Glucosylceramide synthase activity in murine epidermis: quantitation, localization, regulation, and requirement for barrier homeostasis

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Abstract Ceramides, which derive from the hydrolysis of glucosylceramide (GlcCer), are the predominant lipid species in the stratum corneum and are critical for epidermal permeability barrier homeostasis. UDP-glucose:ceramide glucosyltransferase (GlcCer synthase) (EC 2.4.1.80) catalyzes the glucosylation of ceramide to form GlcCer. Recently, we demonstrated a progressive increase in GlcCer synthase expression during fetal barrier development, while others have reported increased GlcCer synthase activity with differentiation of cultured human keratinocytes. To further delineate the role of GlcCer synthase in barrier homeostasis, we determined GlcCer synthase activity and localization in hairless mouse epidermis, both under basal conditions and after acute barrier perturbation. Under basal conditions, GlcCer synthase activity localizes predominantly (~80%) to the dithiothreitol-separated outer epidermis; i.e., 6.2 ± 0.6 versus 1.2 ± 0.1 pmol/min/mg for outer vs. lower epidermis, respectively ($P < 0.0001$). Although acute barrier disruption does not up-regulate epidermal GlcCer synthase activity at any time point up to 24 h, GlcCer synthase is required for barrier homeostasis: topical d,1-*threo*-1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol (P4), a specific GlcCer synthase inhibitor, applied immediately after acute barrier disruption, causes a delay in barrier recovery attributable to specific enzyme inhibition. These findings demonstrate first, that GlcCer synthase activity predominates in the outer epidermis, consistent with an increased formation of GlcCer during barrier ontogenesis and maintenance. Second, GlcCer synthase activity is required for normal permeability barrier homeostasis. Third, baseline epidermal GlcCer synthase activity appears to accommodate acute challenges to the barrier.—**Chujor, C. S. N., K. R. Feingold, P. M. Elias, and W. M. Holleran.** Glucosylceramide synthase activity in murine epidermis: quantitation, localization, regulation, and requirement for barrier homeostasis. *J. Lipid Res.* 1998. **39:** 277–285.

Supplementary key words epidermis • ceramides • sphingolipids • glucosylceramides • UDP-glucose:ceramide d-glucosyltransferase • GlcCer synthase

Together with cholesterol and free fatty acids, ceramides (Cer) comprise the predominant lipid species in the stratum corneum (SC) (1), and they are required for normal epidermal permeability barrier function (2, 3). These ceramides are thought to derive from the hydrolysis of lamellar body-derived glucosylceramides (GlcCer) to Cer, after their extrusion into the SC interstices (4, 5). However, additional Cer could result from the hydrolysis of sphingomyelin (SM), which is present both in lamellar bodies and in the plasma membrane of cell layer in the stratum granulosum, a structure that disappears during cornification.

Glucosylceramides are formed from ceramide and UDP-glucose by the microsomal enzyme, UDP-glucose:ceramide d-glucosyltransferase (GlcCer synthase: EC 2.4.1.80), the first committed step in the biosynthesis of all glycosphingolipids. Originally described by Basu, Kaufman, and Roseman (6), GlcCer synthase activity has been localized to the cytosolic surface of the *cis*-Golgi (7–9), and the human cDNA sequence has been recently published (10). Recently, GlcCer synthase activity was reported to increase with differentiation of human keratinocytes in vitro (11), and we have shown that GlcCer synthase expression increases during fetal barrier development (12). Yet, nothing is known about either the regulation of GlcCer synthase in epidermis in relation to permeability barrier homeostasis, and direct evidence for the requirement of GlcCer synthase for normal epidermal permeability barrier homeostasis also is lacking. To address these points, we first localized GlcCer synthase activity in murine epidermis in relation to differentiation in vivo; second, we determined whether GlcCer synthase activity is

Abbreviations: Cer, ceramide; GlcCer, glucosylceramide; GlcCer synthase, UDP-glucose:ceramide d-glucosyltransferase; TEWL, transepidermal water loss; SPL, sphingolipids.

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regulated by acute changes in barrier status; and third, we ascertained whether application of a selective inhibitor of GlcCer synthase alters the kinetics of barrier recovery in vivo. Our findings show that GlcCer synthase activity increases with epidermal differentiation, but baseline GlcCer synthase activity apparently suffices to accommodate acute challenges to the permeability barrier; and that GlcCer synthase is important for normal permeability barrier homeostasis.

MATERIALS AND METHODS

Materials

[Glucose-U-^{14}C]UDP-glucose (287 mCi/mmol) and [^{3}H]-l-serine were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). d,l-*threo*-1-Phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol·HCL (PPPP or P4), and d,l-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol·HC1 (PDMP) were obtained from Matreya, Inc. (Pleasant Gap, PA). Ceramide (type IV), UDP-glucose, 3-[N-morpholino] propane sulfonic acid (MOPS), 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), dithiothreitol (DTT), and silica gel 60 were purchased from Sigma Chemicals (St. Louis, MO). All other reagents were of the highest commercially available grade. Protein reagents and bovine serum albumin (BSA) were obtained from Bio-Rad (Richmond, CA).

Animals/experimental design

Male hairless mice (Cr1:SKH1-hr strain, 7–8 weeks old) were purchased from Charles River laboratories (Wilmington, MA). They were fed Purina mouse diet and water ad libitum, and were 8–12 weeks old at the time of study. Transepidermal water loss (TEWL) was measured with an electrolytic water analyzer (Meeco, Warrington, PA), as described previously (2, 3, 13). Disruption of the permeability barrier was achieved by tape-stripping (3–4×) one flank of each animal until TEWL rates $\geq$3.0 mg/cm^2 per h. This is a well-established method for disruption of the permeability barrier (2, 3, 13–16), and is performed while animals are anesthetized under approved institutional protocols. The opposite flank of each animal was left untreated, and served as a control for the functional, morphological, and biochemical studies described below.

Sets of 3–6 mice each were treated, as described above, and whole epidermal samples were collected at various time points after tape stripping; i.e., 0, 2.5, 5, 8, 12, and 24 h after tape stripping for morphological and biochemical studies. For the latter, the undersurface of skin samples was scraped with a scalpel blade to remove subcutaneous fat, and immersed in 10 mm ethylenediaminetetraacetic acid (EDTA) in Ca^{2+}- and Mg^{2+}-free Dulbecco's PBS at 37°C for 30–40 min. After incubation, whole epidermis was separated from the dermis by gentle scraping with a scalpel blade. Epidermal sheets then were weighed, minced into small pieces ($<$1 mm^3), and either stored in plastic tubes at −70°C until used, or immediately processed and analyzed for GlcCer synthase activity, as described below. The results were expressed as the ratio of GlcCer synthase activity of the treated flank to that of the untreated flank of the same animal.

Preparation of epidermal fractions

Outer versus lower epidermal preparations were collected after separation with 10 mm dithiothreitol (DTT), as described previously (14). Briefly, excised skin pieces (without subcutaneous fat) were incubated in 10 mm DTT in Dulbecco's phosphate-buffered saline at 37°C for 60 min. The outer epidermis, comprising primarily the stratum corneum (SC), stratum granulosum (SG) and stratum spinosum (SS), was isolated in one piece by gentle peeling with a scalpel blade, while the lower epidermis, comprising primarily the stratum basale (SB), was obtained by scraping the denuded surface (14). In separate experiments, whole epidermis was separated from dermis after incubation in 10 mm EDTA in Ca^{2+}- and Mg^{2+}-free Dulbecco's PBS, pH 7.4, at 37°C for 30–40 min (3, 13). Whole, outer, and lower epidermal samples were processed as above.

Microsome isolation

Samples were thawed on ice, and 1 ml of homogenization buffer (0.25 m sucrose, 0.05 m MOPS, 1 mm, 1 mm EDTA, 1 mm EGTA, and 0.5 mm PMSF, pH 7.4) was added. Crude homogenates were prepared using a Polytron PCU2 tissue homogenizer (Kinematica GmbH, Lucerne, Switzerland) followed by sonication using a Fisher Sonic Dismembranator (Model 300, Artec Systems Corporation, Farmingdale, NY), as described previously (2, 15). Crude homogenates were first centrifuged at 10,000 *g* for 10 min, and then at 100,000 *g* for 60 min, both at 4°C. The resulting high-speed pellets (i.e., crude microsomal fractions) were resuspended in storage buffer containing 0.25 m sucrose, 0.2 m MOPS, pH 6.5, and stored at −70°C until use. No loss of enzyme activity was observed upon freezing or subsequent thawing and activity was stable for over 3 months at −70°C. The protein content was determined by the method of Bradford (17), using bovine serum albumin as standard.

UDP-glucose:ceramide glucosyltransferase (GlcCer synthase) assay

The assay for GlcCer synthase activity was performed according to the method of Matsuo, Nomura, and Imokawa (18), with modifications. Briefly, the solid-phase ceramide substrate was prepared by adsorbing 20 μg ceramide (Type IV, Sigma) in chloroform onto 1 mg silica gel 60 (5 μm diameter). The total assay volume of 110 μl contained, at a final concentration, 45.9 μm [glucose-U-^{14}C]UDP-glucose (60 mCi/mmol), 50 mm MOPS (pH 6.5), 5 mm $MnCl_2$, 2.5 mm $MgCl_2$, 1 mm NADH, 5 mm dimercaptopropanol, 1% w/v CHAPS, and 60 μg protein. The reaction mixture was incubated at 37°C for 30 min, with occasional mixing to suspend the ceramide–silica gel complex. The reaction was terminated by the addition of 0.2 ml of ice-cold PBS and centrifuged at 10,000 *g*, 4°C for 3 min. The resultant pellets were washed by resuspending four times each in 0.2 ml PBS followed by recentrifugation. The pellet was transferred into a scintillation vial with four 0.1-ml additions of PBS; 10 ml of scintillation fluid was added, and radioactivity was counted using a liquid scintillation counter (Beckman LS-1800 Scintillation Counter, CA). Quantitation of the radiolabeled product formed from the labeled UDP-glucose substrate was calculated based upon the specific activity of the sugar nucleotide. Enzyme specific activity is expressed as pmoles of radiolabeled product formed per min per mg protein (pmol/min/mg).

Inhibition studies

The sensitivity of epidermal GlcCer synthase to a specific inhibitor, P4 (19, 20), was first assessed in vitro. Microsomal protein (60 μg) was preincubated with varying concentrations of P4 (range: 0–100 μm) for 5 min at 37°C; reactions then were initiated with the radiolabeled UDP-glucose. Stock P4 solution [10 mm] and appropriate dilutions were prepared in the enzyme storage buffer (pH 6.5, 25°C) immediately prior to use. Results are expressed as the percent of control GlcCer synthase activity; i.e., with no added inhibitor. To then determine whether topically applied P4 was able to inhibit GlcCer synthase activity in vivo, the effect of increasing concentrations of topically applied P4 on GlcCer synthase activity in whole epidermis was determined. The flanks of hairless mice first were tape-stripped, as described above, and TEWL levels were determined again (i.e., ⩾3.0 mg/cm^2/h). Based on the in vitro data, the effect of P4 on in vivo GlcCer synthase activity was assessed in the concentration range between 0.1 μm and 10 mm of P4. Either a solution of P4 in vehicle (30 μl) or vehicle alone (propylene glycol–ethanol, 7:3, v/v) was applied to 5 cm^2 areas of flank skin. One hour later, whole epidermal samples were collected by EDTA treatment (see above), microsomes were prepared, and GlcCer synthase activity was assayed as above. As a control for possible chemical toxicity of P4, the structurally related, but less potent, GlcCer synthase inhibitor PDMP was used.

To determine the effects of reduced GlcCer formation (i.e., GlcCer synthase inhibition) on permeability barrier repair after an acute challenge, three groups of mice were tape-stripped. Each group received either topical vehicle (30 μl) or inhibitor (P4 or PDMP; 300 nmols in vehicle) applied to the treated skin area (5 cm^2). TEWL was then measured every 2 h after P4 application.

Finally, to further demonstrate that the observed effects of P4 on barrier recovery were not a consequence of nonspecific cellular toxicity, [^{3}H]-l-serine incorporation into TCA-precipitable material in the outer epidermis was determined. Serine was chosen to simultaneously estimate protein and lipid (i.e., phosphatidyl-serine) syntheses. Animals were tape-stripped and treated with topical vehicle (30 μl) or P4 (300 nmols), as above. Two h later, animals were killed and flank skin samples were excised and incubated in organ culture for 4 h with [^{3}H]serine (5 μCi in 1.0 ml KGM). During the last h, DTT (to 10 mm) was added to the medium and then rinsed with KGM (4°C, ×3). After separation of outer from lower epidermis using gentle scraping (as above), outer epidermal samples were homogenized and then sonicated in 1 ml PBS containing 0.5 mm PMSF, to which an equal volume of TCA (20% by weight) was added. Precipitated material was collected by vacuum filtration, rinsed with 5% TCA, and incorporated tritium was determined by liquid scintillation spectrometry.

Statistical analysis

Statistical significance was determined using the Student's two-tailed *t*-test. Results are presented as mean ± standard error of the mean (SEM), unless otherwise indicated.

RESULTS

GlcCer synthase activity is abundant in epidermis and concentrated in the outer epidermis

To localize GlcCer synthase activity under basal conditions, we isolated and assayed microsomal preparations from whole epidermis, as well as in the outer and lower layers of the nucleated epidermis. GlcCer synthase activity in whole epidermis was 8.2 ± 0.7 pmol/

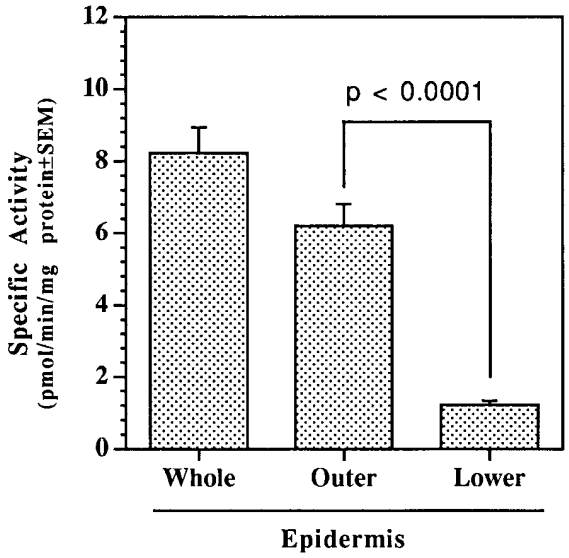

Fig. 1. Localization of GlcCer synthase activity in the epidermis. Mean GlcCer synthase activity (±SEM; n ⩾ 11 animals in duplicate) in microsomal fractions from whole epidermis isolated using EDTA/PBS, outer and lower epidermal layers separated using the DTT method. GlcCer synthase activity is predominantly localized to the outer epidermal layers.

min per mg of protein (n = 30, ±SEM) (**Fig. 1**), levels at least equivalent to that reported in other tissues (11, 12, 18, 19, 21). We next measured GlcCer synthase activity in the outer versus lower epidermis. Activity is concentrated predominantly in the outer epidermis (Fig. 1), where it accounts for ~80% of epidermal specific activity (outer versus lower epidermis 6.2 ± 0.6 versus 1.2 ± 0.1 pmol/min per mg of protein, respectively (n = 11; $P < 0.0001$). An equivalent ratio was obtained for total enzyme activity (i.e., 80% outer vs. 20% lower; $P < 0.0001$). These results demonstrate that epidermis is highly enriched in GlcCer synthase, and that a majority of epidermal GlcCer synthase activity is associated with the outer, differentiating layers of epidermis.

GlcCer synthase activity does not change significantly in response to acute barrier disruption

Previous studies have demonstrated that a more proximal step in the synthesis of Cer; i.e., the generation of sphingoid base by serine palmitoyltransferase, is regulated specifically by barrier requirements (3). Therefore, we next assessed GlcCer synthase levels at various time points after barrier disruption by tape stripping. Although variations in enzyme activity occurred among different experimental groups of animals, the ratios of GlcCer synthase specific activity for treated versus untreated flanks for each group (n = 3–6 animals) were not significantly altered at 2.5, 5, 8.5, 12, and 21.5 h; i.e., the ratio of activity in the treated versus untreated control epidermis remained near unity (not shown). These results demonstrate that GlcCer synthase activity in whole epidermis is not up-regulated in response to barrier disruption by tape-stripping.

As the majority of GlcCer synthase activity is concentrated in the outer epidermis (i.e., 70–80%), we next investigated whether changes in distribution could occur during the recovery response. GlcCer synthase activity was assessed between 2 and 3 h after barrier disruption by tape stripping. Although a slight elevation in GlcCer synthase activity appeared to occur in the outer epidermis (~20%) in comparison to the untreated outer epidermis, the results did not achieve statistical significance (not shown; $P > 0.3$). Moreover, GlcCer synthase activity in the treated, lower epidermis did not change (1.089 ± 0.112 and 1.061 ± 0.076 for treated and untreated, respectively). Furthermore, the ratios (treated/untreated) of GlcCer synthase activity for the outer epidermis (1.245 ± 0.162) and for the lower epidermis (1.031 ± 0.119) were not affected by barrier disruption. These results show that epidermal GlcCer synthase activity does not change significantly in specific epidermal layers after barrier disruption.

P4 inhibits epidermal GlcCer synthase activity and delays barrier recovery

As the baseline levels of GlcCer synthase activity appear to accommodate acute epidermal barrier insults, we next asked whether inhibition of epidermal GlcCer synthase activity could affect permeability barrier function. P4 is the most active congener of the PDMP-type GlcCer synthase inhibitors, which are specific and reversible (19, 20). We first determined the optimal dose of P4 for inhibition of GlcCer synthase activity in an in vitro, cell-free system, and then ascertained the optimal dose in intact epidermis after topical applications. Whereas 1-phenyl-2-(palmitoylamino)-3-morpholino-1-propanol (PPMP) reportedly inhibits GlcCer synthase activity by 40% in cultured human keratinocyte lysates at 10 μm (11), at the same concentration P4 inhibited epidermal microsomal GlcCer synthase activity by 86.6 ± 0.6% (**Fig. 2A**). However, a higher dose of topical P4 (>1 mm) was required in order to achieve a comparable extent of in vivo inhibition (i.e., 68.4–78.0% vs. vehicle control; $P < 0.0001$) of GlcCer synthase activity (Fig. 2B). The difference between in vitro and in vivo (topical) doses likely reflects a limited inhibitor penetration to the site of enzyme action.

We next assessed whether an optimal, inhibitory dose of P4 alters the kinetics of barrier recovery after disruption by tape-stripping. As seen in **Fig. 3**, topical P4 (10 mm) significantly delayed barrier recovery at various time points from 0–6 h. Whereas vehicle-treated animals dem-

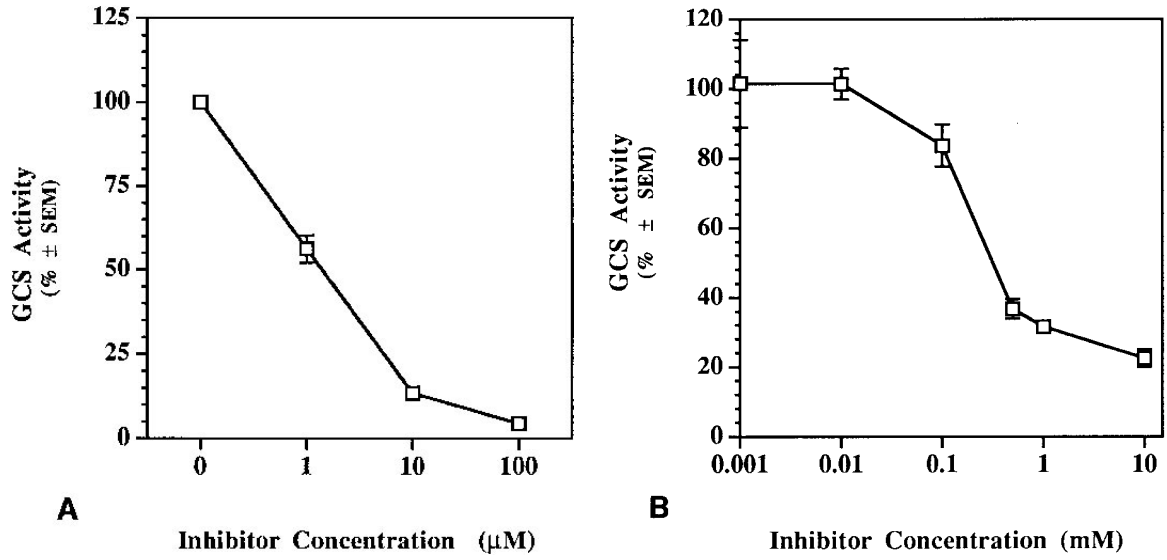

Fig. 2. Inibition of GlcCer synthase activity by P4. A: In vitro inhibition of GlcCer synthase activity by P4. Plot of relative % inhibition as P4 concentration was varied from 0 to 100 μm. Each point represents mean relative % inhibition based on triplicate determinations using microsomes of untreated epidermis (±SEM). B: In vivo inhibition of GlcCer synthase activity. The barrier was disrupted by tape-stripping followed by topical application of various concentrations of P4 to the skin. After 1–2 h, whole epidermis were isolated, microsomes were prepared, and GlcCer synthase activity was determined as described in Materials and Methods. Data are mean relative % inhibition ± SEM; n = 3 animals in duplicate for each group.

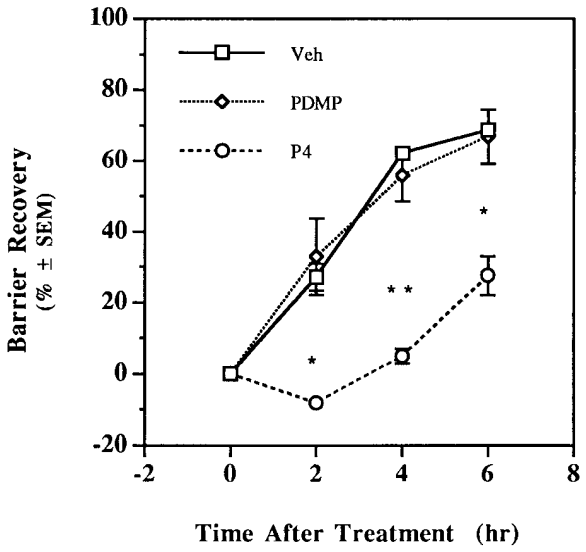

Fig. 3. Effect of P4 and PDMP on barrier recovery after barrier disruption by tape-stripping. The barrier was disrupted by tape-stripping followed by topical application of 10 mm P4, or PDMP, or vehicle (propylene glycol–ethanol 7:3) alone. TEWL measurements were taken at the indicated time points. Results are presented as the percent change from TEWL at time 0 and are mean ± SEM; n = 3 animals for each group. P4 significantly delayed early recovery (*$P < 0.002$ at 2 and 6 h; **$P < 0.0001$ at 4 h) while the less potent inhibitor, PDMP, did not alter barrier recovery.

onstrated >50% recovery at 4 h, TEWL levels remained significantly elevated (i.e., little-to-no barrier recovery) in P4-treated animals (Fig. 3; $P < 0.0001$ vs. vehicle control). Similarly, P4-treated sites again showed a delayed recovery 6 h after barrier disruption; i.e., 27 vs. 69% recovery for P4 vs. vehicle-treated animals, respectively ($P < 0.002$). In addition, no significant difference in recovery between vehicle- and P4-treated epidermis was evident at later time points (i.e., 24 and 48 h; not shown).

A number of additional experiments were performed to eliminate nonspecific toxicity as the cause for the P4-induced delay in barrier recovery. First, we used a structural analog of P4, d,l-threo-l-phenyl-2-decanoylamino-3-morpholino-l-propanol.HCl (PDMP), which is a less potent inhibitor of GlcCer synthase (19). Whereas an equimolar topical dose of PDMP (i.e., 10 mm or 300 nmol) resulted in no inhibition of in vitro GlcCer synthase activity (data not shown), PDMP also caused no delay in permeability barrier recovery after tape-stripping (Fig. 3). Finally, as a further test of nonspecific toxicity, topical P4 did not alter the rate of incorporation [^{3}H]serine into the TCA-precipitable protein (and lipid) in comparison to vehicle-treated control epidermis (not shown), suggesting that synthetic processes in the outer epidermis are not affected. Together, these results strongly suggest that the P4-induced permeability barrier alterations are not secondary to nonspecific chemical

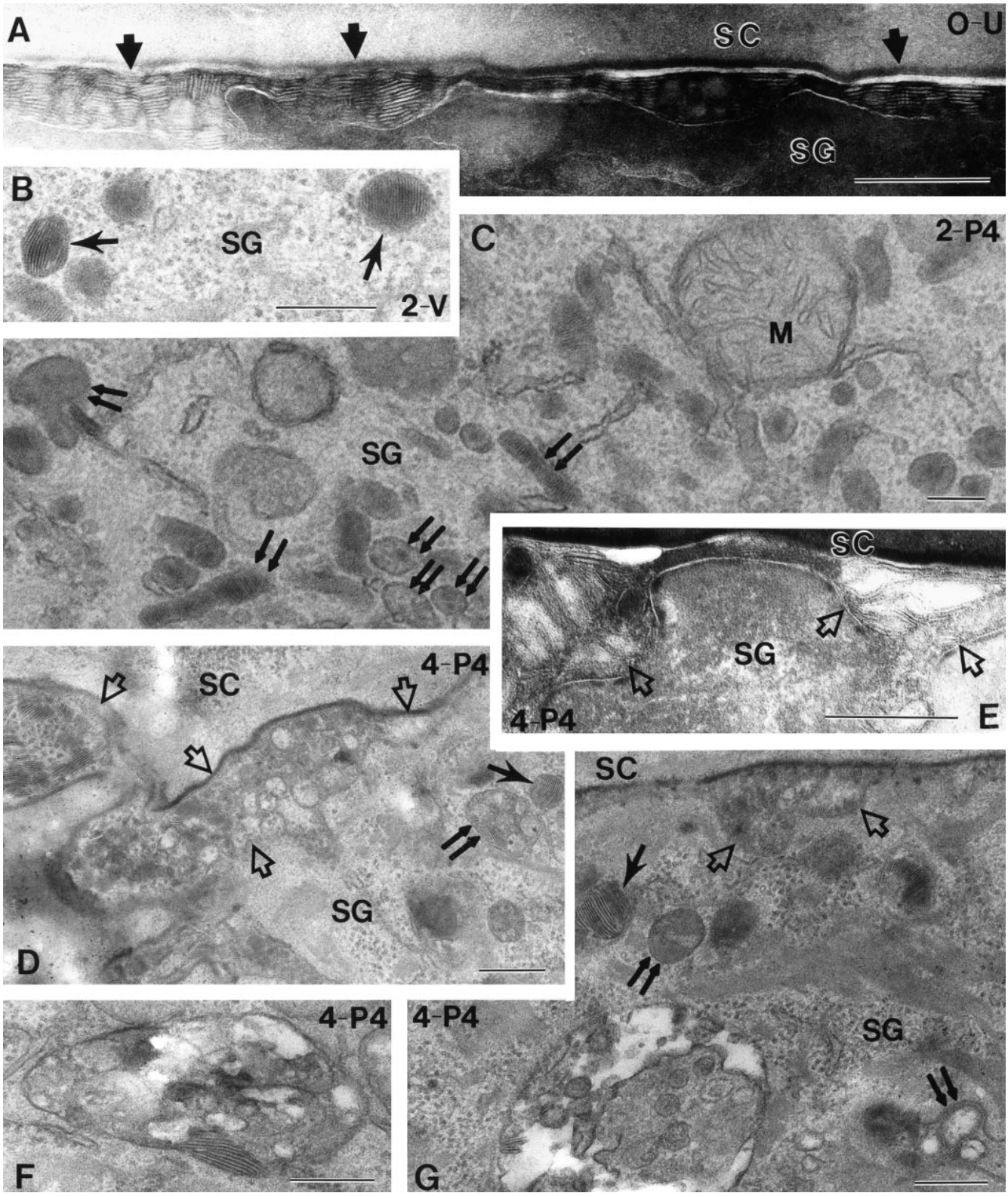

Fig. 4. P4 disrupts the lamellar body secretory system and SC extracellular lamellae. A: Immediately before barrier disruption, normal deposition of lamellar body contents occurs at the stratum granulosum (SG)-stratum corneum (SC) interface (solid fat arrows). B: Two hours after tape-stripping plus vehicle application, most lamellar bodies display normal shape and internal contents (single, thin arrows). C: Two hours after tape-stripping treatment plus a single P4 application, the numbers of lamellar bodies appear normal, but many display

toxicity, but rather due to inhibition of epidermal GlcCer synthase activity. Moreover, these results show that the generation of GlcCer is required for normal permeability barrier homeostasis.

P4 treatment results in marked abnormalities of the lamellar body secretory system

To assess the mechanism of the P4-induced barrier abnormality, we next examined the epidermal ultrastructure of inhibitor- versus vehicle-treated epidermis at various time points after barrier disruption. Untreated epidermis displayed normal deposition of secreted lamellar body contents at the SG–SC interface (**Fig. 4A**, solid arrows). In vehicle-treated epidermis, lamellar body number and contents appear normal-to-increased by 2 h (Fig. 4B). In contrast, P4-treated epidermis displayed abnormal lamellar body contents by 2 h (Fig. 4C). In contrast, mitochondrial ultrastructure appeared normal (Fig. 4C). These changes became more striking by 4 h, when most, but not all lamellar bodies appeared abnormal, coalescing into large multivesicular bodies (Figs. 4D, F, G, double arrows; normal lamellar bodies = 4D, 4G, single arrows). Finally, abnormal lamellar content was present at the SG–SC interface in 4-h samples (Figs. 4D, E, open arrows). These results show that P4 alters the formation, internal contents, and secreted contents of epidermal lamellar bodies.

DISCUSSION

Ceramides and certain sphingosine derivatives regulate a variety of cellular processes (22–24), including the epidermis (25). In the epidermis, ceramides are required as bulk components of the extracellular lamellar matrix (2, 3, 26, 27). Glucosylceramides provide a critical precursor pool for these ceramides (4, 5); consequently, much attention has focused on the production of GlcCer, which serves as a precursor of most glycolipids. The enzyme GlcCer synthase is responsible for the biosynthesis of GlcCer, and has been characterized recently in extracutaneous tissues (7–10). Moreover, two lines of evidence suggest that GlcCer synthase is important for the epidermal differentiation: *1*) its activity correlates directly with the extent of differentiation of cultured human keratinocytes (11); and *2*) its activity increases in parallel with early stages of fetal barrier development (12).

In the present study, we demonstrated that GlcCer synthase activity is localized predominantly to the outer epidermis, consistent with reports that glucosylceramide levels peak in the stratum granulosum (28–31). In contrast to GlcCer synthase, serine palmitoyltransferase levels are nearly equivalent in the lower and the outer epidermis (16), and 3-hydroxy-3-methylglutaryl-coenzyme A reductase localizes predominately to the lower epidermis (14). As with GlcCer synthase, several lipid processing enzymes including glycosidases (32–34), sphingomyelinase (33, 35), phospholipase A (35, 36), triacylglycerol hydrolase (33), and steroid sulfatase (37) accumulate in the outer epidermis. GlcCer synthase activity may provide a pool of the precursor GlcCer, required for the formation of mature lamellar bodies (see below), whose contents ultimately are extruded into the extracellular space of the SC for the formation and maintenance of competent epidermal permeability barrier. However, unlike serine palmitoyltransferase (16) and HMG-CoA reductase (14), GlcCer synthase activity does not seem to be regulated by permeability barrier requirements. Furthermore, in contrast to HMG-CoA reductase, a negligible-to-minor increase in GlcCer synthase activity occurs after acute barrier disruption by tape-stripping, and this limited change is localized to the outer epidermis.

As acute barrier disruption by tape stripping does not significantly alter the levels of GlcCer synthase, we were interested in knowing whether blocking GlcCer synthase activity by P4 would influence epidermal permeability barrier recovery after acute barrier disruption by tape stripping. P4 delayed epidermal permeability barrier recovery, suggesting that GlcCer synthase, through the generation of barrier lipid precursor, GlcCer, plays an important role in epidermal barrier function. These results suggest that production of epidermal ceramides via sphingomyelin synthesis and degradation does not suffice and cannot compensate. In addition, we have previously demonstrated the critical role of glucosylceramide-to-ceramide hydrolysis in the formation and maintenance of the barrier (4, 5, 38). Thus, interruption of the generation of SC Cer at any point in the pathway is detrimental to epidermal homeostasis. Furthermore, these results are consistent with the glucosylation of Cer to GlcCer being required for "targeting" of barrier ceramides to the lamellar body delivery pathway in the epidermis.

A potential link between sphingolipid biosynthesis

a distorted shape and/or abnormal internal contents (double-thin arrows). D–G: Four hours after tape-stripping plus a single P4 application, when the barrier abnormality is maximal, large multivesicular organelles are present in the cytosol throughout the nucleated layers, some of which contain lamellar contents (F, G). Most, but not all lamellar bodies display abnormal internal contents (D, E, fine double arrows), and the SC–SC interface reveals deposition of abnormal lamellar body contents (D, E, open, fat arrows). A–C, E–G, OsO_4 post-fixation; D, RuO_4 post-fixation, Error bar = 0.2 μm; A, ×95,000; B, ×70,000; C, ×40,000; D, ×40,000; E, ×95,000; F, ×60,000; G, ×50,000.

and secretory pathways also has been uncovered using PDMP. In cultured human skin fibroblasts, low dose PDMP (2.5–10 μm) inhibited GlcCer synthase, while higher doses (>25 μm) not only inhibited sphingolipid synthesis, including GlcCer and sphingomyelin, but also delayed lipid and protein transport, and induced Golgi swelling and fragmentation (39). These results suggest that both early (GlcCer synthase) and late Golgi compartments are altered in PDMP-treated cells, presumably due to reduced glycosylation required for normal membrane trafficking (39). Therefore, the P4-induced delay in epidermal barrier recovery reported here may involve not only direct inhibition of GlcCer synthase but also subsequent alterations in secretory pathways. The abnormalities in epidermal LB morphology (c.f., Fig. 4) are consistent with multiple effects of PDMP in vivo.

In summary, the in vivo data presented here demonstrate that GlcCer synthase activity predominates in the outer epidermis, consistent with an increased requirement for GlcCer synthase activity during barrier ontogenesis and maintenance. Second, baseline epidermal GlcCer synthase activity appears to suffice to accommodate acute challenges to the barrier, as only minor changes occur in epidermal enzyme activity after barrier disruption. Third, evidence is also provided in favor of a possible involvement of GlcCer synthase in epidermal barrier function. Finally, these results underlie a potentially critical role for glucosylation events in the normal membrane trafficking of the epidermis.

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