Differential expression of prostaglandin-H synthase isoenzymes in normal and activated keratinocytes *in vivo* and *in vitro*

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Normal mouse epidermis constitutively expresses prostaglandin-H synthase 1 (PGHS-1) but no PGHS-2. Acute inflammation and epidermal hyperplasia, (hyperplastic transformation), as evoked in adult mouse skin *in vivo* by wounding or by the phorbol ester phorbol 12-myristate 13-acetate (PMA), resulted in a transient induction of PGHS-2 expression while PGHS-1 remained unchanged. Under conditions of a stationary epidermal hyperplasia, as in neonatal mouse epidermis, PGHS-1, but not PGHS-2, expression was observed. Induction of 'balanced hyperproliferation' by 4-*O*-methyl-phorbol 12-myristate 13-acetate (4-*O*-methyl-PMA) did not lead to PGHS-2 expression. When keratinocytes were isolated from neonatal mouse skin and separated by Percoll density-gradient centrifugation according to their stage of differentiation, PGHS-1 mRNA expression and protein were found to be highest in the differentiated cells compared with those from the proliferative compartment. A similar distribution of PGHS-1 mRNA was found in keratinocytes from adult mice, whereas PGHS-1 protein was equally distributed in all cell types. Contrary to the situation in intact epidermis, PGHS-2 mRNA but no protein was detected in all cell fractions. Established keratinocyte lines constitutively expressed both isoenzymes at different ratios. In the mouse line MSCP5 an almost exclusive expression of PGHS-2 was found, which was further enhanced by PMA treatment. These data indicate that the expression of PGHS-2 in mouse epidermis is specifically related to the emergency reaction of hyperplastic transformation.

INTRODUCTION

Permanently regenerating tissues such as epidermis maintain a constant mass and size by precisely balancing cell gain and loss. As long as the mechanisms controlling this tissue homoeostasis are intact every increase in cell number in response to mitogenic stimuli will be matched by a corresponding increase in cell loss ('balanced hyperproliferation'). A disturbance of tissue homoeostasis resulting in inflammation and epidermal hyperplasia ('hyperplastic transformation', [1]) is observed upon irritation and damage of the skin. Depending on the stimulus either one or the other response can be induced selectively. Thus, skin massage as well as the non-irritant phorbol ester 4-*O*-methyl-phorbol 12-myristate 13-acetate (4-*O*-methyl-PMA) induce balanced hyperproliferation, whereas skin wounding or the tumour promoter phorbol 12-myristate 13-acetate (PMA) elicit hyperplastic transformation [2]. Upon repeated stimulation by PMA the hyperplastic state becomes stationary, indicating a re-establishment of homoeostatic control. A stationary hyperplastic morphology is also characteristic for the epidermis of the newborn mouse [2,3].

In contrast to balanced hyperproliferation hyperplastic transformation has been shown to depend critically on an immediate stimulation of prostaglandin E_2 (PGE_2) synthesis in the epidermis and can be prevented by inhibitors of the cyclo-oxygenase activity such as indomethacin [4].

Mammalian cells and tissues express two forms of prostaglandin-H synthases (PGHS), PGHS-1, and PGHS-2 [5–12]. These enzymes catalyse the oxygenation of arachidonic acid to the endohydroperoxide prostaglandin G_2 (cyclo-oxygenase reaction), and its reduction to the hydroperoxide prostaglandin H_2, the precursor of prostaglandins. The cyclo-oxygenase activity is inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin [8,13].

The PGHS isoforms are differentially regulated. PGHS-1 has been found to be constitutively expressed in all tissues so far analysed [5,13], whereas PGHS-2 mRNA expression requires induction by growth factors [14–17], PMA [6,17,18], lipopolysaccharide [19] or hormones *in vitro* [11,20,21].

In vivo PGHS-2 expression has been seen in the brain of stressed rats [12] and in hormone-stimulated pre-ovulatory follicle cells [20,21]. Hence, although expression of both PGHS isoforms has been previously investigated in a variety of tissues [22,23] no information is available yet on their expression in epidermis *in vivo* and keratinocytes in culture. Considering the well-defined (patho)physiological functions of prostaglandins in skin we set out to investigate the expression pattern of PGHS isoenzymes in developing and adult mouse epidermis *in vivo* and upon stimulation of epidermal hyperproliferation and hyperplasia.

EXPERIMENTAL

Materials

PMA was kindly provided by Dr. E. Hecker, German Cancer Research Centre. 4-*O*-Methyl-PMA was purchased from Sigma, Munich.

cDNA probes

The 1.73 kb coding sequence of mouse PGHS-1 cloned in a pBR322-derived vector SP 65 1.8-1 was kindly provided by Dr. DeWitt, Michigan State University, East Lansing, U.S.A. [24], the 2.1 kb coding sequence of mouse PGHS-2 (TIS-10) cloned in a pBluescript II KS vector by Dr. Hershman, University of

Abbreviations used: GAPDH, glyceraldehyde phosphate dehydrogenase; KLH, keyhole limpet haemocyanin; PGHS, prostaglandin-H synthase; PGE_2, prostaglandin E_2; PMA, phorbol 12-myristate 13-acetate; 4-*O*-methyl-PMA, 4-*O*-methyl-phorbol 12-myristate 13-acetate.

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California, Los Angeles, U.S.A. [6], and the 1 kb cDNA of glyceraldehyde phosphate dehydrogenase (GAPDH) cloned into pBR322 at the *Pst*1 site was provided by Dr. I. Loncarevic, German Cancer Research Centre, Heidelberg, Germany.

Cells

MSCP5 keratinocytes were kindly provided by Dr. A. Balmain, Beatson Cancer Research Centre, Glasgow, U.K. [25]. The cells were cultured in 4× minimal essential medium supplemented with 10% (v/v) fetal-calf serum, containing 1% penicillin and 1% streptomycin (4 × MEM/FCS, Biochrom, Berlin, Germany). PGHS expression was determined in subconfluent, growth-arrested cells. To this end, cells were seeded (3×10^5/100 mm diam. Falcon dish) in 4 × MEM/FCS. After 24 h the culture medium was replaced by fresh 4 × MEM/2.5% FCS, and after another 48 h by 4 × MEM containing 0.25% FCS. 48 h after the last medium change cells were stimulated with 10^{-6} M PMA in fresh 4× MEM/0.25% FCS. At various time points after treatment the culture medium was removed and cells were washed twice with PBS and subjected to various extraction procedures.

Animals

Female NMRI mice (outbred strain from Deutsche Versuchstieranstalt, Hannover, Germany) aged 1–10 days *post natum* or adult 7-week-old animals were used. The animals were kept under an artificial day/night rhythm and were fed Altromin Standard food pellets (Altromin, Lage, Germany) with sterile water available *ad libitum*. Three days prior to treatment the back skin of adult mice was shaved with electric clippers. Removal of the horny layer from adult epidermis was done by means of cosmetic sandpaper under histologic control as published previously [26]. PMA (10^{-4} M) and 4-*O*-methyl-PMA (4×10^{-3} M) were dissolved in acetone, and 100 μl of these solutions were topically applied to shaved neonatal or adult mouse back skin, respectively. At various time points after treatment mice were killed by cervical dislocation, the skin was dissected and epidermis was scraped off the skin specimens by means of a scalpel at 4 °C or −70 °C.

Separation and fractionation of epidermal cells from neonatal and adult mouse skin by Percoll density-gradient centrifugation

For assessment of PGHS expression levels in the individual layers, epidermis of 1–3-day-old mice was isolated by cold trypsinization. After mechanical disaggregation the keratinocyte suspension was centrifuged on a discontinuous Percoll density gradient. Four fractions were obtained as described previously [27]. On top of the Percoll gradient, mainly squamous and large granular cells banded (fraction 1), at intermediate density, granular and spinous cells accumulated (fraction 2), and at the highest density, basal keratinocytes were resolved into fractions 3 and 4. Adult dorsal epidermis was separated from dermis in thin-split sections by cold trypsinization as previously described in detail. The keratinocyte suspension obtained was separated into four cell fractions F1–F4 (see above) by means of discontinuous Percoll gradient centrifugation [28]. After collection of the individual fractions, cells were washed free from Percoll with PBS. Cells were frozen at −70 °C or extracted immediately.

Preparation of anti-PGHS antibodies

Rabbits were immunized with purified antigens emulsified with complete Freund's adjuvant. Two booster injections were given in 4-week intervals with incomplete Freund's adjuvant. Sera were collected 14 days after each immunization. 100 μg of ram seminal vesicle PGHS-1 (Oxford Biochemical Research, Oxford, MI, U.S.A.) or 1 mg of denatured PGHS-2 peptide–keyhole limpet haemocyanin (KLH) complex were injected (KLH, Imject-Kit, Pierce, BA Oud-Beijerland, Netherlands). The 18-amino-acid-containing oligopeptide H-ASASHSRLDDINPTVLIK-OH (corresponding to the unique amino acids 556–573 of the authentic mouse PGHS-2 sequence, [6]) was conjugated via an additional N-terminal cysteine to maleimide-activated KLH. The polyclonal antisera were tested for their immunoreactivity in enzmye-immunoassays and in immunoblotting using PGHS-1 and PGHS-2 peptides as antigens. No cross-reactivity of the anti-PGHS-2 serum with purified PGHS-1 protein could be detected. Furthermore, the antiserum against PGHS-2 was compared with a commercially available anti-PGHS-2 serum.

Preparation of a particulate fraction

For detection of PGHS protein a particulate fraction was prepared from subconfluent, growth-arrested cells or epidermal tissue samples. Cells were washed twice with ice-cold PBS, lysed in homogenization buffer [50 mM Tris/HCl (pH 7.6), 1 mM diethyldithiocarbamate, 2 mM EDTA (pH 8.0), 0.1% Tween 20, 0.2 mg/ml α-macroglobulin, 1 mM PMSF and 10 μg/ml leupeptin] and sonicated. The homogenates were then centrifuged with 100000 *g* at 4 °C for 60 min. The sedimented particulate fraction was resuspended in extraction buffer (homogenization buffer supplemented with 1% Tween 20) and incubated for a further 15 min on ice. For Western-blot analysis protein from the 13000 *g* (4 °C, 25 min) supernatant was used.

Protein determination

Protein concentrations were determined by means of the Bio-Rad DC Protein-Assay using BSA as standard.

Western-blot analysis

Proteins from the particulate fraction were electrophoresed in discontinuous SDS/polyacrylamide gels with a 7.5% separating gel [29]. The proteins were electroblotted on to poly(vinyl difluoride) (PVDF) membranes as described before [30] and stained with Ponceau Red to check the efficiency of the transfer. Thereafter the membranes were handled according to the protocol recommended by the supplier of the TROPIX-Western light chemiluminescence detection system (Serva, Heidelberg). Both anti-PGHS antibodies were used at a dilution of 1:4000, and the anti-(rabbit IgG) alkaline phosphatase-linked antibody (Sigma, Munich, Germany) was used at a dilution of 1:10000. Protein sizes were estimated by comparison with co-blotted molecular-mass standard proteins (SDS-6H, Sigma, Munich, Germany).

Immunoprecipitation

Cells were lysed and homogenized in homogenizing buffer (see above). After centrifugation (100000 *g*, 4 °C, 1 h), 1 ml of the particulate fraction was resuspended in homogenization buffer and incubated with 60 μl of Protein G–Sepharose CL4B (Pharmacia, Freiburg, Germany) and 5 μl of antibodies specific for PGHS-1 or PGHS-2 for 1 h at 4 °C. Immunoprecipitates were washed five times in homogenization buffer lacking Tween 20. The precipitates were denatured in 30 μl of 2× Laemmli sample buffer, and boiled for 5 min prior to PAGE.

Isolation of RNA

Total RNA was extracted from cells by the acid–guanidine thiocyanate–phenol–chloroform extraction method as described previously [31]. Frozen epidermal tissue samples were homogenized in Trisolv-solution (AGS, Heidelberg, Germany) by means of a Braun dismembrator.

^{32}P-labelling of cDNA fragments

Isolated cDNA-inserts of PGHS-1 and PGHS-2 were labelled with [α-^{32}P]dCTP (Amersham, specific radioactivity 110 TBq/mmol) using a random-primed DNA-labelling kit (Boehringer, Mannheim). The specific activity of the DNA was $(1–2) \times 10^9$ c.p.m./μg on average. For Northern blots PGHS cDNAs were used at 1×10^7 c.p.m./ml and GAPDH cDNA at $(1–2) \times 10^6$ c.p.m./ml.

Northern-blot analysis

Samples (10–20 μg) of total RNA were denatured at 67 °C for 15 min in 66% formamide, 40 mM Mops, 10 mM sodium acetate, 1 mM EDTA, 8% formaldehyde, 10 μg/ml ethidium bromide. Gel electrophoresis was carried out through a 1% agarose gel containing 2.2 M formaldehyde, 40 mM Mops, 10 mM sodium acetate and 1 mM EDTA. RNA was photographed and transferred to GENEscreen membranes (Du Pont–New England Nuclear, Dreieich, Germany) according to the instructions of the supplier and a protocol described previously [32]. After UV-cross-linking of RNA to the filters (Stratagene UV-cross-linker) they were prehybridized at 60 °C in 0.5 M Na_2HPO_4, pH 7.2, 1 mM EDTA, 1% BSA, 7% SDS for 4 h. Hybridization was performed in the same solution with the ^{32}P-labelled cDNA probes for 16–24 h at 60 °C. Thereafter filters were washed once in 2 × SSC/0.1% SDS (SSC: 0.15 M NaCl/0.015 M sodium citrate) at room temperature and once at 60 °C for 20 min, then in 0.5 × SSC/1% SDS for 20 min at 60 °C and in 0.1 × SSC/1% SDS for a further 20 min. The filters were autoradiographed using Kodak XAR-5 films with intensifying screens at −70 °C. Each filter was rehybridized to ^{32}P-labelled GAPDH- or 7 S RNA- specific cDNAs to verify that all lanes were loaded with an equal amount of RNA. The autoradiographs were scanned with a colour densitometer (Apple Macintosh) using the Ofoto- and Bioscan software. The signals were integrated and normalized to the value obtained in the corresponding lane for GAPDH.

RESULTS

Expression of PGHS in normal mouse epidermis *in vivo*

Epidermis of neonatal and adult mice constitutively expressed the 2.7 kb mRNA and the 70 kDa protein of PGHS-1 (Figures

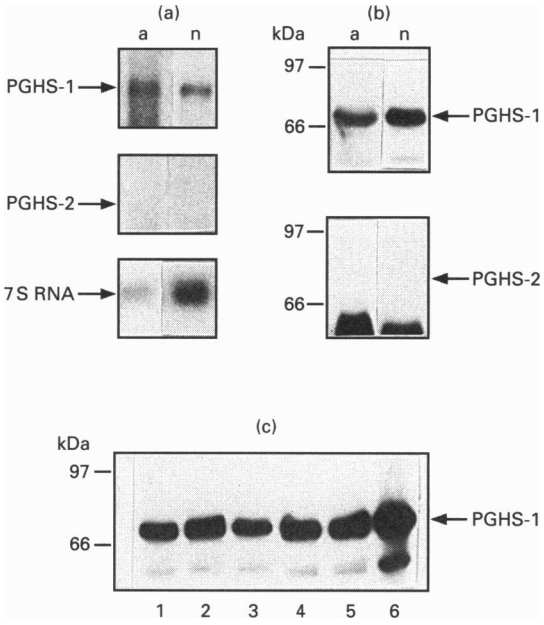

Figure 1 PGHS isoenzyme levels in normal adult and neonatal mouse epidermis

Total RNA and protein were obtained from adult (a) and neonatal (n, 5-day-old) mouse epidermis. Northern-blot analysis (**a**) was performed using ^{32}P-labelled PGHS-1- or PGHS-2-specific cDNA probes. Filters were rehybridized with a ^{32}P-labelled 7 S RNA-specific probe to control the loadings of RNA per lane. The films were exposed for 7 days. The positions of the PGHS-1 and -2 and GAPDH mRNAs are indicated. Immunoblot analysis (**b**, **c**) was performed using 75 μg of protein from the particulate fractions of adult (a) and neonatal (n; 5-day-old mice) epidermis and probing with PGHS-1- and PGHS-2-specific antisera. Kinetics of the PGHS-1 protein expression (**c**) in the epidermis of 1-, 3-, 5-, 7-, or 10-day-old mice (lanes 1 to 5) as compared with the PGHS-1 content of adult epidermis (lane 6). The molecular-mass standards are given in kDa. The positions of PGHS-specific immunosignals are indicated. Note that there are varying levels of PGHS-1 protein in the epidermis of adult mice (**b**, lane a and **c**, lane 6).

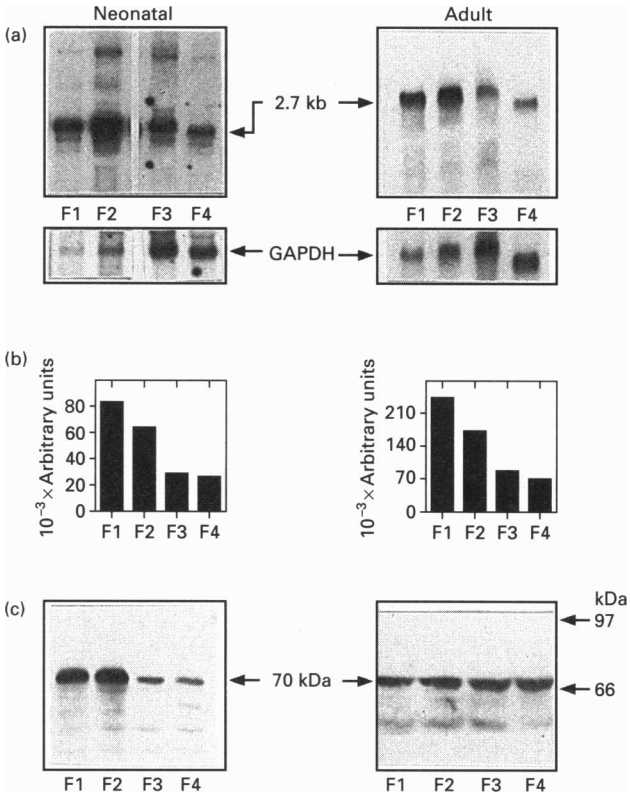

Figure 2 Cell-layer-dependent PGHS-1 expression in neonatal and adult mouse epidermis

Keratinocytes isolated from neonatal or adult epidermis and fractionated by Percoll density-gradient centrifugation were analysed for PGHS-1 mRNA (**a**, **b**) and protein (**c**) by Northern- and Western blotting, respectively. Quantification of 2.7 kb PGHS-1 mRNA signal intensities normalized to GAPDH signal intensities is given in (**b**). F1, squamous and large granular cells; F2, granular and spinous cells; F3 and F4, basal proliferative cells.

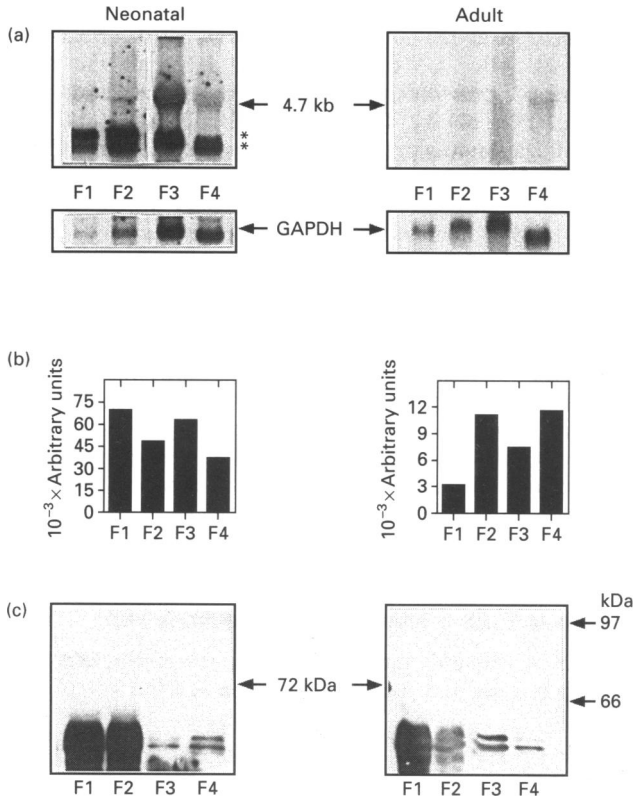

Figure 3 Cell-layer-dependent PGHS-2 expression in neonatal and adult mouse epidermis

Keratinocytes isolated from neonatal or adult epidermis and fractionated by Percoll density-gradient centrifugation were analysed for PGHS-2 mRNA (**a**, **b**) and protein (**c**) content by Northern- and Western blotting, respectively. The positions of the relevant species are indicated. Asteriks indicate specific low-molecular-mass mRNA species. The quantitative evaluation of 4.7 kb PGHS-2 mRNA signal intensities normalized to GAPDH signal intensities is given in (**b**). Here, PGHS-2 and GAPDH cDNAs were co-hybridized. F1, squamous and large granular cells; F2, from granular and spinous cells; F3 and F4, basal proliferative cells.

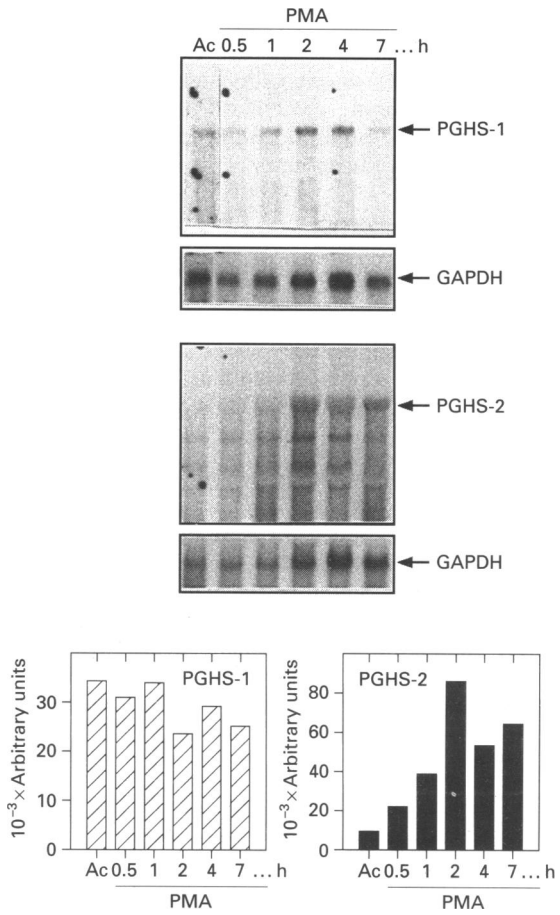

Figure 4 PGHS isoenzyme mRNA levels in normal and PMA-stimulated adult mouse epidermis

Total RNA was extracted from mouse epidermis treated *in vivo* with acetone (Ac, control) for 2 h or with 10 nmol PMA for the times indicated. Northern-blot analysis was performed using ^{32}P-labelled PGHS-1- or PGHS-2-specific cDNA probes. Filters were hybridized with a ^{32}P-labelled GAPDH-specific probe to control the loadings of RNA per lane. The films were exposed for 7 days. The arrows indicate the positions of the 2.7 kb PGHS-1 and the 4.7 kb PGHS-2 mRNA. Quantification of the PGHS signal intensities in relation to the GAPDH signal intensities is shown in the lower panel.

1a and 1b). No signals for PGHS-2 mRNA and protein were found (Figures 1a and 1b). Expression of PGHS-1 protein was already found in the epidermis of 1-day-old animals and remained relatively constant for the next 10 days (Figure 1c), i.e. when the adult skin phenotype was developing [33]. No PGHS-2 protein could be detected within this time period (data not shown).

Expression of PGHS in keratinocytes from different cell layers of epidermis

In order to investigate a cell-layer-specific expression of PGHS, keratinocytes were isolated from mouse skin and separated according to their state of differentiation into four fractions. Fraction 1 contained squamous and late granular cells, fraction 2 granular and spinous cells, and fractions 3 and 4 proliferatively active basal keratinocytes [27,28]. Using neonatal mouse epidermis, the expression of PGHS-1 mRNA and protein was found to increase with terminal differentiation, i.e. from layers 3/4 to 1. In adult mouse epidermis a similar distribution was observed only for the mRNA, while PGHS-1 protein was more or less uniformly distributed in all fractions (Figures 2a–2c).

In contrast to the situation in intact tissue, a strong expression of PGHS-2 mRNA was observed in all keratinocyte fractions from neonatal epidermis (Figures 3a and 3b). In addition to the major 4.7 kb PGHS-2 mRNA species, two additional mRNA signals of 2.2 and 2.0 kb were monitored under stringent conditions of hybridization using the PGHS-2 cDNA probe. These low-molecular-mass species were particularly expressed in the differentiated cells layers. Whether they represent degradation products or differential splicing products is not known. Despite the high mRNA steady-state concentrations PGHS-2 protein could not be detected in any of the keratinocyte fractions (Figure 3c). As in the intact tissue of adult mice, the PGHS-2 mRNA (Figures 3a and 3b) was barely detectable in keratinocyte fractions from adult epidermis and a signal for the PGHS-2 protein was not obtained (Figure 3c).

Expression of PGHS in stimulated mouse epidermis

Hyperplastic transformation of adult epidermis was evoked by removal of the horny layer or application of the phorbol ester

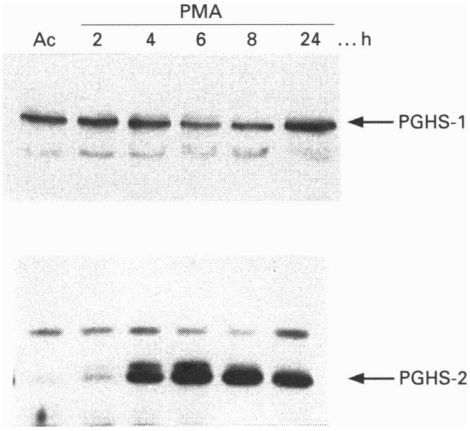

Figure 5 PGHS isoenzyme protein levels in normal and PMA-stimulated adult mouse epidermis

The animals were treated as described in the legend of Figure 4. Protein of the particulate fraction extracted from epidermis was analysed for PGHS-1 (upper panel) and PGHS-2 (lower panel) contents by Western blotting.

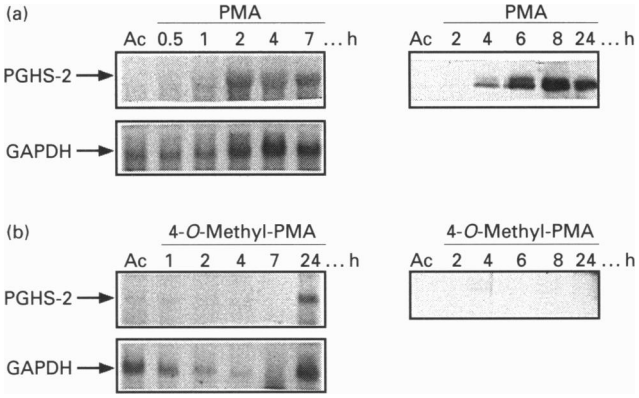

Figure 7 Effect of 4-*O*-methyl-PMA on PGHS-2 expression in mouse epidermis *in vivo*

Total RNA and protein (from the particulate fraction) was extracted from mouse epidermis treated *in vivo* with acetone for 2 h (Ac), or with 10 nmol PMA (**a**) or 400 nmol 4-*O*-methyl-PMA (**b**) for the times indicated. Left-hand panel: Northern-blot analysis. Right-hand panel: Western-blot analysis. The positions of the relevant signals are indicated.

PMA. A strong induction of PGHS-2 expression was observed after a single topical application of PMA *in vivo*. The 4.7 kb signal of PGHS-2 mRNA became visible within 30–60 min, peaking at 2 h, and remaining elevated up to 7 h (Figure 4). Only after 24 h had the signal declined to basal levels. PGHS-2 protein increased steadily reaching a maximum after 6 h and declining thereafter (Figure 5). The anti-PGHS-2 antibody recognized two protein species of 70 and 72 kDa, respectively. The 72 kDa species appeared later and disappeared earlier than the 70 kDa species. Similarly, removal of the horny layer induced a strong expression of PGHS-2 protein with a somewhat delayed onset but a longer duration when compared with the phorbol ester (Figure 6). PGHS-1 expression remained unchanged upon challenge with both stimuli (Figures 4–6). In neonatal epidermis (days 1–10 *post-natum*) PMA was unable to induce PGHS-2 expression (data not shown).

PGHS-2 expression was specifically related to hyperplastic transformation rather than to mitogenic stimulation (balanced hyperproliferation, see the Introduction), as shown by the experiments with 4-*O*-methyl-PMA. As compared with PMA this phorbol ester neither induced PGHS-2 protein expression, despite a late increase of mRNA expression 24 h after treatment (Figures 7a and 7b), nor influenced the constitutive expression of PGHS-1 (data not shown).

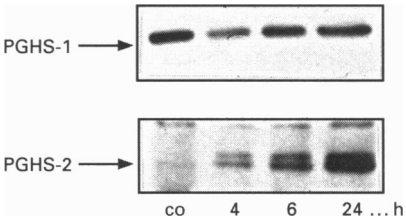

Figure 6 PGHS isoenzyme levels in mouse epidermis after tissue damage *in vivo*

The horny layer from adult mouse skin was removed with cosmetic sandpaper according to Bertsch et al. [26]. Animals were killed at the times indicated. Proteins from the particulate fraction were analysed by Western blotting using isoenzyme-specific antibodies. Untreated mice served as controls. The positions of the PGHS isoenzyme signals are indicated.

Expression of PGHS-2 isoenzymes in mouse keratinocytes *in vitro*

Mouse keratinocytes grown in culture were found to express both PGHS isoenzymes constitutively although at different levels (Table 1). Among those keratinocytes, the immortalized MSCP5 cell line [25] was found to be unique in that it expressed PGHS-2 mRNA and protein constitutively at a high level, whereas the expression of PGHS-1 was found to be very low (Figures 8a and 8b, lane Ac). These results were confirmed by Western-blot analysis of immunoprecipitates using PGHS-isoenzyme-specific antibodies (Figure 8c). Treatment of MSCP5 cells with PMA induced a strong but transient increase of the steady-state concentration of PGHS-2 mRNA and protein (Figures 8a and 8b). With respect to PGHS-1 a slight increase of its mRNA was observed while its protein levels remained below the level of detection (Figure 8a and data not shown).

DISCUSSION

Skin provides a model suitable for investigating different types of hyperproliferative tissue responses to exogenous stimuli. While balanced hyperproliferation appears to represent an acceleration of normal tissue regeneration, hyperplastic transformation is a typical emergency reaction to irritation and injury, i.e. a component of the wound response [1,34]. The possibility of evoking each response selectively offers a unique opportunity to study different mechanisms of mitogenesis including the involvement of endogenous factors. Thus skin has a distinct advantage over most of the *in vitro* systems generally employed to investigate the mechanism of cell growth control.

Prostaglandin formation by keratinocytes has been shown to provide a critical condition of hyperplastic transformation but not of balanced hyperproliferation [35,36]. The experiments presented here were designed to study epidermal prostaglandin synthesis under different conditions at the level of PGHS isoenzyme expression.

In normal epidermis of adult mice a constitutive expression of PGHS-1 was found while no expression of PGHS-2 was de-

Table 1 Expression levels of PGHS isoenzymes in different mouse keratinocyte cell lines

Proteins from the particulate fractions prepared from subconfluent cultures were analysed by Western blotting using isoenzyme-specific antisera and the TROPIX-chemiluminescence detection method. PGHS-1 and -2 signals were quantified by a colour densitometer (Apple Macintosh) using the Ofoto- and Bioscan software.

Cell line	Ratio PGHS-1 to PGHS-2
Hel-30 [51]	1[a]:0.1[a]
PDV/C57 [52]	1[b]:1.9[b]
308 [53]	1[b]:1.2[b]
MCA3D [54]	1[b]:2.0[b]
CarcB [55]	1[b]:0.3[b]
MSCP5 [25]	1[c]:287[a]

[a] 1 min development of hyperbond films.
[b] 30 s development of hyperbond films.
[c] 3 min development of hyperbond films.

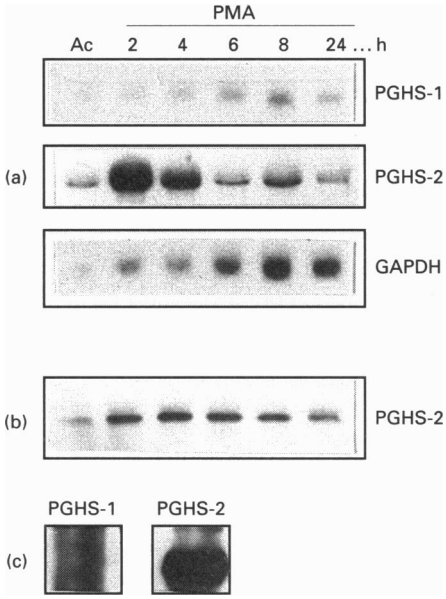

Figure 8 Expression of PGHS in normal and PMA-treated MSCP5 mouse keratinocytes

The cells were treated with acetone for 2 h (Ac) or with PMA (10^{-6} M) for the times indicated. (**a**) Northern-blot analysis; (**b**) Western-blot analysis (protein from particulate fraction); (**c**) Western-blot analysis of protein immunoprecipitated from the particulate fraction of MSCP5 cells with PGHS-1- or PGHS-2-specific antisera.

tectable, thus confirming data obtained for other tissues [5,6,8]. Induction of balanced hyperproliferation (by 4-*O*-methyl-PMA) did not lead to a substantial alteration of PGHS expression whereas upon induction of hyperplastic transformation (by PMA or mechanical damage), a rapid and transient increase of PGHS-2 mRNA and protein occurred, indicating that PGHS-2 is encoded by an immediate early-response gene. This has, indeed, been convincingly demonstrated for fibroblasts treated with phorbol ester in the presence or absence of protein synthesis inhibitors [37] as well as for other mitogen-treated cell types [10,15]. Also expression of other early immediate genes such as c-*fos* and c-*myc* was observed only upon hyperplastic transformation but not in the course of balanced hyperproliferation [38]. The endogenous factors responsible for the PGHS-2 induction by mechanical damage are presently unknown. Potential candidates are cytokines such as interleukin-1 and the autocrine keratinocyte mitogen-transforming growth factor-α which both induce PGHS-2 expression in keratinocyte cultures (unpublished work). Moreover, both factors are released by activated keratinocytes [39–41].

When, upon repeated PMA treatment, epidermis is developing a stationary hyperplasia, i.e. a homoeostatic equilibrium, PGHS-2 expression becomes completely down-regulated, indicating an adaptation of the tissue to prolonged stimulation [42]. A similar situation was observed in neonatal epidermis, which is in a physiological state of stationary hyperplasia, and did not respond to PMA by PGHS-2 expression. Non-responsiveness of neonatal mouse skin has also been reported for other PMA effects such as hyperproliferation, induction of ornithine decarboxylase, inflammation, and tumour promotion [33]. Thus, PGHS-2 expression and prostaglandin production seem to be involved in triggering hyperplastic transformation, rather than in the maintenance of the hyperplastic state.

The constitutive expression of PGHS-1 was neither altered in the course of mouse skin ontogenesis nor upon induction of hyperplastic transformation or balanced hyperproliferation. This indicates that the expression of PGHS-1 and PGHS-2 is controlled by different mechanisms. A result like this was rather unexpected since PMA-induced gene expression is thought to depend on the presence of PMA-responsive elements in the gene promoter [43], which have indeed been found in both PGHS genes [44,45].

The stratified architecture of epidermis allows it to show a correlation between molecular events and stages of differentiation. Previously a differentiation-dependent distribution of PGE_2 formation was reported for mouse epidermis [46,47]. Here we have shown PGHS-1 expression to correlate with terminal differentiation in neonatal skin, thus supporting data on prostaglandin synthesis reported by Cameron et al. [47]. However, in adult mouse epidermis such a correlation was only seen for PGHS-1 mRNA while PGHS-1 protein appeared to be equally distributed in all cell layers, indicating differences in the post-transcriptional regulation of PGHS-1 in neonatal and adult mice. In contrast to the intact tissue, isolated keratinocytes expressed PGHS-2 mRNA (but no protein) in addition to PGHS-1, while subsequent cultivation led to the expression of both PGHS-2 mRNA and protein. In this respect disintegration of the tissue and subsequent cultivation of the basal keratinocytes resemble wounding [42].

Moreover, keratinocyte lines expressed both PGHS isoenzymes, although with different ratios. The MSCP5 line is especially noteworthy in that it almost exclusively expressed PGHS-2. The level of constitutive PGHS-2 expression in MSCP5 cells could be further increased by PMA treatment. Hence, this line is expected to provide a suitable model for studies on the function of PGHS-2 as well as for testing isoenzyme-specific inhibitors [48–50].

In summary, our data indicate a close correlation between differential PGHS expression and distinct physiological states of the skin. In particular, PGHS-2 expression seems to be specifically related to the emergency response to wounding and irritation rather than to simple cellular hyperproliferation and may be responsible for the increased tissue levels of PGE_2 and prostaglandin $F_{2\alpha}$ which are required for the development of epidermal hyperplasia and wound healing [35]. A dysregulation of PGHS expression has been recently shown to occur in the course of skin tumour development [42].

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