (HETE) acids (20, 35, 18, 7, and 7% of total metabolites, respectively). Arachidonic acid metabolism was stereoselective for 11S,12R- and 14S,15R-EET, and 11S-, 12R-, and 15R-HETE. The CYP2B19 metabolites 11,12- and 14,15-EET are endogenous constituents of murine epidermis and are present in similar proportions to that generated by the enzyme in vitro, suggesting that CYP2B19 might be the primary enzymatic source of these EETs in murine epidermis.

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† To whom correspondence should be addressed: Dept. Biochemistry & Medicine/Dermatology, Vanderbilt University School of Medicine, 607 Light Hall, Nashville, TN 37232-0146. Tel.: 615-322-3318; Fax: 615-322-4349; E-mail: diane.keeney@vanderbilt.edu.

‡ The abbreviations used are: E, embryonic day; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; LDL, low density lipoprotein; TPA, phorbol 12-myristate 13-acetate; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s); kb, kilobase(s); HPLC, high performance liquid chromatography.

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intracellular free Ca$^{2+}$ concentrations are greatest in the granular cell layer where CYP2B19 is expressed and lowest in the basal cell layer (13).

**EXPERIMENTAL PROCEDURES**

**RNA Isolation and Polymerase Chain Reaction**—Total RNA was isolated as described (14). For reverse transcription-polymerase chain reaction (RT-PCR), total RNA was primed with oligo(dT)$_{18}$ reverse-transcribed and amplified using a GeneAmp RNA PCR kit (Perkin-Elmer). CYP2B subfamily-specific oligonucleotides 1 and 2 (Table I) were used to screen embryonic RNAs. The PCR products were size-fractionated on agarose gels, visualized with ethidium bromide, subcloned, and DNA sequenced. Oligonucleotides 3–6 were used for tissue distribution studies.

**In Situ Hybridization and Northern Analysis**—Mice (CD-1 outbred strain) obtained from Charles River and rats (Sprague-Dawley) obtained from Harlan Sprague Dawley were handled according to protocols approved by Vanderbilt University. Tissue preparation and in situ hybridization methods were described (14). Specific hybridization was distinguished by comparing silver grain development produced by sense and antisense cRNAs applied to consecutive sections on the same slide. For Northern analyses, total RNA was size-fractionated on formaldehyde containing 1% agarose gels and transferred by capillarity to GeneScreen Plus membranes (DuPont). Membranes were baked at 80 °C and stained with methylene blue to assess RNA integrity. The following murine cDNAs were used for in vitro transcription to generate $^{[35]}$S-labeled cRNAs for hybridization or were random labeled with $[^{32}]$P-dCTP for Northern blot analyses: CYP2B19 (GenBank accession number AF047829, 558–981 and 2381–3639 bp), keratin 10 (number V00830, 1727–1944 bp), keratin 14 (number M13806, 681–1063 bp), loricrin (number M34398, 1518–1684 bp), profilaggrin (number J03458, 128–188 bp), and low density lipoprotein (LDL) receptor (number X64414, 2109–2272 bp). For Northern data analyses, radioactivity on the washed membranes was quantitated using a PhosphorImager and the volume integration method of ImageQuant software (Molecular Dynamics). In every sample lane, the relative counts (average from two scans) corresponding to each radiolabeled cDNA were normalized with the 18S ribosomal RNA signal. The normalized values were compared to the relative expression of glycolytic enzymes (glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and enolase) for each developmental stage, as previously described (16).

**RESULTS**

**Identification of a Novel CYP Monooxygenase in Fetal Mouse Skin**—Mouse embryo RNAs were screened by RT-PCR to identify CYP genes expressed during fetal development. Degener-
from developing limbs (E15.5). This nucleotide sequence was more closely related to two rat genes, CYP2B12 (7, 8) and CYP2B15 (24), than to previously identified murine genes. Authenticity of this partial cDNA was confirmed by the specific hybridization of antisense CYP2B19 cRNA to fetal mouse epidermis. Specific hybridization was not observed with any other tissue or organ. Silver grains representing CYP2B19 mRNA localized to the uppermost, suprabasal cells in fetal epidermis. "Arrows" indicate epidermis of the forelimb (fl) and body wall (bw). B and C, CYP2B19 mRNA localizes to the uppermost, suprabasal cells in fetal epidermis. D and E, loricrin mRNA localizes to the same cell layer as CYP2B19. F and G, keratin 10 mRNA localizes uniformly to all suprabasal cells. "Arrows" (B–G) indicate the position of basal cells at the basement membrane. Bar in F is equivalent to 1.6 mm (A) or 0.1 mm (B–G).

FIG. 1. In situ localization of CYP2B19 mRNA to fetal mouse skin, and comparison with loricrin and keratin 10 mRNAs. Fetal mouse tissues obtained at E15.5 (A) or E16.5 (B–G) were hybridized with antisense cRNA encoding CYP2B19 (A–C), loricrin (D and E), or keratin 10 (F and G). Specific hybridization is represented by silver grains which appear black in brightfield (B, D, and F) and white in darkfield (A, C, E, and G). Results for sense cRNA are not shown. A, CYP2B19 mRNA localizes to fetal mouse epidermis. Arrows indicate epidermis of the forelimb (fl) and body wall (bw). B and C, CYP2B19 mRNA localizes to the uppermost, suprabasal cells in fetal epidermis. D and E, loricrin mRNA localizes to the same cell layer as CYP2B19. F and G, keratin 10 mRNA localizes uniformly to all suprabasal cells. Arrows (B–G) indicate the position of basal cells at the basement membrane. Bar in F is equivalent to 1.6 mm (A) or 0.1 mm (B–G).

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Spontaneously, CYP2B19 is expressed in the same cellular layer as loricrin, a marker of late differentiation of epidermal keratinocytes (granular cell layer) (1). Loricrin mRNAs localized to the most differentiated (uppermost) suprabasal cells in fetal (E16.5) epidermis (Fig. 1, B and C). Specific hybridization was not observed in basal cells or in many of the intermediate cells. Temporally, CYP2B19 mRNA was first detected at E15–E15.5, coincident with the appearance of suprabasal cells in fetal epidermis. Expression levels appeared to increase proportionately with increases in the number of differentiated keratinocytes, as the epidermis thickens between E15 and term (~E19).

Spatially, CYP2B19 is expressed in the same cellular layer as loricrin, a marker of late differentiation of epidermal keratinocytes (granular cell layer) (1). Loricrin mRNAs localized to the most differentiated (uppermost) suprabasal cells in fetal (E16.5) epidermis (Fig. 1, B and C). Considering its much higher expression level than CYP2B19, relatively few silver grains representing loricrin mRNA were present over intermediate cells. A different pattern was observed for keratin 10, a marker of newly differentiating keratinocytes (spinous cell layer) (1). Keratin 10 was expressed at moderately high and uniform levels in all suprabasal cells (Fig. 1, F and G). None of these mRNAs were detectable in basal cells. These data demonstrate that CYP2B19, like loricrin, is a specific marker for the most differentiated, nucleated keratinocytes in murine epidermis. The coincident onset of CYP2B19 expression and the appearance of loricrin-expressing suprabasal keratinocytes suggest a function for this cytochrome P450 enzyme in the stratification stage during development of fetal mouse epidermis.

Isolation and Characterization of a Full-length CYP2B19 cDNA—Sequence analyses of RT-PCR-generated CYP2B clones led to identification of low levels of CYP2B10 mRNA in mouse skin (not shown). CYP2B10 is a predominant CYP2B in mouse skin having 16-hydroxylase activity with androgens, like testosterone (25). CYP2B10 mRNAs were represented in the mouse skin cDNA library, so a CYP2B19 hybridization probe and stringency conditions were chosen to minimize cross-hybridization with CYP2B10. Several CYP2B19 cDNA clones were isolated (Fig. 2) that contained the novel CYP2B sequence originally amplified from mouse embryo RNA. Except for a missing ATG start codon, clone 2-1B-1A is full-length (2.7 kb). It contains an open reading frame encoding a 492-amino acid protein, 1.2 kb of 3'-untranslated sequence, and a polyadenylated tail. A start codon was likely missing because the second codon contains an EcoRI site, the same restriction site used to prepare the cDNA library. An ATG start codon was found in a 2-kb clone (5-2A-1A) containing the first 0.96 kb of the open reading frame, up to the putative exon 6/7 boundary. Exon/intron boundaries are conserved within CYP gene subfamilies (26). The remainder of this clone might encode intron 6 because the next 50 bp are 90% identical to intron 6 of CYP2B15 (24). Only the first 50 bp of the 5'-end of this intron have been reported. Sequence data obtained beyond this point were un-
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In rat skin (Fig. 4, C and D), CYP2B15 localized to suprabasal cells in the epidermis and sebocytes in the preputial gland, the large paired sebaceous glands beneath the genital skin of rodents. Sectioned close to the midline, sebaceous tissue expressing CYP2B15 is shown surrounding a central collecting duct that directs the flow of sebum to the skin surface. Sebocytes in other types of sebaceous glands also express CYP2B15: meibomian glands in the eyelid, anal glands in anogenital skin, and sebaceous glands associated with hair follicles (not shown). A serially sectioned rat vibrissae follicle (Fig. 4, E and F) shows a single layer of CYP2B15-expressing keratinocytes, which outlines the hair canal. An adjacent section cut obliquely (Fig. 4G) exposes a relatively large surface of inner root sheath expressing CYP2B15.

The tissue distribution of CYP2B19 and CYP2B15 was analyzed by RT-PCR. For CYP2B19 mRNAs in mouse tissues, products of expected size were obtained using RNAs isolated from all cutaneous tissues examined, but not from mouse adrenal gland, ovary, testis, vesicular gland, uterus, liver, lung, kidney, small intestine, colon, caecum, spleen, brain, heart, tongue,ibia, or femur (not shown). No products were observed in the absence of reverse transcriptase. Similar results were obtained for CYP2B15 mRNAs in rat tissues. Products of expected size were amplified with RNA isolated from all cutaneous tissues examined, but not from rat adrenal gland, ovary, uterus, heart, liver, kidney, or spleen (not shown). In situ hybridization studies also confirmed that CYP2B19 is not detectable in the keratinocytes of mucosal epithelia such as the rectum, colon, vagina, uterus, cornea, and conjunctiva (not shown). Hence, CYP2B19 and CYP2B15 appear truly specific for skin keratinocytes.

CYP2B19 and CYP2B15 are predicted to be orthologous because their expression patterns are indistinguishable and their 3′-untranslated sequences are more identical to each other than to that of CYP2B12. Importantly, CYP2B12 expression patterns differ (8) (Table II), being limited to sebocytes, a subset of differentiated skin keratinocytes. The inability to detect epidermal and follicular CYP2B12 expression by in situ hybridization further suggests that the orthologues will be CYP2B19 and CYP2B15.

CYP2B19 Is an Arachidonic Acid Monoxygenase—Arachidonic acid was demonstrated to be a substrate for recombinant CYP2B19. Turnover numbers estimated for CYP2B19 with [1-14C]arachidonic acid averaged 3.5 min⁻¹ (Fig. 5A). Metabolites generated by CYP2B19 were resolved by reversed-phase HPLC (Fig. 5B). Five fractions (P1–P5) were collected and analyzed further, and characterized structurally (Table III). All five P450 metabolites were produced enantioselectively, attesting to their enzymatic origin. Fraction P5 had the same retention time in reversed-phase HPLC as a mixture of authentic 11,12- and 8,9-EET. Further analysis confirmed that P5 contained only 11,12-EET and that the major enantiomer was 11S,12R-EET. Fraction P4 had the same retention time as authentic 14,15-EET and contained nearly exclusively 14S,15R-EET. Fraction P3 contained a mixture of 11-, 12-, and 15-HETE, in which the predominant enantiomers were 11S-, 12R-, and 15R-HETE. The radioactive material in P1 had a retention time similar to that of a mixture of dihydroxyeicosatrienoic acids and likely arose by hydration of the EETs. The radioactive material in P2 remains to be identified. Importantly, ω- or ω-1 hydroxylated arachidonic acids were not detected. In summary, EETs accounted for approximately two-thirds of the identified metabolites, while HETEs accounted for the remaining one-third. Significantly, CYP2B19 is the first cytochrome P450 identified in skin that generates 12R-HETE.
Abnormally high concentrations of 12R-HETE are present in hyperproliferative epidermis, in psoriasis and related dermatoses (5, 6, 28, 29), implicating a role for the enzymatic source of this eicosanoid in skin diseases.

**Differentiation Induced by Calcium Up-regulates Cyp2b19**

**Gene Expression in Vitro**—Northern analysis was used to investigate whether normal mouse epidermal keratinocytes express CYP2B19 in vitro and whether mRNA levels correlate temporally with those of two other gene markers of late differentiation, loricrin and profilaggrin. Proliferating keratinocyte cultures were induced to differentiate by increasing extracellular Ca\(^{2+}\) concentrations from 0.05 mM (promotes basal cell phenotype) to 1 mM (induces granular cell phenotype) as described (12). Higher Ca\(^{2+}\) concentrations were less effective in up-regulating genes specifically associated with late differentiation of epidermal keratinocytes. For example, at 36 h after increasing Ca\(^{2+}\), message levels for CYP2B19, profilaggrin, loricrin and filaggrin (12) peaked at 36 h after inducing differentiation and showed a 9-fold increase at 48 h. CYP2B19 mRNA levels were up-regulated in parallel with that of profilaggrin, achieving a 15-fold increase by 48 h, while little change was observed in LDL receptor mRNA levels. Some spontaneous differentiation occurred in keratinocytes maintained in low Ca\(^{2+}\) (0.05 mM) medium (5-fold increase at 48 h for profilaggrin and CYP2B19 mRNAs). This is not surprising since the cultures become confluent over the time course of treatment, and this factor alone causes keratinocytes to differentiate (30). These studies demonstrate a close correlation between CYP2B19 and profilaggrin mRNA levels, two markers of late differentiation, whether differentiation is induced by Ca\(^{2+}\) or occurs spontaneously.

Results in Fig. 6 are consistent with data from another study in which mRNA levels for five genes were measured after the same calcium induction protocol, over a 36-h time course (Table IV). These data demonstrate the specificity of Ca\(^{2+}\) (0.125 mM) to up-regulate genes specifically associated with late differentiation of epidermal keratinocytes. For example, at 36 h after increasing Ca\(^{2+}\), message levels for CYP2B19, profilaggrin, and loricrin ranged from 500 to 700% of the 0-h control values, while those of cytokeratin 14 (a basal cell cytokeratin marker) or occurs spontaneously.

**Table II**

| Gene      | Constitutive | Epidermis | Hair follicles | Sebaceous glands | Substrate | Metabolites |
|-----------|--------------|-----------|----------------|------------------|-----------|-------------|
| Cyp2b19   | Yes          | Yes       | Yes            | Yes              | Arachidonic acid | EETs and HETEs |
| CYP2B15   | Yes          | Yes       | Yes            | Yes              | ND\(^a\)   | ND          |
| CYP2B12\(^b\) | Yes        | No        | No             | Yes              | Arachidonic acid | EETs        |

\(^a\) ND, not determined. 
\(^b\) Data reported in Ref. 8.
leading to expression of the Cyp2b19 gene.

The concentration of extracellular Ca\(^{2+}\) affected the time course and extent of up-regulation of CYP2B19 mRNA (Fig. 7), as demonstrated previously for the cytokeratin genes 1 and 10, and profilaggrin (12). Fig. 7, C and D, shows that 0.125 mM Ca\(^{2+}\) was optimal for up-regulation of both CYP2B19 and profilaggrin mRNA in normal mouse epidermal keratinocytes, during 72 h of treatment. The increase in message appears slightly later in these cultures compared with those represented in Fig. 6 and Table IV, but the data are otherwise consistent among different primary cell preparations.

Murine Epidermis Contains Endogenous EETs—To determine whether the EETs generated by recombinant CYP2B19 are present in mouse skin, we extracted epidermis from neonatal mice and analyzed its EET content by gas chromatography/mass spectrometry. Of 36 ng total EET recovered, 25% (9.7 ng) was 14,15-EET, 41% (14.6 ng) was 11,12-EET, and 32% (11.6 ng) was 8,9-EET. Similar ratios of 11,12-EET:14,15-EET were calculated for EETs generated by recombinant CYP2B19 (1.75:1) and endogenous EETs in mouse epidermis (1.5:1), suggesting that CYP2B19 may participate in the endogenous formation of these eicosanoids in epidermis. The endogenous 8,9-EET suggests the presence of an additional epoxygenase, but we were unable to identify another major CYP2B in mouse skin as a possible source of 8,9-EET, as we did in rat skin (i.e. CYP2B12 and CYP2B15) (8).

**DISCUSSION**

CYP2B19 is an arachidonate monooxygenase expressed exclusively in differentiated skin keratinocytes. It is a specific marker of the most differentiated, nucleated keratinocytes in cutaneous tissues. Other differentiation-specific keratinocyte markers are either intermediate filaments (cytokeratins) or enzymes involved in formation of cornified cell envelopes. CYP2B19 differs from previously identified keratinocyte markers because it catalyzes the formation of lipid mediators that are likely to participate in keratinocyte signaling pathways.

A role for CYP2B19 in lipid metabolism was suspected initially because differentiated keratinocytes in the epidermis and sebaceous glands are highly enriched in the lipid precursors that ultimately form the water permeability barrier in the epidermis and sebum in sebaceous glands (34). The arachidonic acid epoxygenase activity of recombinant CYP2B19 suggested to us that this enzyme potentially functions in an intracellular signaling pathway and unlikely has a direct role in the biosynthesis of structural lipids or sebum. CYP2B19 expression is always detected when differentiated keratinocytes are present, from the ontogeny of fetal epidermis throughout postnatal development. Hence, it will be important in future studies to prove whether CYP2B19 catalytic activity and signals generated by its metabolites are required for the terminally differentiated (granular cell) phenotype of keratinocytes in skin.
Based on in situ hybridization, RT-PCR, and Northern analyses, expression of CYP2B19 and CYP2B15 is restricted to a single cell type, the differentiated keratinocytes in skin, as is the homologous cytochrome P450, rat CYP2B12 (8). The cell type specificity of these three skin-specific CYP2Bs is unparalleled among mammalian CYP genes. In the CYP2B15 gene (24), a sequence motif upstream (~2827 bp) of the putative “TATA” box conforms to the consensus sequence (AAPuC-CAA) found in the 5’-region of epidermal cytokeratin genes (35). CYP2B19, CYP2B15, and CYP2B12 are specific for skin keratinocytes, unlike previously described keratinocyte markers. For example, loricrin is also present in the granular cell layer of oral, esophageal, and forestomach mucosa (36). It is not apparent why epidermis would require a keratinocyte-specific cytochrome P450 to epoxidize arachidonic acid. No other arachidonic acid epoxygenase has been shown to be specific for a single cell type. Perhaps differentiated keratinocytes have a unique requirement for the EET and HETE products of CYP2B19, or perhaps the Cyp2b19 gene contains regulatory elements required to target expression specifically to the most differentiated, nucleated keratinocytes in skin. The potential to form 12R-HETE in the skin by a CYP-dependent mechanism is novel and physiologically relevant. A cytochrome P450 enzyme has been suspected as the source of this eicosanoid in human skin since Wollard (28) identified 12R-HETE as the predominant stereoisomer in psoriatic lesions. This finding, which also proved true for related dermatoses (6, 29), ruled out platelet type 12-lipoxygenase as the source because this enzyme generates only 12S-HETE. Isolated human epidermal cells form EETs and HETEs, including 12R-HETE, from arachidonic acid (37). These activities were stimulated by NADPH and inhibited

| TABLE IV |
|---------------------------------------------------------------|
| **Relative mRNA Levels of five genes expressed in normal murine epidermal keratinocytes, during 36 hours of treatment in primary culture** |
| Hours of treatment | 0 h | 12 h | 24 h | 36 h | Vehicle | 36 h |
|-------------------|-----|------|------|------|---------|------|
| CYP2B19 | 100 | 100 | 100 | 100 | 100 | 100 |
| Profilaggrin | 100 | 100 | 100 | 100 | 100 | 100 |
| Loricrin | 100 | 100 | 100 | 100 | 100 | 100 |
| MK14 | 100 | 100 | 100 | 100 | 100 | 100 |
| LDL Rc | 100 | 100 | 100 | 100 | 100 | 100 |
| Ca²⁺ (0.125 mM) | | | | | | |
| 0 h | 100 | 100 | 100 | 100 | 100 | 100 |
| 12 h | 166 | 378 | 385 | 86 | 79 | 79 |
| 24 h | 181 | 234 | 301 | 94 | 79 | 79 |
| 36 h | 526 | 501 | 736 | 119 | 87 | 87 |
| Vehicle | | | | | | |
| 36 h | 150 | 188 | 276 | 123 | 80 | 80 |
| PAF (100 nM) | | | | | | |
| 0 h | 100 | 100 | 100 | 100 | 100 | 100 |
| 12 h | 166 | 221 | 167 | 121 | 110 | 110 |
| 24 h | 198 | 199 | 77 | 140 | 106 | 106 |
| 36 h | 245 | 271 | 180 | 175 | 135 | 135 |
| Vehicle | | | | | | |
| 36 h | 205 | 210 | 92 | 151 | 108 | 108 |
| TPA (100 nM) | | | | | | |
| 0 h | 100 | 100 | 100 | 100 | 100 | 100 |
| 12 h | 64 | 22 | 28 | 76 | 99 | 99 |
| 24 h | 48 | 36 | 93 | 87 | 98 | 98 |
| 36 h | 34 | 22 | 65 | 115 | 106 | 106 |
| Vehicle | | | | | | |
| 36 h | 238 | 288 | 602 | 169 | 130 | 130 |

\[ \text{Ca}^{2+} (0.125 \text{ mM}) \]

\[ 12R-HETE \]

\[ 12S-HETE \]

\[ \text{MK14, mouse cytokeratin 14.} \]

\[ \text{LDL Rc, mouse low density lipoprotein receptor.} \]

\[ \text{Some degradation present in this sample lane.} \]
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Fig. 7. Northern analysis of effects of divalent cations on mRNA levels of CYP2B19 and profilaggrin, two markers of late differentiation, in normal murine epidermal keratinocytes. At 80% confluence, primary keratinocyte cultures were treated with media containing cations as indicated: A and B, Mg\(^{2+}\) (5 mM) or Sr\(^{2+}\) (0.125 mM and 5 mM) were added to media containing 0.05 mM Ca\(^{2+}\). C and D, Ca\(^{2+}\) was added to media at 1.25, 0.5, and 0.125 mM final concentration (0.05 mM Ca\(^{2+}\) for 0 h culture conditions). Data are expressed as % of 0 h control values, normalized for the relative amount of cyclophilin mRNA.

by CO, hallmarks of cytochrome P450 enzyme activities. It is likely that the arachidonic acid monoxygenase activities in human epidermis will be catalyzed by an orthologue of murine CYP2B19. Recently, a 12R-lipoxygenase was identified in human skin (38). It will be important to demonstrate whether this novel lipoxygenase is expressed in the same cutaneous cell types as CYP2B19, since it is very likely that orthologues corresponding to both enzymes exist in human and murine skin. The ultimate physiological and biochemical functions of HETEs and EETs in normal epidermal keratinocytes remain to be established. A critical question is whether any specific eicosanoid metabolite(s) resulting from CYP2B arachidonate monoxygenase activities are involved in epidermal hyperproliferation and scaling skin phenotypes in murine and human skin.

Murine epidermis contains endogenous 11,12- and 14,15-EET in similar proportion to that generated in vitro by recombinant CYP2B19. The endogenous epidermal EET profile supports the hypothesis that CYP2B19 may be responsible for endogenous 11,12- and 14,15-EET synthesis in mouse epidermis and that this biosynthesis is largely restricted to the upper, granular cell layer of the epidermis. This is because CYP2B19 is only expressed in epidermal keratinocytes that have committed to differentiate and passed through the spinous (newly differentiating) cell layer. The physiological reasons for such highly regulated and compartmentalized biosynthesis of eidermal EETs during the terminal stages of keratinocyte differentiation remain to be determined.

The general importance of fatty acid metabolism in keratinocyte differentiation was demonstrated by studies of essential fatty acid-deficient epidermal keratinocytes. Essential fatty acid supplementation induced differentiation of these keratinocyte cultures (39, 40), and altered the metabolism and cellular levels of active retinoids (41), another key regulator of keratinocyte growth and differentiation. It is easy to imagine that CYP2B19-dependent arachidonic acid metabolites might function in vivo by participating in Ca\(^{2+}\)-dependent intracellular signaling pathways, since EETs are well known to mediate the mobilization of Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\), and H\(^{+}\) in renal and endothelial cells (9, 10). Also, ongoing studies have shown that purified EET regioisomers induce intracellular Ca\(^{2+}\) mobilization responses in keratinocyte-derived cell lines. Clearly changes in intracellular Ca\(^{2+}\) concentrations are somehow critical to the later stages of keratinocyte differentiation in the skin, whether effected by changes in extracellular Ca\(^{2+}\) concentrations in vitro (42, 43) or by endogenous regulatory mechanisms in vivo. Although the in vivo function of CYP2B19 remains to be proven, it is conceivable that its arachidonic acid monoxygenase activities might serve in a signaling pathway that ultimately leads to the biosynthesis of barrier lipid precursors or programmed cell death. Since CYP2B19 expression can be induced by Ca\(^{2+}\) in primary epidermal keratinocytes in a differentiation-specific manner, it is possible to investigate potential functions of this enzyme, and effects of its specific metabolites, in an in vitro system. Future studies will be aimed at proving whether CYP2B19 catalytic activities are required for the terminally differentiated phenotype of skin keratinocytes.

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