Stress alters cutaneous permeability barrier homeostasis

MITSUHIRO DENDA,1 TORU TSUCHIYA,1 PETER M. ELIAS,2 AND KENNETH R. FEINGOLD2,3
1Shiseido Research Center, Yokohama, 236-8643 Japan; Departments of 2Dermatology and 3Medicine (Metabolism Section), University of California, San Francisco; and 2Dermatology and 3Medical Services, Department of Veterans Affairs Medical Center, San Francisco, California 94121

Denda, Mitsuhiro, Toru Tsuchiya, Peter M. Elias, and Kenneth R. Feingold. Stress alters cutaneous permeability barrier homeostasis. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R367–R372, 2000.—Recent studies have shown that psychological stress can influence cutaneous barrier function, suggesting that this form of stress could trigger or aggravate skin disease. In the present study, we demonstrate that transfer of hairless mice to a different cage delays barrier recovery rates. Pretreatment with a phenothiazine sedative, chlorpromazine, before transfer of animals restored the kinetics of barrier recovery toward normal, suggesting that psychological stress is the basis for this alteration in barrier homeostasis. To determine the mechanism linking psychological stress to altered barrier recovery, we first demonstrated that plasma corticosterone levels increase markedly after transfer of animals to new cages and that pretreatment with chlorpromazine blocks this increase. Second, we demonstrated that the systemic administration of corticosterone delays barrier recovery. Finally, we demonstrated that pretreatment with the glucocorticoid receptor antagonist RU-486 blocks the delay in barrier recovery produced by systemic corticosterone, change of cage, or immobilization. These results suggest that psychological stress stimulates increased production of glucocorticoids, which, in turn, adversely affects permeability barrier homeostasis.


MATERIAL AND METHODS

Animals. All experiments were performed on 5- to 6-wk-old male hairless mice (HR-1, Hoshino, Japan). They were housed in a 12:12-h light-dark environment, with lights on at 0700, at a temperature of 22–25°C and a relative humidity of 40–70% throughout the experiment. Five animals were kept in the same cage (22.5 × 33.8 × 14.0 cm3) together for 10–14 days. The mice were then moved to a different cage of the same size, material, and structure in the same room as their prior cage. Mice in the control group were handled in the same way and then returned to the cage in which they had been kept initially. In some experiments, the immobilization model of psychological stress was employed, as described previously (4). These studies were approved by the Animal Research Committee of the Shiseido Research Center in accordance with National Research Council guidelines (14).

Methods of barrier disruption and evaluation of barrier function. Permeability barrier function was evaluated by measurement of transepidermal water loss (TEWL) with an electrolytic water analyzer, as described previously (4) (Meeco, Warrington, PA). Barrier disruption was achieved by sequential applications of cellophane tape or acetone-soaked cotton balls on the animals’ flank skin, as described previously (5). Each procedure was terminated when TEWL measurements reached 7–10 mg·cm−2·h−1. Each barrier disruption and subsequent TEWL measurements were carried out under anesthesia with pentobarbital sodium (50 mg/kg). Barrier

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpregu.org R367
recovery rates are expressed as percent recovery because of day-to-day variations in the extent of barrier disruption. In each animal, the percent recovery was calculated by the following formula: 1 - [(TEWL immediately after treatment - TEWL at indicated time)/(TEWL immediately after treatment - baseline TEWL)]×100%.

Plasma corticosterone levels. Blood samples were obtained immediately within 1 min after animals were killed between 0800 and 0900. Trunk blood was collected into heparinized beakers and centrifuged (3,500 rpm × 20 min). The plasma was separated and frozen at -20°C until assayed. Plasma corticosterone levels were determined by RIA, using a commercially available RIA kit (TKRC1, Diagnostic Products).

To confirm that introduction to a new environment alone alters barrier homeostasis, we next carried out another experiment in which acetone treatment instead of tape stripping was used to disrupt the barrier. Acetone treatment and tape stripping produce equivalent disruptions of the permeability barrier, and barrier repair occurs via identical pathways. In this study, the control group was housed five per cage for 11 days, whereas the stress group was housed five per cage for 10 days and then separated into individual cages 1 day before study. As shown in Fig. 2, barrier recovery was delayed when animals were moved to a new environment for the final 24 h before study. This experiment confirms that a change in environment adversely affects barrier homeostasis. Together, these results demonstrate first, that moving animals to a new cage environment produced short-term alterations in rates of barrier recovery, independent of crowding. Second, crowding is an additional factor that aggravates barrier homeostasis.
To determine if the abnormality in barrier homeostasis induced by a change in environment is due to psychological stress, we next determined the effects of chlorpromazine administration before transfer of animals to new cages. As shown in Fig. 3, prior administration of chlorpromazine reduces the adverse effect of a change in environment on barrier homeostasis, further suggesting that psychological stress is an important negative determinant of barrier homeostasis.

We next performed a series of studies to determine the metabolic basis for the barrier abnormality. Psychological stress is well recognized to increase plasma corticosterone levels in rodents. One day after transfer of animals to a new cage environment, plasma corticosterone levels were markedly increased, and in animals maintained under crowded conditions, i.e., five or ten per cage, this increase persisted for at least 6 days (Fig. 4). We did not see any order effect among the mice maintained under crowded conditions with respect to barrier repair or serum corticosterone levels. Moreover, the stress-induced increase in plasma corticosterone could be blocked by pretreatment with chlorpromazine (Fig. 5). Although the difference between vehicle-treated group and chlorpromazine-treated group was not quite statistically significant (P = 0.06), the significant difference between stress-exposed animals and untreated controls disappeared after chlorpromazine treatment. These results suggest that a stress-induced increase in plasma glucocorticoids could be the metabolic basis for the alterations in barrier homeostasis.

As a test of the potential role of corticosterone, we next determined the effect of the systemic administration of corticosterone on barrier recovery after tape stripping. As shown in Fig. 6, systemic corticosterone treatment resulted in a marked delay in barrier recovery that was dose dependent. In contrast, the topical application of corticosterone to untreated skin for either 1 or 2 days before acute disruption did not affect barrier recovery, whereas topical application of corticosterone for 3 days caused a slight delay in barrier recovery at 3 h (Fig. 7). However, topical application of corticosterone for 3 days delayed barrier recovery on both the corticosterone-treated side and the vehicle-treated side (Fig. 7), suggesting that the inhibition of barrier recovery is due to systemic effects of absorbed drug rather than local cutaneous effects of glucocorticoids. These findings suggest further that elevated endogenous corticosteroids are responsible for the abnormalities in barrier homeostasis induced by psychological stress.

To determine whether increased corticosterone production accounts for altered barrier homeostasis, we...
next treated animals with the GR antagonist RU-486. Systemic RU-486 administration did not alter either baseline TEWL or barrier recovery rates (not shown). As a positive control, RU-486 treatment blocked the ability of systemic corticosterone administration to delay barrier recovery (Fig. 8A). Most importantly, treatment with RU-486 prevented the delay in barrier recovery induced by a change in cage environment (Fig. 8B). Moreover, RU-486 also prevented the delay in barrier recovery induced by immobilization, another model for inducing stress in rodents (Fig. 8C). These results suggest that glucocorticoids are an important mediator of the alterations in barrier homeostasis induced by environmental factors that produce stress.

DISCUSSION

The present study demonstrates that psychological stress induced either by housing large numbers of animals in the same cage or by moving animals to a new cage environment results in abnormalities in barrier function. This derangement in barrier homeostasis also occurs when animals are immobilized, another manipulation that induces stress (4). Thus three different manipulations that produce psychological stress lead to abnormalities in barrier homeostasis. That psychological stress is an important component in causing the barrier abnormality was shown by the effects of chlorpromazine, a sedative. Pretreatment with chlorpromazine blocked the adverse effects of...
either immobilization (4) or changes in environment on barrier homeostasis. The present study also provides mechanistic insights about the metabolic basis for the stress-induced abnormality. Glucocorticoids were shown to be a key mediator linking psychological stress with derangements in barrier homeostasis. First, both immobilization and moving animals to a new cage environment increased plasma corticosterone levels. Second, systemic treatment with glucocorticoids produced abnormalities in barrier recovery similar to those observed after psychological stress alone. Last, and most importantly, inhibition of glucocorticoid action by treatment with the GR antagonist RU-486 prevented the abnormality in barrier homeostasis produced by either immobilization or movement of animals to a new cage environment. Thus stress increases plasma corticosterone levels and, by yet to be elucidated mechanisms, the increase in endogenous glucocorticoids adversely affects barrier homeostasis. Because increased endogenous glucocorticoid production is induced by a variety of different stresses including trauma, surgery, infection, and inflammation, it is likely that barrier homeostasis will be compromised in a wide variety of situations. However, glucocorticoids did not adversely affect barrier homeostasis when applied topically unless sufficient glucocorticoids were absorbed to influence both local and distant sites, indicating that the topically applied glucocorticoids were having systemic effects. It is possible, however, that topical applications of a more potent glucocorticoid would have local effects on barrier homeostasis. Sheu et al. (23) reported that long-term topical corticosteroids induced skin barrier abnormalities. How systemic glucocorticoids adversely affect barrier homeostasis remains to be elucidated. Moreover, proopiomelanocortin-related peptides that could be regulated by glucocorticoids, such as corticotropin-releasing hormone and ACTH-melanocortin-stimulating hormone, are produced in the skin (24) and could play a role in permeability barrier homeostasis. Further studies are required to determine the mechanisms by which glucocorticoids regulate barrier homeostasis.

Although our results suggest that stimulation of glucocorticoids by stress plays a major role in the alteration in barrier homeostasis, it is possible that other factors also contribute. For example, group-caged animals, depending on social rank, have variations in serum testosterone levels. Previous studies have shown that testosterone stimulates epidermal lipid synthesis (8), and this could have an effect on barrier homeostasis. Experiments determining the effect of testosterone on barrier homeostasis have not yet been carried out.

The stress-induced derangements in barrier homeostasis shown here are analogous to the well-recognized perturbations in wound healing induced by psychological stress (12). We previously demonstrated that immobilization-induced stress reduces epidermal DNA synthesis and also lipogenesis in sebaceous gland (25, 26). Elevated levels of endogenous or exogenous glucocorticoids also are well known to delay wound healing (2). Moreover, Padgett et al. (17) recently showed that the delay in wound healing induced by immobilization could be prevented by treatment with the GR antagonist RU-40555. Thus the increase in circulating glucocorticoids could have adverse effects on several aspects of cutaneous homeostasis, and these may include both wound healing and barrier repair. On the other hand, Ramsing and Agner (19) demonstrated that after SDS irritation, topical application of steroids resulted in a faster recovery of the skin barrier function. Steroids are well recognized to reduce inflammation, and it is likely that the enhancement of barrier recovery was due to the anti-inflammatory effects of steroids.

It has been recognized for many years that psychological stress is an important factor associated with the onset and exacerbation of a number of cutaneous diseases (1, 7, 9, 21, 22). The abnormality in barrier homeostasis induced by psychological stress could have adverse effects on cutaneous function, leading to pathophysiological alterations. Barrier disruption is associated with both an increase in keratinocyte proliferation, which can result in epidermal hyperplasia (5, 18), and an increase in cytokine production and secretion (15, 27), which can result in cutaneous inflammation. In previous studies, we demonstrated that even relatively small disruptions of the barrier induced epidermal hyperplasia and cytokine secretion when the barrier was disrupted repeatedly (5). We also reported that the epidermal proliferative response and mast cell degranulation induced by barrier disruption were amplified drastically by environmental low humidity (3). Thus even relatively minor defects in barrier homeostasis could potentially produce cutaneous abnormalities, including hyperplasia and/or inflammation. Moreover, certain skin diseases, such as psoriasis and atopic dermatitis, are associated with abnormal barrier homeostasis (10, 16). Psychological stress superimposed on disease-specific abnormalities in barrier homeostasis could potentially further exacerbate the barrier abnormality in these disorders, aggravating or exacerbating disease. Our observations that psychological stress increases systemic glucocorticoid levels provide a pathophysiological link not only between psychological stress but also any type of stress associated with elevated endogenous glucocorticoids and abnormalities in barrier homeostasis. Delineating the pathways linking psychological stress to barrier dysfunction could result in rational treatment strategies aimed at blocking the stress-induced cascade.

Perspectives

A number of common dermatologic disorders, including atopic dermatitis, psoriasis, and dyshidrotic eczema, are exacerbated by psychological stress (1, 7, 9, 13). Alterations in permeability barrier function frequently occur in these disorders (10), and by stimulating cytokine production and inducing epidermal hyperplasia, barrier dysfunction may play a role in triggering or sustaining these cutaneous abnormalities. Stress reduction has been shown to accelerate lesion resolution in psoriatic patients treated with ultraviolet light (11). The present study suggests that one mechanism by which stress could exacerbate skin disorders is to perturb permeability barrier homeostasis. Additionally, the present study suggests that the stress-induced
stimulation of glucocorticoids by pathways that remain to be elucidated plays an important role in these alterations in permeability barrier homeostasis. It therefore may be possible to improve or ameliorate certain cutaneous disorders by specifically blocking the pathways by which stress produces defects in permeability barrier homeostasis. This strategy could result in novel therapeutic approaches to treat cutaneous disorders.

Address for reprint requests and other correspondence: M. Denda, Shiseido Research Center 2, 2–12–1 Fukuura, Kanazawa-ku, Yokohama, 236–8643 Japan (E-mail: mitsuhiro.denda@to.shiseido.co.jp).

Received 20 May 1999; accepted in final form 8 September 1999.

REFERENCES


