Glucocorticoid blockade reverses psychological stress-induced abnormalities in epidermal structure and function

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Choi, Eung-Ho, Marianne Demerjian, Debra Crumrine, Barbara E. Brown, Theodore Mauro, Peter M. Elias, and Kenneth R. Feingold. Glucocorticoid blockade reverses psychological stress-induced abnormalities in epidermal structure and function. Am J Physiol Regul Integr Comp Physiol 291: R1657–R1662, 2006. First published July 20, 2006; doi:10.1152/ajpregu.00010.2006.—Many cutaneous disorders are adversely affected by psychological stress (PS), but the responsible mechanisms are poorly understood. Recent studies have demonstrated that PS decreases epidermal proliferation and differentiation, impairs permeability barrier homeostasis, and decreases stratum corneum integrity. PS also increases the production of endogenous glucocorticoids (GC), and both systemic and topical GC cause adverse effects on epidermal structure and function similar to those observed with PS. We therefore hypothesized that increased endogenous GC in PS mediates its adverse effects. To test this hypothesis, we used two independent approaches, administering either RU-486, a GC receptor antagonist that inhibits GC action, or antalarmin, a corticotropin-releasing hormone (CRH) receptor antagonist that prevents increased GC production in the face of PS. Inhibition of either GC action or production prevents the PS-induced decline in epidermal cell proliferation and differentiation, impairment in permeability barrier homeostasis, and decrease in stratum corneum (SC) integrity. Moreover, the pathophysiological basis for the abnormality in permeability barrier homeostasis; i.e., decreased lamellar body production and secretion, is restored toward normal by inhibition of GC action. Similarly, the mechanistic basis for the decrease in SC integrity, i.e., a reduction in corneodesmosomes, is also normalized by inhibition of GC action. Thus many of the adverse effects of PS on epidermal structure and function can be attributed to increased endogenous GC and conversely, approaches that either reduce GC production or action might benefit cutaneous disorders that are provoked or exacerbated by PS.

RU-486; corticotropin-releasing hormone; stratum corneum integrity; barrier homeostasis

PSYCHOLOGICAL STRESS (PS) is well recognized to provoke, exacerbatе, or propagate many cutaneous dermatoses, including psoriasis and atopic dermatitis (20, 31, 35). Many of the disorders that are adversely affected by PS are also associated with abnormal permeability barrier function (17, 18). Studies in both rodents and human have demonstrated that PS perturbs permeability barrier homeostasis. While basal permeability barrier function is normal, the kinetics of barrier recovery following acute barrier disruption are delayed (2, 7, 8, 16). This delay in barrier recovery is accounted for by a decrease in the production and secretion of lamellar bodies (LB) (5) with a resultant decrease in the formation of the extracellular lamellar membranes that mediate epidermal permeability barrier function (10). Epidermal lipid synthesis is essential for providing the lipids required for the formation of LB (11). Accordingly, the decrease in LB secretion in PS animals is explained by a decrease in epidermal lipid synthesis (5). Of note, the abnormality in barrier homeostasis induced by PS can be normalized by the topical applications of a mixture of barrier lipids, which normalize LB formation and secretion (5). In addition to adversely affecting barrier homeostasis, PS also decreases keratinocyte proliferation, impairs epidermal differentiation, and decreases stratum corneum (SC) integrity, defined as the resistance of SC to sequential tape stripping (5). The decrease in SC integrity is associated with a reduction in both the density and size of corneodesmosomes (CD) in the lower SC (13). Interestingly, coinadministration of topical lipids not only normalized LB production and barrier function in the face of PS but also increased the density and size of CD, restoring SC integrity toward normal (5). Thus the decrease in epidermal lipid synthesis induced by PS not only accounts for the defect in permeability barrier homeostasis, but it also impairs SC integrity (5).

Although the mechanisms by which PS induces these cutaneous abnormalities remain unknown, both systemic and topical glucocorticoid (GC) treatment produce comparable abnormalities in epidermal structure, function, and homeostasis (23). Prolonged treatment with GC decreases epidermal proliferation and differentiation (9, 26, 32, 33). Additionally, more recent studies by our laboratory demonstrated that GC treatment also impairs both permeability barrier homeostasis and SC integrity and cohesion (23). Moreover, the mechanisms that account for the abnormalities in GC-treated animals appear similar to those that account for the abnormalities induced by PS. Specifically, GC treatment decreases epidermal lipid synthesis, leading to a reduction in both LB formation and secretion, as well as a decrease in the quantities of extracellular lamellar membranes and a decrease in the density of CD in the SC (23). Furthermore, as in PS animals, topical application of a mixture of barrier lipids largely corrects the GC-induced structural and functional abnormalities (23). These results are consistent with the hypothesis that GC could mediate the abnormalities in cutaneous function produced by PS.

In support of this hypothesis, numerous studies have shown that PS increases endogenous GC production (6, 7, 14, 15).

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This abnormality occurs at the level of the hypothalamus, initially with an increase in corticotropin-releasing hormone (CRH) leading to increased ACTH secretion from the pituitary gland, which then stimulates the adrenal gland to produce excess GC (34). Further support for this hypothesis has come from studies using RU-486, an inhibitor of GC action at the cellular level. Treatment of PS animals with RU-486 reverses the delayed kinetics of permeability barrier recovery in PS animals (7, 8). Together, these results led us to hypothesize that PS stimulates increased endogenous GC resulting in the spectrum of adverse cutaneous effects described above.

To test this hypothesis, we employed two independent approaches to block GC in a recently described PS model (5); systemic administration of either RU-486 to inhibit GC action, or antalarmin, a CRH receptor antagonist, to prevent the increase in GC production that occurs during PS. Both approaches prevent emergence of the abnormalities in cutaneous structure, function, and homeostasis induced by PS. These findings provide strong support for the hypothesis that increased endogenous GC mediate the adverse epidermal effects of PS.

MATERIALS AND METHODS

Animal model. Female hairless mice (SkH1/Hr), 8–10 wk of age, were purchased from Charles River Laboratories (Wilmington, MA). All animal experiments described in this study were conducted in accordance with accepted standards of humane animal care, under protocols approved by the local animal research committee at San Francisco Veteran Affairs Medical Center. All mice were maintained in our animal care facility in a temperature- and humidity-controlled room and fed standard laboratory chow and tap water ad libitum. Before beginning experiments, cohorts of four animals each were kept in separate cages for at least 14 days. Psychological stress was produced as described previously (5). Briefly, groups of six animals at a time were transferred to a 12.5 cm diameter, 12.5 cm high, transparent glass jar for 48 h and exposed to continuous visible light and radio noise. Control mice were kept in ordinary cages without exposure to light and radio noise. All animals continued to have free access to food and water ad libitum.

GC receptor antagonist and CRH receptor inhibitor treatment. Three groups of animals were studied: 1) control (not stressed), 2) PS injected with vehicle, and 3) PS injected with RU-486 or antalarmin. RU-486 (mifepristone; Sigma-Aldrich, St. Louis, MO) was administrated intraperitoneal at a dose of 6 mg/kg (1 mg/ml of propylene glycol) (1) and antalarmin hydrochloride (Sigma-Aldrich) was administrated intraperitoneal at a dose of 20 mg/kg (1) 1 h before stress (8:00 AM) and 24 h after stress (9:00 AM). Because GCs increase rapidly after stress, we elected to administer RU-486 and antalarmin 1 h before the stress. Also, because GC hormones have a diurnal variation peaking around 8:00–9:00 AM, RU-486 and antalarmin were administered again at that time, 24 h after the first injection.

Measurement of serum corticosterone. At 48 h after continuous PS, blood samples were obtained immediately after animals were killed between 8:00 AM and 9:00 AM. Blood was centrifuged for 15 min. The serum was separated and frozen at -80°C until assayed. Serum corticosterone level was measured with a commercially available ELISA kit (ALPCO Diagnostic, Windham, NH) according to manufacturer’s protocol.

Functional studies. Transepidermal water loss (TEWL) was measured as parts per million per centimeter squared per hour with an electrolytic water analyzer (Meeco, Warrington, PA) as described previously (19). SC integrity, defined as resistance to mechanical disruption, was assessed as the rate of change in TEWL with repeated tape stripping. TEWL was measured after each sequential stripping with 22 mm D-squame 100 tapes (CuDerma, Dallas, TX) as described previously (13, 21). Baseline TEWL was not altered by PS. Barrier recovery was determined by measuring TEWL immediately after and at 6 h following acute barrier disruption (TEWL levels > 400 ppm) by tape stripping, as described previously (12, 19, 27). The barrier was disrupted to a similar degree in all groups of animals. We used different sets of animals for the barrier recovery and SC integrity experiments.

Immunohistochemical staining. Skin biopsy samples were taken (n = 5 from each group) and processed for proliferation cell nuclear antigen (PCNA) immunostaining and immunohistochemical staining for differentiation markers, including involucrin, loricrin, filaggrin, as well as desmoglein-1 (DSG1). Six-micrometer sections were prepared for all specimens. Binding of the PCNA primary antibody was detected by ABC-peroxidase from Vector, utilizing diaminobenzidine as the substrate (Vector, Burlingame, CA). Affinity-purified rabbit antibodies specific for mouse involucrin, loricrin, and filaggrin were obtained from BabCo (Richmond, CA); antibody for DSG1 was a gift from Dr. John Stanley, University of Pennsylvania; affinity-purified biotinylated goat antirabbit IgG was purchased from Vector. Immunohistochemical staining for the differentiation proteins and DSG1 was detected by the ABC-peroxidase method, as above (25). Negative controls without primary antibodies showed no immunolabeling. Five pictures for each sample were taken. For the PCNA experiments, stained nuclei were counted as positive and the number of positive cells per length of basal epidermis was calculated. For the involucrin, filaggrin, loricrine, and DSG1 immunohistochemical studies, an investigator without knowledge of the origin of the specimen analyzed the intensity of immunostaining. A representative picture for each group was chosen for publication.

Electron microscopic studies. Skin biopsy samples were taken from treated and control mice (n = 5 from each group). Samples were minced to <0.5 mm³, fixed in modified Karnovsky’s fixative overnight, and postfixed in 0.5% ruthenium tetroxide (RuO₄) and 2% aqueous osmium tetroxide (OsO₄) containing 1.5% potassium ferrocyanide (22, 28). After postfixation, all samples were dehydrated in graded ethanol solutions and embedded in an Epon-epoxy mixture. Ultrathin sections were examined with or without further lead citrate contrasting, in Zeiss 10A electron microscope (EM; Carl Zeiss, Thornwood, NY) operated at 60 kV.

Quantitative EM analysis. To exclude subjective bias in these morphologic studies, both CD and LB number (density) and secretion in EM pictures were analyzed by an objective method, as described previously (5). Briefly, four EM pictures of low magnification (>5,000) from each sample were analyzed for LB quantitation. The number of protrusions (invagination along the SC-SC interface) was quantitated and assessed planimetrically as the number per unit length of SC-SC interface. To assess LB densities, LB images in the cytosol of the uppermost two layers of the SG were counted and expressed as average number per unit area of cytosol. For CD quantitation, CD length, selected at random from the first and second cell layers of the lower SC, was measured. The ratio of the total length of intact CD to the total length of cornified envelopes was determined by planimetry, as described previously (21, 24, 30).

Statistical analysis. All data were expressed as means ± SE. Statistical analyses were performed using paired and unpaired Student’s t-tests.

RESULTS

GC receptor blockade reverses the PS-induced decrease in keratinocyte proliferation and differentiation. As reported previously (5), PS inhibits epidermal proliferation, reflected by a decrease in PCNA-positive cells in the basal layer of the epidermis, as well as decreases the expression of epidermal differentiation-related proteins, involucrin, loricrin, and filaggrin (Fig. 1). In contrast, coadministration of RU-486 with PS...
RU-486 reverses the decrease in keratinocyte proliferation, differentiation, and desmoglein-1 (DSG1) expression induced by psychological stress (PS). PS results in a decrease in proliferation cell nuclear antigen (PCNA)-positive cells in the basal layer of the epidermis (≈38% decrease) (A, B, C, and C-1). However, coadministration of RU-486 with PS reverses the PS-induced decline in PCNA immunostaining, which suggests that the PS-induced decrease in epidermal proliferation is mediated by increased endogenous glucocorticoids (GC). PS decreased expression of epidermal differentiation-related proteins, involucrin (INV; D, E, and F), loricrin (LOR; G, H, and I), and filaggrin (FIL; J, K, and L). In contrast, coadministration of RU-486 with PS reversed the PS-induced decline in involucrin, loricrin, and filaggrin immunostaining. Expression of DSG1, a protein component of corneodesmosomes (CD), declines during PS, an abnormality that again is reversed by RU-486 treatment (M, N, and O). Statistical significance in PCNA immunostaining determined by Student’s t-test (n = 5). Scale bar = 100 µm. Veh, vehicle.
reverses the PS-induced decline in PCNA, involucrin, loricrin, and filaggrin immunostaining (Fig. 1). Together, these results suggest that the PS-induced decrease in epidermal proliferation and differentiation is mediated by increased endogenous GCs.

**GC Receptor blockade reverses the PS-induced abnormalities in permeability barrier homeostasis.** Previous studies have shown that the PS-induced delay in permeability barrier recovery following acute disruption is blocked by cotreatment with RU-486 (7). The delay in barrier recovery in PS animals is associated with a significant decrease in the density of LB in the SG cytosol, as well as decreased secretion of LB contents at the stratum corneum-stratum granulosum (SG-SC) interface (Figs. 2 and 3, A and B). Again, RU-486 cotreatment prevented the PS-induced abnormalities in the LB production, and it also appears to normalize secretion (Figs. 2 and 3, A and B). Together, these results show that GC-receptor blockade normalizes LB production and secretion, even in the face of ongoing PS.

**GC receptor blockade normalizes SC integrity in the face of PS.** We next assessed whether RU-486 prevents the reduction in SC integrity induced by PS. As in the previous studies (5), PS again induced an impairment in SC integrity (Fig. 4). Yet, coadministration of RU-486 to PS rodents reverses the decrease in SC integrity induced by PS (Fig. 4; $P < 0.001$). Previous studies have shown further that the decrease in SC integrity in PS can be attributed to a reduction in the density of CD (5). Again, the expected decrease in CD density that occurs in PS animals is prevented by cotreatment with RU-486 (Figs. 2 and 3C; $P < 0.05$). Finally, expression of DSG1, a protein component of CD, declines during PS, an abnormality that again is reversed by RU-486 treatment (Fig. 1, M–O). Together, these results show that the PS-induced impairment in SC integrity is mediated by the action of excess GC induced by PS (7).

**Inhibition of GC production prevents PS-induced abnormalities in epidermal structure and function.** Although the above studies with RU-486 strongly suggest that the abnormalities in the epidermis that occur during PS are mediated by elevations in GC acting through the GC receptor, RU-486 has other effects that extend beyond inhibition of GC action. To ensure that the normalization of epidermal structure and function observed with RU-486 in PS animals are not due to other effects of RU-486, we next used antalarmin, an inhibitor of CRH action, which blocks the increase in plasma GC induced by PS (36). In these experiments, PS again increased plasma corticosterone levels (7), an increase that was blocked by cotreatment with antalarmin (Fig. 5; $P < 0.002$). More importantly, both the PS-induced delay in permeability barrier recovery and the PS-induced impairment in SC integrity are completely reversed by antalarmin cotreatment (Fig. 5). Additionally, coadministration of antalarmin with PS also reverses the PS-induced inhibition of epidermal proliferation and differentiation (The online version of this article contains supplemental data showing PCNA, involucrin, loricrin, and filaggrin immunostaining). These results provide further evidence that

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![Figure 2](image2.png)

**Fig. 2.** Structural changes induced by PS are recovered by coadministration of RU-486 with PS. PS animals (B) show a significant decrease in the density of lamellar bodies (LB) in the SG cytosol (arrowheads), as well as decreased secretion of LB contents at the stratum corneum-stratum granulosum (SG-SC) interface (short tailed arrows) compared with control animals (A). Also CD in SC are decreased by PS (long-tailed arrows). RU-486 cotreatment (C) prevented the PS-induced abnormalities in the LB production and secretion, and CD. Scale bar = 2 μm.

![Figure 3](image3.png)

**Fig. 3.** Decrease of LB density and secretion and CD density by PS are recovered by coadministration of RU-486 with PS. The delay in barrier recovery in PS animals is associated with a significant decrease in the density of LB in the SG cytosol, as well as as decreased secretion of LB contents at the SG-SC interface. Again, RU-486 cotreatment prevented the PS-induced abnormalities in the LB production (A) and normalized secretion (B). These results show that GC-receptor blockade normalizes LB production and secretion in the face of PS. As in a previous study, the expected decrease in CD density that occurs in PS animals is prevented by treatment with RU-486 (C). Statistical significance was determined by Student’s t-test ($n = 5$).
the abnormalities in epidermal structure and function induced by PS are mediated by increased endogenous GC production.

DISCUSSION

In previous studies, we have shown that PS inhibits epidermal proliferation, decreases epidermal differentiation, impairs permeability barrier homeostasis, and reduces SC integrity and cohesion (5). We and others have described similar derangements in epidermal structure and function following systemic or topical GC treatment (23). Moreover, PS is well known to lead to an increase in GC production. Specifically, PS stimulates hypothalamic CRH production leading to an increase in ACTH secretion by the pituitary, resulting in an increase in the synthesis and secretion of GC from the adrenal glands (6, 15). We therefore hypothesized that the abnormalities in epidermal structure and function that occur in response to PS are mediated by excess GC. In the present study, we prove this hypothesis by two different experimental approaches; i.e., inhibition of GC action with RU-486, a GC receptor antagonist, and by inhibition of the excess GC production with antalarmin, a CRH receptor antagonist. Both approaches prevent the abnormalities in SC structure and function induced by PS. Specifically, treatment with either RU-486 or antalarmin prevents the decrease in epidermal proliferation, the decrease in epidermal differentiation marker expression, the impairment in permeability barrier homeostasis, and the reduction in SC integrity and cohesion that occurs with PS. That two very different approaches for decreasing the effects of excess GC resulting from PS were able to normalize the abnormalities in epidermal structure and function provides strong support for our hypothesis that the abnormalities seen are mediated by excess GC and were not due to other potential effects of these drugs.

In previous studies, our laboratory has shown that the abnormality in permeability barrier homeostasis induced by PS is due to decreased LB formation and secretion, whereas the abnormality in SC integrity and cohesion is due to a decrease in the density of CD (5). In the present study, we demonstrate that inhibition of GC action or production restores both LB formation and secretion, as well as the density of CD in SC animals. Thus the pathophysiological basis for the abnormality in permeability barrier function, i.e., decreased LB secretion and the abnormality in SC integrity and cohesion, i.e., decreased CD density, that together account for the abnormalities in SC function induced by PS are also mediated by the increase in endogenous GC that are produced during PS.

Many skin disorders, including psoriasis and atopic dermatitis, are initiated, exacerbated, or propagated by PS (20, 31, 35). The abnormalities in epidermal proliferation, epidermal differentiation, permeability barrier homeostasis, and SC integrity and cohesion could all contribute to the adverse effects of PS on cutaneous function. Moreover, the ability of inhibiting GC action to prevent the abnormalities in epidermal structure and function induced by PS suggests a possible new therapeutic strategy for these disorders. Specifically, in patients in whom PS adversely impacts cutaneous disease, inhibition of GC production or action might improve the cutaneous disease.

The present study and previous studies by our and other laboratories clearly demonstrate that excess GC from either an endogenous or exogenous source can result in abnormalities in epidermal structure and function. It should be recognized that PS is not the only circumstance in which endogenous GC production is increased. Cushing’s syndrome from a variety of causes, such as pituitary adenomas, adrenal adenomas, adrenal carcinoma, and ectopic tumor production of ACTH, can lead to excess endogenous GC production (4). However, these abnormalities are relatively uncommon. More importantly a large number of acute and chronic medical disorders lead to increased endogenous GC production (29). Additionally, high doses of exogenous GC are used to treat a variety of diseases, particularly inflammatory disorders (3). Thus although we have focused our studies on the effects of PS, it is likely that other
forms of stress, which also increase GC production, will have similar adverse impacts on SC structure and function. We would speculate that in chronic medical disorders an increase GC production could decrease epidermal proliferation and differentiation and that there would be impairments in permeability barrier homeostasis and SC integrity and cohesion. Similar to PS, these abnormalities could exacerbate cutaneous diseases.

In conclusion, the present study demonstrates that PS induces epidermal abnormalities by increasing GC production and suggests that therapeutic strategies designed to reduce GC production or action may be beneficial in selected patients with cutaneous disorders that are exacerbated by PS or other conditions associated with excess GCs.

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