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New strategies to improve skin barrier homeostasis

Mitsuhiro Denda*

Shiseido Life Science Research Center, 2-12-1 Fukuura, Kanazawa-ku, Yokohama 236-8643, Japan

Abstract

The uppermost thin layer, stratum corneum, plays a crucial role as a water impermeable barrier. After acute damage, it recovers automatically, but with aging or psychological stress, the recovery is delayed. Frequent damage, or damage under a dry environment, induces epidermal hyperplasia or inflammation. A specific protease inhibitor, histamine antagonist, and some magnesium salts have been demonstrated to accelerate the barrier recovery. These treatments also mitigated the epidermal hyperplasia induced by repeated barrier disruption or the damage under a dry condition. For the delay of the barrier repair induced by psychological stress, a glucocorticoid receptor antagonist or reduction of the stress by some specific odorant was significantly effective. Recently, the ion flux in the epidermis was found to be crucial for the barrier homeostasis. An external negative electric field accelerated the skin barrier recovery. These new methods to improve skin barrier homeostasis could be useful strategies to solve skin problems.

Keywords: Keratinocyte; Epidermis; Stratum corneum; Ion; Psychological stress; Skin care; Vanilloid receptor; VR1

Contents

Abbreviations: t-AMCHA, *trans*-4-(aminomethyl) cyclohexane carboxylic acid; PPARα, peroxisome-proliferator-activated receptor α; VR1, vanilloid receptor subtype 1

*Tel.: +81-45-788-7268; fax: +81-45-788-7277.

E-mail address: mitsuhiro.denda@to.shiseido.co.jp (M. Denda).

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1. Introduction

The stratum corneum plays a crucial role as a water impermeable barrier. It is composed of two components, i.e. protein-rich nonviable cells and intercellular lipid domains [1]. When the barrier function is damaged by a surfactant, organic solvent, or tape stripping, a series of homeostatic systems is accelerated and the barrier function recovers its original level [1]. Firstly, exocytosis of lipid-containing granules, lamellar bodies, is accelerated and the inside lipids are secreted into the intercellular domain between the stratum granulosum and stratum corneum and form the water impermeable membrane [1]. Then, lipid synthesis and processing are accelerated and finally the barrier function recovers to its original level [1]. However, acute disruption of the barrier also results in an increase in epidermal DNA synthesis [2] and cytokine production [3]. Even if the damage of the barrier is relatively small, when it is repeated [4] or under low environmental humidity [5], the damage induces obvious epidermal hyperplasia and inflammation. Various kinds of dermatoses, such as atopic dermatitis, psoriasis, and contact dermatitis, are associated with barrier dysfunction [6]. Moreover, recent studies suggested that environmental or intrinsic factors affect cutaneous barrier homeostasis. Psychological stress delays barrier recovery after artificial barrier disruption [7,8]. Glucocorticoids in serum might mediate skin homeostasis through the central nervous system [8]. There is a circadian rhythm in the stratum corneum barrier homeostasis [9]. With aging, the stratum corneum barrier becomes fragile and recovery is delayed [10]. Thus, improvement of the barrier function would be needed to prevent skin problems for people in today's world.

Several methods to accelerate the skin barrier repair by regulation of non-lipid factors such as enzymes and ions have been reported [11–13] (Table 1). Acceleration of the barrier repair improved skin conditions such as epidermal hyperplasia [11,13]. Studies on the biochemical and biophysical functions associated with the epidermal barrier homeostasis are important for clinical dermatology. In the present article, I describe several new methods to improve skin barrier homeostasis from different viewpoints of skin biology.

2. Biochemical methods

2.1. Topical application of lipids

As described above, lipids play a crucial role for the water impermeable barrier function of the skin.

Table 1
Strategies to regulate skin barrier recovery rate

Lipids (Refs. [14,15])	Optimized mixture of physiologic lipids (ceramide, cholesterol, fatty acid)	Accelerate
	Single lipid or double mixture of physiologic lipids	Delay
Protease inhibitors (Ref. [11])	Trypsin-like serine protease inhibitor	Accelerate
	Plasminogen activator inhibitor	Accelerate
Salts (Ref. [12,27])	Calcium	Delay
	Potassium	Delay
	Magnesium	Accelerate
	Magnesium + calcium	Accelerate
Histamine receptor antagonists (Ref. [13])	H1 receptor antagonist	Accelerate
	H2 receptor antagonist	Accelerate
	H3 receptor antagonist	No effect
	Histamine or histamine releaser	Delay
Nuclear hormone receptor activator (Ref. [19])	PPARα activator	Accelerate
External electric potential (Ref. [31])	Positive potential	Delay
	Negative potential	Accelerate

Damaged barrier function can be restored by topical application of a water impermeable substance such as petrolatum [14]. In this case, the petrolatum stays in the stratum corneum and forms a water impermeable membrane. However, Man et al. demonstrated that a topically applied mixture of stratum corneum lipids, i.e. ceramide, cholesterol and free fatty acids, was incorporated in the nucleated layer of epidermis and accelerated repair of the barrier function after its damage [15]. They were the first to report a method to accelerate the barrier recovery by regulating endogenous factors in the epidermis. Interestingly, when they applied ceramide, cholesterol, or free fatty acid alone, or a mixture of two of these, the barrier recovery was delayed. Only when they applied the mixture of all three lipids, at a specific relative ratio, was the barrier recovery accelerated [15]. These results suggest that a balance of the three lipids is crucial for the skin barrier homeostasis.

In the case of aging, a different treatment might be necessary because of the different metabolism of aged skin. Previously, Ghadially et al. demonstrated that skin barrier function in elderly subjects was destroyed more easily than that in young individuals [10]. Moreover, barrier recovery rate after barrier disruption was significantly slower for the elderly subjects than that for the younger ones. The same tendency was observed in both humans and hairless mice. The authors also suggested that synthesis of cholesterol is reduced more than that of other lipids, i.e. ceramide and fatty acids in the aged mice. The delay of barrier recovery with aging was improved by topical application of cholesterol [16] or mevalonic acid [17], because the delay of the aged skin was caused by a decrease of cholesterol synthesis.

2.2. Protease inhibitor

Denda et al. previously demonstrated [11] that *trans*-4-(aminomethyl) cyclohexane carboxylic acid (t-AMCHA), an anti-fibrinolytic agent that activates plasminogen, improved the barrier homeostasis and whole skin condition. After barrier disruption, proteolytic activity in the epidermis increased within 1–2 h. This increase was inhibited by t-AMCHA. Topical application of t-AMCHA or trypsin-like serine protease inhibitors accelerated the barrier recovery. Moreover, topical application of t-AMCHA mitigated epidermal hyperplasia induced by repeated barrier disruption. These findings suggested that manipulations that injure the stratum corneum activate the plasminogen/plasmin system and the increase of the extracellular protease activity is detrimental to barrier repair and may induce pathologic changes in the skin. Kitamura et al. also reported [18] the efficacy of this agent to dry skin. The protease balance might be important for the barrier homeostasis and skin pathology.

2.3. Nuclear hormone receptor activator

Feingold and co-workers demonstrated an important role of nuclear hormone receptor on epidermal differentiation and stratum corneum barrier formation. Activation of PPARα by farnesol also stimulated the differentiation of epidermal keratinocytes [19,20]. Cornified envelope formation, involcrin and transglutaminase protein and mRNA levels were also increased by the activation of PPARα. Interestingly, the inflammatory response was also inhibited by the treatment [21]. They also showed that topical application of PPARα activators accelerated the barrier recovery after tape stripping or acetone treatment and prevented the epidermal hyperplasia induced by repeated barrier disruption [19]. Regulation of the nuclear hormone receptor would open a new possibility for improvement of the cutaneous barrier.

2.4. Histamine receptor antagonist

Histamine receptors are related to skin barrier function [13]. Three different types of histamine receptors, H1, H2 and H3 have been reported. Firstly, topical application of histamine H1 and H2 receptor antagonists accelerated the barrier repair. Histamine itself, H2 receptor agonist, and histamine releaser delayed the barrier repair. Histamine H3 receptor antagonist and agonist did not affect the barrier recovery rate. Topical application of the H1 and H2 receptor antagonists prevented the epidermal hyperplasia induced by barrier disruption under low humidity. The mechanism of the relationship between the histamine receptors and the barrier repair process has not been elucidated yet.

3. Psychological stress and barrier homeostasis

As described above, psychological stress adversely affects the barrier homeostasis. Three different models of psychological stress have been proposed, i.e. immobilization, crowded environment and exchanging the living place [8,9]. In each case, the barrier recovery rate delayed after barrier disruption. The plasma corticosterone level was increased by the stress and it was reduced by application of a sedative drug [9]. The delay of barrier repair induced by psychological stress was also prevented by application of a sedative drug or glucocorticoid receptor antagonist [9]. These results suggest that psychological stress stimulates increased production of glucocorticoids, which, in turn, adversely affects skin barrier homeostasis (Fig. 1). The effect of psychological stress on skin barrier homeostasis in humans has also been reported [22]. Reduction of psychological stress might accelerate the skin barrier repair process. Previously, several articles demonstrated that some odorants have an effect of reducing the stress like a sedative drug [23,24]. The odorants, which have a sedative effect, prevented the delay in skin barrier recovery induced by psychological stress in both mice and humans [22,25]. These results suggest the possibility of a new skin care strategy by specific odorant inhalation.

4. Ion dynamics in the epidermis

4.1. Ions

Lipid metabolism is regulated by a series of enzymes in the epidermis [1] and each of them has their optimal condition such as pH [26] and other ion balance [12]. For example, the pH value of the healthy stratum corneum is kept acidic because the lipid processing enzymes have an acidic optimal pH. Mauro et al. [26] demonstrated that topical application of a basic buffer after barrier disruption delayed the repair process because the basic condition perturbs the lipid processing.

Other ions such as calcium and magnesium [12,27–30] also play important roles in the lipid metabolism in the epidermis. Previous studies demonstrated that the topical application of calcium or potassium reduced the barrier repair [27,28] and magnesium and a mixture of calcium and magnesium salts accelerated the repair process [12]. Topical application of an aqueous solution containing 10 mM magnesium chloride, magnesium sulfate, and magnesium lactate accelerated barrier repair. Application of magnesium bis(dihydrogen phosphate) or magnesium chloride in PBS solution did not affect the barrier recovery rate. Application of 10 mM calcium chloride aqueous solution delayed the barrier repair

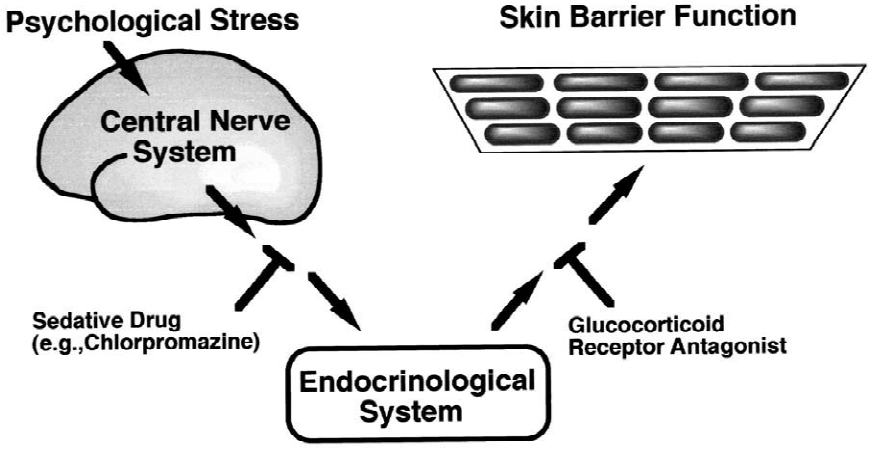

Fig. 1. Psychological stress increases glucocorticoid in the serum and delays the skin barrier repair consequently. Reduction of the stress by a sedative drug or specific odorant prevents the delay of the barrier repair. Glucocorticoid receptor antagonist also blocks the delay.

but a mixture of calcium chloride and magnesium chloride accelerated barrier recovery when the calcium to magnesium molar ratio was lower than 1. Application of the mixture also improved the condition of dry, scaly skin induced by SDS treatment. These results suggest an important role of these ions in barrier homeostasis.

4.2. Electric potential

The heterogeneous field formed by ions such as calcium, magnesium and potassium might be crucial for the terminal differentiation and barrier formation in the epidermis (Fig. 2). Recently, the external electric potential has been shown to regulate the ion

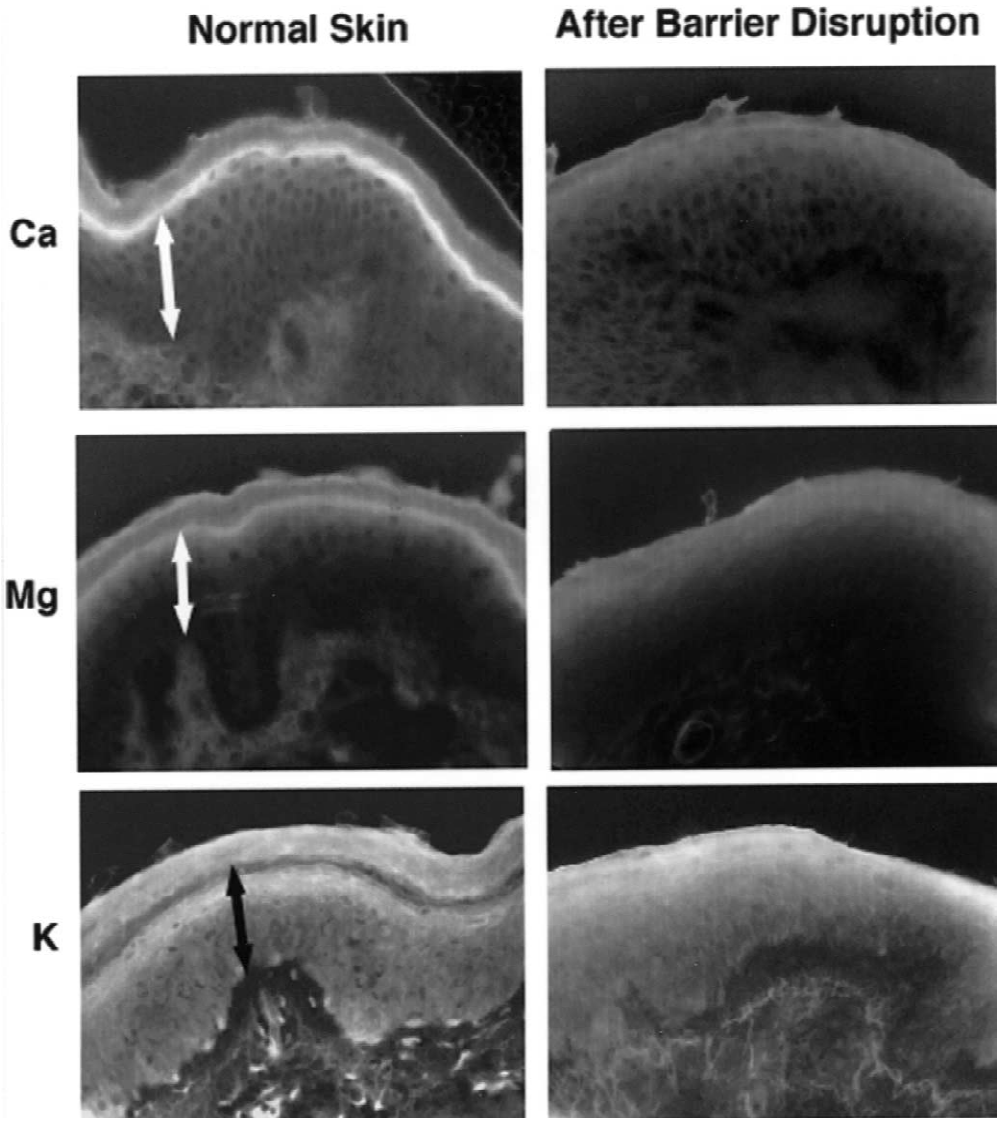

Fig. 2. Heterogeneous ionic field exists in the epidermis and the specific gradation of calcium, magnesium and potassium disappeared immediately with barrier disruption. Arrows show the living layer of the epidermis. Arranged from Ref. [29].

gradation in the epidermis and skin barrier homeostasis consequently [31]. Application of a negative electric potential on hairless mice skin after barrier disruption significantly accelerated the barrier recovery. On the other hand, application of a positive potential delayed the barrier repair. Under a negative potential, the extent of epidermal lamellar body exocytosis into the stratum corneum/stratum granulosum interface increased. Magnesium and calcium ion concentrations in the uppermost epidermis were obviously higher in the portion where the positive electric potential was applied. Methods to improve skin barrier homeostasis without topical application of any bioactive chemical materials might be novel therapeutic approaches to cure the skin disorders associated with abnormal barrier function or ion dynamics.

4.3. Thinking epidermis

The epidermis is an intelligent organ in its barrier function. When the barrier is damaged, it recovers automatically [1]. When the skin is exposed to low environmental humidity, the stratum corneum becomes thick and the barrier function stimulated to adapt to its environmental condition [32]. The basic mechanism of the smart system has not been clarified yet, but ion dynamics in the epidermis plays an important role as a signaling system as described above. Recently, the existence and function of vanilloid receptor subtype 1 (VR1) in epidermal keratinocytes has been demonstrated [33,34] (Fig. 3). Originally, VR1 was discovered as a polymodal pain receptor in the nerve system. Previous studies also showed that several other receptors that play important roles in the nerve system exist in keratinocytes. Many of them are associated with ion dynamics in the central or peripheral nerve system [35–37]. Both the central nerve system and epidermis are ectoderm-derived organs. The basic signaling mechanism of the two systems might be similar to each other. As described above, skin barrier function is also regulated by ion dynamics. Thus, the receptors might play a crucial role in skin

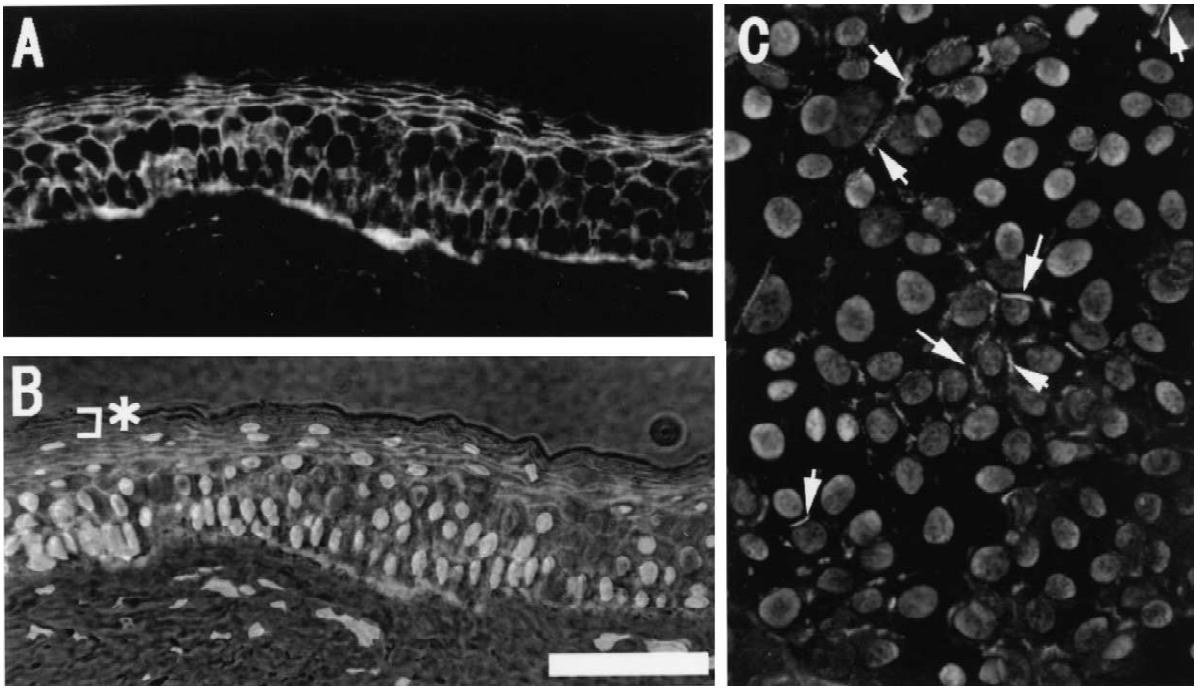

Fig. 3. Pain receptor VR1 is localized in human epidermal keratinocytes. (A) Skin tissue staining with VR1 antiserum. (B) Merged image with A, DAPI staining, and bright field image. Asterisk shows stratum corneum. (C) Cultured human keratinocytes staining with VR1 antiserum (arrows). Bar, 100 μm. Arranged from Refs. [33,34].

barrier homeostasis. The area between dermatology and neuroscience should be an important scientific field in the near future.

5. Conclusion

In the history of skin care, occlusion or moisturization with artificial materials have been used to improve the skin condition, and the efficacy of these treatments has been suggested biologically [5]. However, such treatments potentially perturb the homeostasis of the skin. On the other hand, recovery of the original, endogenous skin function by acceleration of its homeostatic process results in natural healthy skin without side effects. Methods to accelerate barrier repair might open new possibilities for future skin care systems.

References

[1] K.R. Feingold, P.M. Elias, The environmental interface: regulation of permeability barrier homeostasis, in: M. Loden, H.I. Maibach (Eds.), Dry Skin and Moisturizers. Chemistry and Function, CRC Press, Boca Raton, FL, 2000, pp. 45–58.
[2] E. Proksch, K.R. Feingold, M.Q. Man, P.M. Elias, Barrier function regulates epidermal DNA synthesis, J. Clin. Invest. 87 (1991) 1668–1673.
[3] L.C. Wood, S.M. Jackson, P.M. Elias, K.R. Feingold, Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice, J. Clin. Invest. 90 (1992) 482–487.
[4] M. Denda, L.C. Wood, S. Emami, C. Calhoun, B.E. Brown, P.M. Elias, K.R. Feingold, The epidermal hyperplasia associated with repeated barrier disruption by acetone treatment or tape stripping cannot be attributed to increased water loss, Arch. Dermatol. Res. 288 (1996) 230–238.
[5] M. Denda, J. Sato, T. Tsuchiya, P.M. Elias, K.R. Feingold, Low humidity stimulates epidermal DNA synthesis and amplifies the hyperproliferative response to barrier disruption: implication for seasonal exacerbations of inflammatory dermatoses, J. Invest. Dermatol. 111 (1998) 873–878.
[6] K.A. Grice, Transepidermal water loss in pathologic skin, in: A. Jarrett (Ed.), The Physiology and Pathophysiology of the Skin, Academic Press, London, 1980, pp. 2147–2155.
[7] M. Denda, T. Tshuchiya, J. Hosoi, Immobilization-induced and crowded environment-induced stress delay barrier recovery in murine skin, Br. J. Dermatol. 138 (1998) 780–785.
[8] M. Denda, T. Tsuchiya, P.M. Elias, K.R. Feingold, Stress alters cutaneous permeability barrier homeostasis, Am. J. Physiol. 278 (2000) R367–R372.
[9] M. Denda, T. Tsuchiya, Barrier recovery rate varies time-dependently in human skin, Br. J. Dermatol. 142 (2000) 881–884.
[10] R. Ghadially, B.E. Brown, S.M. Sequeira-Martin, K.R. Feingold, P.M. Elias, The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and asenescent murine model, J. Clin. Invest. 95 (1995) 2281–2290.
[11] M. Denda, K. Kitamura, P.M. Elias, K.R. Feingold, *Trans*-4-(aminomethyl)cyclohexane carboxylic acid (t-AMCHA), an anti-fibrinolytic agent, accelerates barrier recovery and prevents the epidermal hyperplasia induced by epidermal injury in hairless mice and humans, J. Invest. Dermatol. 109 (1997) 84–90.
[12] M. Denda, C. Katagiri, N. Maruyama, Some magnesium salts and a mixture of magnesium and calcium salts accelerates skin barrier recovery, Arch. Dermatol. Res. 291 (1999) 560–563.
[13] Y. Ashida, M. Denda, T. Hirao, Histamine H1 and H2 receptor antagonists accelerate skin barrier repair and prevent epidermal hyperplasia induced by barrier disruption in a dry environment, J. Invest. Dermatol. 116 (2001) 261–265.
[14] M.Q. Man, B.E. Brown, S. Wu-Pong, K.R. Feingold, P.M. Elias, Exogenous nonphysiologic vs. physiologic lipids, Arch. Dermatol. 131 (1995) 809–816.
[15] M.Q. Man, K.R. Feingold, C.R. Thornfeld, P.M. Elias, Optimization of physiologic lipid mixtures for barrier repair, J. Invest. Dermatol. 106 (1996) 1096–1101.
[16] R. Ghadially, B.E. Brown, K. Hanley, J.T. Reed, K.R. Feingold, P.M. Elias, Decreased epidermal lipid synthesis accounts for altered barrier function in aged mice, J. Invest. Dermatol. 106 (1996) 1064–1069.
[17] A. Haratake, K. Ikenaga, N. Katoh, H. Uchiwa, S. Hirano, H. Yasuho, Topical mevalonic acid stimulates de novo cholesterol synthesis and epidermal permeability barrier homeostasis in aged mice, J. Invest. Dermatol. 114 (2000) 247–252.
[18] K. Kitamura, K. Yamada, A. Ito, M. Fukuda, Research on the mechanism by which dry skin occurs and the development of an effective compound for its treatment, J. Soc. Cosmet. Chem. 29 (1995) 133–145.
[19] K.R. Feingold, Role of nuclear hormone receptors in regulating epidermal differentiation, in: Program and Preprints of Annual Scientific Seminar, Society of Cosmetic Chemists, 1999, pp. 30–31.
[20] K. Hanley, L.G. Komuves, D.C. Ng, K. Schoonjians, S.S. He, P. Lau, D.D. Bikle, M.L. Williams, P.M. Elias, K.R. Feingold, Farnesol stimulates differentiation in epidermal keratinocytes via PPARalpha, J. Biol. Chem. 275 (2000) 11484–11491.
[21] M.Y. Sheu, A.J. Fowler, J. Kao, M. Schmuth, K. Schoonjans, J. Auwerx, J.M. Fluhr, M.Q. Man, P.M. Elias, K.R. Feingold, Topical peroxisome proliferator activated receptor-alpha activators reduce inflammation in irritant and allergic contact dermatitis models, J. Invest. Dermatol. 118 (2002) 94–101.
[22] M. Denda, M. Tanida, K. Shoji, T. Tsuchiya, Inhalation of a sedative odorant prevents the delay of cutaneous barrier repair induced by psychological stress, Auton. Nerv. Syst. 37 (2000) 419–424.
[23] T. Tsuchiya, M. Tanida, S. Uenoyama, T. Ozawa, Effects of olfactory stimulation on the sleep time induced by pentobarbital administration in mice, Brain. Res. Bull. 26 (1991) 397–401.

[24] T. Tsuchiya, M. Tanida, S. Uenoyama, Effects of olfactory stimulation with jasmine and its component chemicals on the duration of pentobarbital-induced sleep in mice, Life Sci. 50 (1992) 1097–1102.
[25] M. Denda, T. Tsuchiya, K. Shoji, M. Tanida, Odorant inhalation affects skin barrier homeostasis in mice and humans, Br. J. Dermatol. 142 (2000) 1007–1010.
[26] T. Mauro, S. Grayson, W.N. Gao, M.Q. Man, E. Kriehuber, M. Behne, K.R. Feingold, P.M. Elias, Barrier recovery is impeded at neutral pH, independent of ionic effects: implications for extracellular lipid processing, Arch. Dermatol. Res. 290 (1998) 215–222.
[27] S.H. Lee, P.M. Elias, E. Proksch, G.K. Menon, M.Q. Man, K.R. Feingold, Calcium and potassium are important regulators of barrier homeostasis in murine epidermis, J. Clin. Invest. 89 (1992) 530–538.
[28] G. Menon, L.F. Price, B. Bommannan, P.M. Elias, K.R. Feingold, Selective obliteration of the epidermal calcium gradient leads to enhanced lamellar body secretion, J. Invest. Dermatol. 102 (1994) 789–795.
[29] M. Denda, J. Hosoi, Y. Ashida, Visual imaging of ion distribution in human epidermis, Biochem. Biophys. Res. Commun. 272 (2000) 134–137.
[30] M. Denda, Y. Ashida, K. Inoue, N. Kumazawa, Skin surface electric potential induced by ion-flux through epidermal cell layers, Biochem. Biophys. Res. Commun. 284 (2001) 112–117.
[31] M. Denda, N. Kumazawa, Negative electric potential induces epidermal lamellar body secretion and accelerates skin barrier recovery after barrier disruption, J. Invest. Dermatol. 118 (2002) 65–72.
[32] M. Denda, J. Sato, Y. Masuda, T. Tsuchiya, M. Kuramoto, P.M. Elias, K.R. Feingold, Exposure to a dry environment enhances epidermal permeability barrier function, J. Invest. Dermatol. 111 (1998) 858–863.
[33] M. Denda, S. Fuziwara, K. Inoue, S. Denda, H. Akamatsu, A. Tomitaka, K. Matsunaga, Immunoreactivity of VR1 on epidermal keratinocyte of human skin, Biochem. Biophys. Res. Commun. 285 (2001) 1250–1252.
[34] K. Inoue, S. Koizumi, S. Fujiwara, S. Denda, K. Inoue, M. Denda, Functional vanilloid receptors in cultured normal human epidermal keratinocytes, Biochem. Biophys. Res. Commun. 291 (2002) 124–129.
[35] S.A. Grando, Biological functions of keratinocyte cholinergic receptors, J. Invest. Dermatol. Symp. Proc. 2 (1997) 41–48.
[36] P.E. Stoebner, P. Carayon, G. Penarier, N. Frechin, G. Barneon, P. Casellas, J.P. Cano, J. Meynadier, L. Meunier, The expression of peripheral benzodiapine receptors in human skin: the relationship with epidermal cell differentiation, Br. J. Dermatol. 140 (1999) 1010–1016.
[37] P.G. Genever, S.J. Maxfield, G.D. Kennovin, J. Maltman, C.J. Bowgen, M.J. Raxworthy, T.M. Skerry, Evidence for a novel glutamate-mediated signaling pathway in keratinocytes, J. Invest. Dermatol. 112 (1999) 337–342.