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Skin Surface Electric Potential Induced by Ion-Flux through Epidermal Cell Layers

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The skin surface electric potential has been widely used for psychological studies because it is sensitive to emotional conditions. We measured the electric potential on the surface of hairless mice skin in organ culture with several physiological factors. Disruption of mitochondrial function and inhibition of ATPase reduced the skin surface potential 50–70%. Calcium, potassium, and sodium channel blockers also reduced the potential. A calcium-specific and potassium ionophore reduced the potential, but the calcium and magnesium ionophore increased it. EDTA decreased the potential but EGTA had no effect. Skin surface barrier disruption reduced the potential and calcium and potassium channel blockers partially prevented the decrease. Substance P and corticotropin-releasing factor decreased the potential, and antagonists blocked the decreases. These results suggest that the ion flux in the nucleated layer of the epidermis induce the skin surface potential and it is influenced by environmental and neuroendocrinological factors.

Key Words: **barrier; epidermis; calcium; magnesium; potassium.**

The electric potential of the skin surface has been widely used for psychophysiological studies because it is sensitive to emotional condition (1). Previous studies have suggested the contribution of sweat gland or diffusion of ions into the stratum corneum on the skin surface potential (2). However, Barker *et al.* demonstrated the existence of a high electric potential on human and guinea pig epidermis (3). They reported that amiloride decreased the potential and suggested that the potential was in the living, not dead, cell layers of the epidermis. Edelberg also suggested that epidermal cells generate a skin surface potential (4), but the mechanism of the endogenous voltage in the epidermis has not been elucidated. The skin surface potential is reduced by disruption of stratum corneum with tape stripping (4). Recently, quick movement of ions in the human epidermis (5) and hairless mice epidermis (6) immediately after disruption of the stratum corneum has been reported. In normal human skin, both calcium and magnesium were localized in granular layer, i.e., the uppermost layer of the epidermis (5). On the contrary, potassium was localized in the spinous layer of the epidermis and showed the lowest concentration in the granular layer. Within 30 min after tape stripping, the localization disappeared (5). From these observations, we hypothesized that a part of the skin surface potential is induced by movement of these ions in the epidermis. In the present study, we studied the relationship between the ion-flux and the electric potential of flank skin sections of hairless mice which do not have sweat glands using ATPase inhibitors, ion channel blockers, ionophores, and chelates. We also studied the alteration of the potential after the disruption of the stratum corneum and the effects of ion-channel blockers on it. Moreover, we also presented the effects of hormone and neuropeptide on the skin surface potential.

METHODS

Reagents. Sodium azide, ouabain, amitripyline, 4-aminopyridine, trifluoperazine, verapamil, nifedipine, ionomycin, valinomycin, A23187, EGTA, and EDTA were purchased from Wako Chemicals (Osaka, Japan). Corticotropin releasing factor (CRF, Sheep) and its antagonist (alpha-helical CRF) were purchased from Sigma (St.Louis, MO). Substance P, and its antagonist (D-Arg1,*p*-Trp7,9,Leu)-Substance P were purchased from Peptide Institute, Inc. (Osaka, Japan). Dulbecco's modified Eagle medium (DMEM) was purchased from GibcoBRL, Life Technologies (NY).

Animals. Male hairless mice, 7–10 weeks old (HR-1, Hoshino, Japan) were used for the present study. Before the experiment, animals were caged separately for at least 4 days. These cages (size:

Abbreviations used: CRF, corticotropin-releasing factor; DMEM, Dulbecco's modified Eagle medium.

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136 × 208 × 115 mm) were maintained in a room kept at a temperature of 21–25°C and at a relative humidity of 40–70%. All experiments were approved by the Animal Research Committee of the Shiseido Research Center in accordance with the National Research Council (NRC) Guide (7).

Transcutaneous potentials. Skin samples were taken from mice flank skin of mice euthanized with diethylether inhalation. Subcutaneous fat was removed gently and a 1.5 × 1.5 cm^2 skin specimen was cut. The skin was placed epidermal side up in 2 ml DMEM solution in a plastic dish 2.2 cm in diameter which was kept at 37°C during the experiment. The reagent for regulation of ions in the present study was added to the medium after a 15-min preincubation. We used silver/silver chloride electrodes for both reference and measurement of skin surface potential. Those electrodes were immersed in glass tubes filled with KCl solution, and the glass tubes were kept in plastic tubes filled with saturated KCl solution. The KCl solution in the glass tube was connected with that in the plastic tube by a salt bridge made of sponge. Saline-agar bridges consisting of flexible 30 cm long and 1 mm in internal diameter were also immersed in each tube. The other side of the salt bridge connected to the reference electrode was immersed in the medium in which the skin section was incubated. Another salt bridge was used for the measurement. For the measurement, a 3 × 3 mm piece of filter paper soaked in saline was attached on the skin and the end of the salt bridge was attached gently to the paper. The difference of the potential between the electrodes was measured by a Keithley model 2000 digital multimeter (System Digital Multimeter, type 2000, Keithley, Cleveland, OH). Before the measurements, we immersed both terminals of the salt bridge in the saline of the same bottle and waited at least 1 h for the measurement system to stabilize.

A 5% sodium azide solution in DMEM was prepared and 100 μl of the solution was added to 2 ml of DMEM in which the skin sections were incubated. ATPase inhibitors, ion channel blockers, and ionophores were first prepared as 10 mM DMEM or ethanol solution and 10 μl of each solution were added to 2 ml of DMEM in which the skin sections were incubated (i.e., final concentration was 50 μM). The same amount of DMEM or ethanol was added for control sections. For EDTA and EGTA, first prepared as 20 mM solutions, was added to DMEM to give a final concentration of 2 mM.

For the studies of CRF and Substance P, first we incubated the skin section for 15 min. Then we measured the skin surface potential as the value of time 0. After the first measurement, we added antagonists of CRF or Substance P at the final concentration of 0.5 μM in DMEM and incubated for the skin section 5 min. Then CRF or Substance P at a final concentration of 0.1 μM was added to DMEM. Then we started to evaluate the effects of each reagent with or without their antagonists.

For the tape stripping study, we used an adhesive cellophane tape (Nichiban, Japan). First, we measured the skin surface potential. Then ion channel blockers were added. After a 5 min incubation, the skin surface stratum corneum was stripped 3 times using new tape each time. Some of the stratum corneum remained. Then the potential was measured.

RESULTS

The skin surface potential showed a negative value on both mouse skin in organ culture as previously reported (3). The potential on mouse skin in organ culture approximately −3 mV. Here, we describe the alteration of the potential as an absolute value. Thus, an original negative potential close to zero, was regarded as a decrease in the potential value. In the untreated control mouse skin in organ culture, the potential started to decrease within 3 h (data not shown). Thus we measured the potential within 2–3 h of culture.

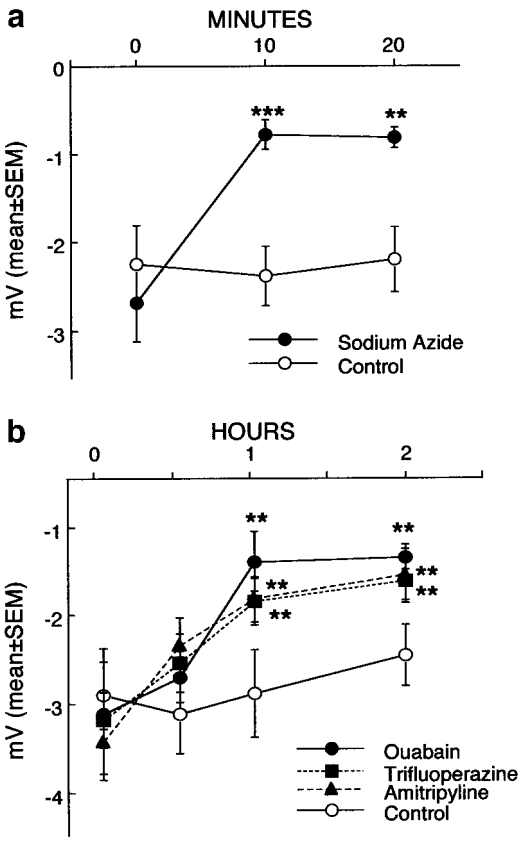

FIG. 1. Effects of sodium azide on the potential of hairless mice skin sections. (a) The skin surface potential decreased 70% within 10 min after the application of sodium azide (**$P < 0.01$, ***$P < 0.005$). The application of Na^+K^+ATP ase inhibitor, ouabain, and indirect inhibitor of Ca^{2+},Mg^{2+} ATPase, i.e., calmodulin inhibitor, trifluoperazine, amitriptyline, decreased the skin surface potential significantly (b) (**$P < 0.01$).

To examine whether the skin surface potential on the mouse skin in organ culture was induced by living cells, we examined the effect of sodium azide which induces cell death, on the potential of hairless mouse skin sections. As shown in Fig. 1a, the skin surface potential decreased approximately 70% within 10 min after the application of sodium azide. To examine whether the potential generation is an energy-requiring process, we examined the effects of ATPase inhibition. The Na^+K^+ATPase inhibitor, ouabain, and calmodulin inhibitor, i.e., indirect inhibitor of Ca^{2+},Mg^{2+} ATPase, trifluoperazine, amitriptyline started to reduce the potential within 30 min and the potential reached 50–70% of the original level within 60 min (Fig. 1b).

To determine the contribution of ion channels for the potential, we examined the effects of various ion channel blockers on the skin surface potentials. As shown in

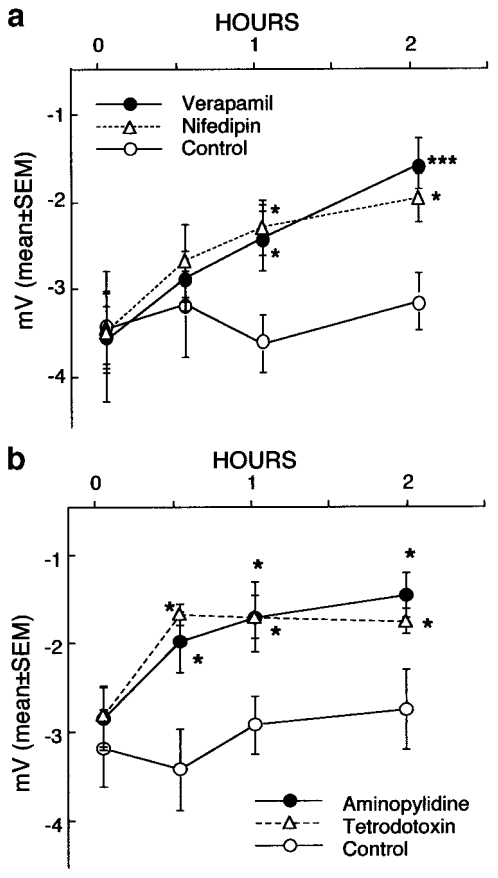

FIG. 2. The effects of various ion channel blockers on the skin surface potentials. (a) Both L-type calcium channel blocker, verapamil, and nifedipin, decrease the potential ($*P < 0.05$). Potassium channel blocker, 4-aminopylidine, and sodium channel blocker, tetrodotoxin, also reduced the potential (b) ($*P < 0.05$).

Fig. 2a, both L-type calcium channel blockers, verapamil and nifedipine started to decrease the potential within 60 min. The potassium channel blocker, 4-aminopylidine, and sodium channel blocker, tetrodotoxin, also reduced the potential. We examined the effects of ionophores and chelates on the potential to study the contribution of the difference in ion concentration between the outside and inside of the cell membrane (Fig. 3). The potassium ionophore, valinomycin, and calcium-specific ionophore, ionomycin, decreased the potential. However, A23187, which binds both calcium and magnesium, increased the skin surface potential by 40% of the original level within 60 min incubation and then the potential recovered to the original level within 2 h (Fig. 3a). EDTA which chelates both calcium and magnesium, did not affect the potential, or even slightly increased it (not significantly different, $P = 0.072$). On the other hand, EGTA which mainly chelates calcium, decreased approximately 30% within 2 h (Fig. 3b).

Both the corticotrophin releasing factor (CRF), and a neuropeptide, Substance P (SP), on the skin surface potential (Fig. 4) decreased the potential within 15 min and the effect was completely blocked by their antagonists.

We studied the effects of skin barrier disruption induced by tape stripping of the stratum corneum which plays a crucial role as the cutaneous water impermeable barrier. The tape stripping decreased the skin surface potential 30–60 min after the barrier disruption (Fig. 5a). Interestingly, the decrease was not the largest immediately after the disruption. After the decrease within 30–60 min, the potential started to recover. The decrease of the skin surface potential was partially prevented by the calcium channel blocker, verapamil, and potassium channel blocker, 4-aminopyridine (Figs. 5b and 5c), but the sodium channel blocker, tetrodotoxin, did not affect the decrease induced by the barrier disruption (Fig. 5c).

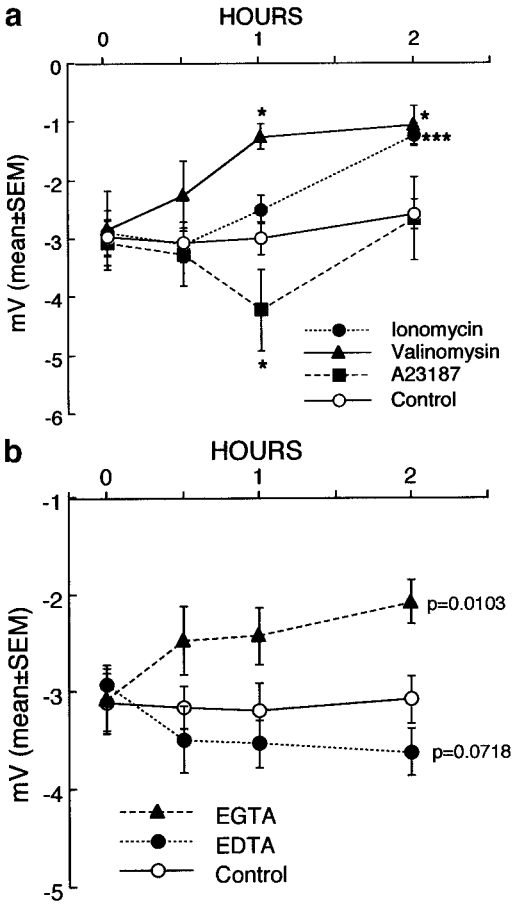

FIG. 3. The effects of ionophore and chelate reagent on the potential. The potassium ionophore, valinomycin, and calcium specific ionophore, ionomycin, decreased the potential, but A23187, which binds both calcium and magnesium, increased the skin surface potential ($*P < 0.05$, $***P < 0.005$) (a). EDTA which chelates both calcium and magnesium, decrease the potential, but not significantly. On the other hand, EGTA which mainly chelates calcium, increased the potential significantly (b).

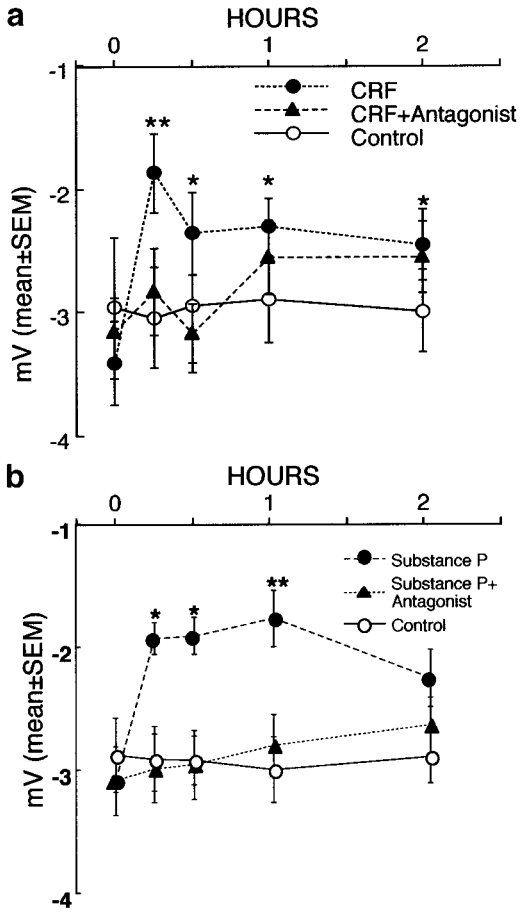

FIG. 4. The effects of a hormone, corticotropin-releasing factor (CRF) (a), and a neuropeptide, Substance P (SP) (b), on the skin surface potential. Both compounds decreased the potential and the effect was blocked by their antagonists (*$P < 0.05$, **$P < 0.01$).

DISCUSSION

The present study showed that the living epidermis contributed significantly to the skin surface potential. Sodium azide which disrupts the mitochondrial function, immediately reduced the potential. This suggests that most of the skin surface potential on the hairless mouse skin section was induced by living cell activity. This was supported by the results of an ATPase inhibitor study. ATP, which is required by ion pump might play an important role for generating the electric potential. The epidermis might consume ATP for the ion pump and induce the ion flux which produces the skin surface potential. A potential of approximately −0.8 mV remained after incubation with sodium azide. This might have been induced by a gradation of ions between dead cell membranes. The dermis in organ culture was not likely to be the source of the potential because it is a gel-like structure mainly composed of collagen fiber.

Previously (5), we found higher concentrations of calcium and magnesium in the uppermost epidermis and a lower concentration of potassium. In the epidermis, chloride ion is the major anion. Thus, calcium and magnesium might have a transference number of less than 0.5 in the epidermis (when the concentration was 0.1 mol/dm^{-3}, Mg^{2+}: 0.375, Ca^{2+}: 0.407, data from Ref. 8). In this case, when calcium or magnesium moves toward the bottom of the epidermis, the skin surface potential becomes negative. A constant energy-requiring process which induces the flux of calcium and magnesium to the surface of the skin in the epidermis may exist. In this process, not only calcium and magnesium, but also potassium and sodium play important roles. Ion channels also might play an important role for the potential. However, present study can not make clear the ion dynamics in cellular level. The ion flux between outside and inside of the keratonocyte cell membrane seemed to the main cause of the potential.

The results using ionophores and chelates suggested the importance of the ratio of calcium and magnesium on the potential. When both a Ca^{2+} flux and Mg flux were induced by A23187, the potential was increased, but when only a Ca^{2+} flux was induced by ionomycin, the potential was decreased. Moreover, chelation of Ca^{2+} and Mg^{2+} in the medium with EDTA did not change the potential and when the relative concentration of Ca^{2+} to Mg^{2+} was decreased by EGTA, the potential was significantly decreased. The relative ratio of Ca^{2+} to Mg^{2+} between outside and inside of the epidermis might be crucial for the potential.

The skin surface potential has long been recognized as a marker of psychological factors. Sweat glands might play an important role in the alteration of the potential under different emotional condition. We do not deny the contribution of the sweat glands on the human skin surface potential alteration under the different psychological conditions. However, in the present study, the mouse skin organ culture which did not have sweat glands showed a significant response against Substance P or CRF. Substance P is released from the peripheral C nerve endings and it induced an intracellular increase in calcium concentration, and translocation of protein kinase C in the epidermis (9). Previously, we demonstrated that a psychological stress affected the skin barrier homeostasis (10). Circadian rhythms were observed on the skin barrier homeostasis (11), but the mechanism of the mediator system between the central nervous system and epidermal homeostasis remains to be elucidated. Neuropeptides secreted from the terminal of the nerve might play an important role on the relation between skin homeostasis and psychological or physiological factors with the ion flux. Sugiura *et al.* reported that the number of Substance P-positive nerve fibers in atopic dermatitis lesions was far less than one-tenth of

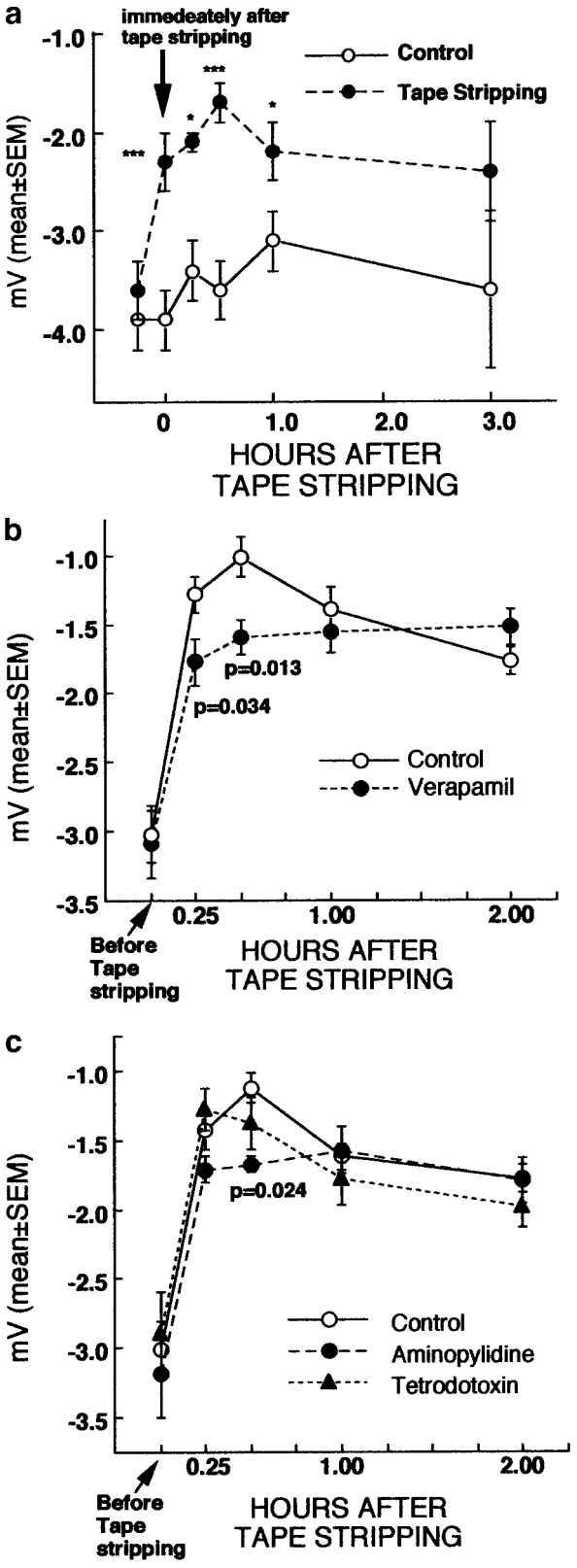

FIG. 5. Tape stripping decreased the skin surface potential 30–60 min on mouse skin samples (a) (*$P < 0.05$, ***$P < 0.005$). The decrease of the skin surface potential was partially prevented by a calcium channel blocker, verapamil, and potassium channel blocker, 4-aminopyridine (b and c), but sodium channel blocker, tetrodotoxine, did not affect the decrease induced by the barrier disruption (c).

the number of protein gene product nerve fibers (12). The nervous system might be associated with dermatoses which are characterized by abnormal metabolism of keratinocytes.

Whether CRF plays a role in the epidermis remains unknown. Slominskli *et al.* demonstrated that CRF-receptor mRNA was transcribed in human keratinocyte (13). The effects of other types of endocrinological factors such as glucocorticoid on the skin surface potential also should be studied. The present findings suggest the involvement of another mechanism for the alteration of the skin surface potential induced by psychological factors.

The role of the negative potential on the skin surface is not clear. Keratinocyte migration was influenced by the electric potential (14). The electric potential has been reported to accelerate the wound healing process (15). The electric potential might be important for the epidermal metabolism or homeostasis, but these phenomena have not been fully elucidated. Whether the potential itself has a role in the epidermal function remains to be investigated.

Tape stripping induced a flux of calcium and magnesium toward the bottom of the epidermis (5). In this case, the skin surface potential would be altered in a positive direction as in our present study because of relatively smaller referential number of both ions rather than the major counter ion, i.e., chloride. In the present study, tape stripping reduced the potential 30 min after the disruption on mouse skin sections. This suggests that the decrease of the potential was due to not only to the loss of the stratum corneum but also the following dynamic electric chemical processing in the epidermis. Part of the decrease of the potential might be induced by the ion flux. The calcium channel and potassium channel might play an important role in this ion movement, but the sodium channel was not directly related to the alteration after the tape stripping. The potassium ion was also transferred after tape stripping, but, it did not induce an electric potential because its transference number was similar to that of chloride ion (KCl 0.1 mol/dm^{-3}, K^+: 0.4898, Cl^-: 0.5102, Ref. 8).

Acute disruption of the skin barrier, which resides at the stratum corneum, by tape stripping or detergents, elicits a homeostatic response in the epidermis which results in a rapid restoration of the barrier function. In addition, acute disruption of the barrier results in an increase in epidermal DNA synthesis (16) and cytokine production (17). The mechanism of the signal transfer after the barrier disruption has not been fully eluci-

dated, but previous findings suggest a significant role of ions such as calcium and potassium ion the keratinocyte differentiation in the barrier homeostasis (18–20). The drastic movement of ions or the alteration of the electric potential after the barrier insults might be an important signal for the homeostatic process.

In conclusion, we demonstrated the skin surface electric potential which was induced by on ion flux such as Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} with ATP consumption in hairless mouse skin in organ culture. Disruption of stratum corneum reduced the potential; and, calcium and potassium channels were associated with the decrease. Substance P and CRF could influence the skin surface potential. These results suggest that ion movement in the epidermis on the skin significantly contributes to the skin surface potential.

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