Impaired Stratum Corneum Hydration in Mice Lacking Epidermal Water Channel Aquaporin-3*

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The water and solute transporting properties of the epidermis have been proposed to be important determinants of skin moisture content and barrier properties. The water/small solute-transporting protein aquaporin-3 (AQP3) was found by immunofluorescence and immunogold electron microscopy to be expressed at the plasma membrane of epidermal keratinocytes in mouse skin. We studied the role of AQP3 in stratum corneum (SC) hydration by comparative measurements in wild-type and AQP3 null mice generated in a hairless SKH1 genetic background. The hairless AQP3 null mice had normal perinatal survival, growth, and serum chemistries but were polyuric because of defective urinary concentrating ability. AQP3 deletion resulted in a >4-fold reduced osmotic water permeability and 2-fold reduced glycerol permeability in epidermis. Epidermal, dermal, and SC thickness and morphology were not grossly affected by AQP3 deletion. Surface conductance measurements showed remarkably reduced SC water content in AQP3 null mice in the hairless genetic background (165 ± 10 versus 269 ± 12 microsiemens (µS), p < 0.001), as well as in a CD1 genetic background (209 ± 21 versus 469 ± 11 µS). Reduced SC hydration was seen from 3 days after birth. SC hydration in hairless wild-type and AQP3 null mice was reduced to comparable levels (90–100 µS) after a 24-h exposure to a dry atmosphere, but the difference was increased when surface evaporation was prevented by occlusion or exposure to a humidified atmosphere (179 ± 13 versus 441 ± 34 µS). Conductance measurements after serial tape stripping suggested reduced water content throughout the SC in AQP3 null mice. Water sorption-desorption experiments indicated reduced water holding capacity in the SC of AQP3 null mice. The impaired skin hydration in AQP3 null mice provides the first functional evidence for the involvement of AQP3 in skin physiology. Modulation of AQP3 expression or function may thus alter epidermal moisture content and water loss in skin diseases.

The water content of the stratum corneum is an important determinant of the appearance, physical properties, and barrier function of the skin (1–3). The stratum corneum, the most superficial layer of skin, consists of layers of flattened corneocytes (dead epidermal cells) embedded in a lipid-rich matrix containing specialized proteins and lipids (4). Abnormalities of stratum corneum hydration are seen in a variety of hereditary and acquired skin diseases such as atopic dermatitis (5), eczema (6), psoriasis (7), senile xerosis (8), and hereditary ichthyosis (9). Hydration of the stratum corneum could in principle be determined by a number of factors including the concentration of water-retaining osmolytes, the water and solute transporting properties of the underlying layers of viable epidermal keratinocytes, and the barrier properties of the stratum corneum. There is evidence for a high concentration of solutes (Na⁺, K⁺, and Cl⁻) and a low concentration of water (13–35%, Ref. 10) in the superficial stratum corneum, producing in the steady-state gradients of both solutes and water from the skin surface to the viable epidermal keratinocytes (11–13). Although transepithelial fluid transporting properties have been studied extensively in various mammalian epithelia, the molecular mechanisms of fluid transport across epidermal keratinocyte layers remain poorly understood, as is the relationship between keratinocyte fluid transport and stratum corneum hydration. It has been proposed that aquaporin-3 (AQP3)1 might facilitate transepidermal water permeability to protect the stratum corneum against desiccation by evaporative water loss from the skin surface and/or to dissipate water gradients in the epidermal keratinocyte cell layer (14).

The integral membrane protein AQP3 has been proposed to be a potentially important transporter of water and solutes across epidermal keratinocytes (14–16). AQP3 was initially cloned from rat kidney (17, 18) and is a member of a family of homologous aquaporin water channels expressed widely in mammalian epithelia and endothelia that facilitate fluid transport. Phenotype studies in aquaporin knockout mice have implicated the involvement of aquaporins in the urinary concentrating mechanism in kidney (19), water movement in lung (20, 21), cerebral water balance (22), exocrine gland secretion (23, 24), and mechano-electric signal transduction (25). AQP3 is a member of a subclass of aquaporins, called aquaglyceroporins, which transport not only water but also glycerol and possibly other small solutes. AQP3-mediated water permeability was reported to be pH-dependent, decreasing at low pH (26). The physiological role of AQP3-mediated glycerol transport has been the subject of considerable speculation but remains unknown. We first reported by immunocytochemistry the expression of AQP3 protein in epidermal keratinocytes in rat skin (15), and subsequently AQP3 was localized in human keratinocytes (14), and in moist noncornified barriers such as the

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1 The abbreviations used are: AQP3, aquaporin-3; RT-PCR, reverse transcription-PCR; PBS, phosphate-buffered saline; µS, microsiemens.
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mucous membranes in the mouth (16). It was reported recently that water permeability of human epidermal keratinocytes was inhibited by mercurials and low pH, consistent with the involvement of AQP3 (14). Additional indirect evidence supporting a role for AQP3 in skin physiology includes the regulation of epidermal cell AQP3 expression by extracellular osmolality, barrier perturbation, and exposure to a dry atmosphere (27, 28).

The purpose of this study was to investigate whether AQP3 is involved in stratum corneum hydration. Because humans with AQP3 deficiency have not been identified and nontoxic inhibitors of AQP3 are not yet available, we assessed skin phenotype in transgenic mice lacking AQP3. For studies in skin, the AQP3 null genotype, originally created in a CD1 genetic background (29), was transferred to the hairless SKH1 background. We found remarkably reduced stratum corneum water content in the skin of AQP3 null mice and investigated possible mechanisms for this defect. An interesting and unexpected finding was that AQP3 deletion remarkably impaired the ability of stratum corneum to become hydrated even when water loss from the skin surface was prevented by occlusion or exposure to a humidified atmosphere.

**EXPERIMENTAL PROCEDURES**

**Mice**—The AQP3 null genotype originally generated in CD1 genetic background (29) was transferred to a hairless background by back-cross breeding of heterozygous AQP3 CD1 mice with SKH1 hairless mice. Hairless AQP3 heterozygous founder mice were bred to generate wild-type, heterozygous, and AQP3 null hairless mice. The mice were main-

**Immunofluorescence, Light Microscopy, and RT-PCR**—Immunofluorescence was done on frozen sections of mouse skin fixed in PBS containing 2% paraformaldehyde and incubated with 1:200 dilution of affinity-purified anti–AQP3 rabbit polyclonal antibody. Slides were washed three times in PBS and incubated for 40 min at room temperature in PBS/bovine serum albumin containing fluorescein isothiocyanate-antiali-

**Urinary Output, Osmolarity, and Serum Chemistries**—Measurements were performed on mice under normal conditions (external temperature 22 ± 2 °C, humidity 40 ± 5%) or on mice treated with various maneuvers including exposure to low (10%) and high (90%) external humidity, tape stripping, and topical occlusion. Urine sorption-desorp-

**Water and Glycerol Permeability Measurements**—For measurement of water permeability, epidermal sheets were freshly isolated from the same area of skin were averaged for each value. Measurements were performed on mice under normal conditions (external temperature 22 ± 2 °C, humidity 40 ± 5%). After neutralization with fetal bovine serum (10% final concentration), the epidermis was mounted in a perfusion chamber (exchange time < 0.2 s) for measurement of osmotic water permeability by spatial filtering micro-

**RESULTS**

To study skin phenotype, the AQP3 null mutation generated in CD1 mice was transferred to a SKH1 hairless genetic back-

**Skin Conductance Measurements**—Stratum corneum hydration was determined by high frequency electrical conductance using a Skicon-200 skin surface hygrometer (IBS). Three independent measurements from the same area of skin were averaged for each value. Measure-

**Immunofluorescence**—Immunofluorescence was done on frozen sections of mouse skin fixed in PBS containing 2% paraformaldehyde and incubated with 1:200 dilution of affinity-purified anti–AQP3 rabbit polyclonal antibody. Slides were washed three times in PBS and incubated for 40 min at room temperature in PBS/bovine serum albumin containing fluorescein isothiocyanate-antiali-

**Electron Microscopy**—Skin samples were fixed in 2% glutaraldehyde and embedded in Epon. 70-nm thick sections were cut on a Reichert-E ultramicrotome and collected on Formvar-coated electron microscopy grids. Sections were stained in 5% uranyl acetate for 3 min and then in lead acetate for 1 min, dried, and observed by electron microscopy (Philips CM 400). For immunogold labeling, skin samples were post-fixed in 2% paraformaldehyde + 0.1% glutaraldehyde and embedded in Unicerol. 70-nm thick sections were preincubated in 20 ml Tris buffer (pH 7.4) containing 0.1% bovine serum albumin, 0.1% fish gelatin, and 0.05% Tween 20 (TBuffer) followed by a 1-h incubation in affinity-purified anti-AQP3 antibody (1:50 dilution) in TBuffer. Grids were incubated in a 1:25 dilution of 10-nm gold-coupled anti-rabbit antibody (Amersham Biosciences) for 1 h, washed six times, stained in 5% uranyl acetate for 3 min and then in lead citrate for 1 min, dried, and observed by electron microscopy.

**Water and Glycerol Permeability Measurements**—For measurement of water permeability, epidermal sheets were freshly isolated from 6–8-week-old wild-type and AQP3 null mice by digesting full thickness skin fragments in 50 ml/m dispace solution (BD Biosciences) at room temperature for 1 h. After washing in cold PBS, the epidermis was peeled off the dermis. Small fragments of the epidermal sheets (~12-mm diameter) were immobilized (surface facing downward) on 18-mm-diameter round coverglasses with superglue. The coverglass was mounted in a perfusion chamber (exchange time < 0.2 s) for measurement of osmotic water permeability by spatial filtering micro-

**Skicon-200 skin surface hygrometer (IBS). Three independent measurements from the same area of skin were averaged for each value. Measurements were performed on mice under normal conditions (external temperature 22 ± 2 °C, humidity 40 ± 5%) or on mice treated with various maneuvers including exposure to low (10%) and high (90%) external humidity, tape stripping, and topical occlusion. Urine sorption-desorp-

**Analytical Results**—Stratum corneum was transferred to a hairless SKH1 hairless genetic background. We found remarkably reduced stratum corneum water content in the skin of AQP3 null mice and investigated possible mechanisms for this defect. An interesting and unexpected finding was that AQP3 deletion remarkably impaired the ability of stratum corneum to become hydrated even when water loss from the skin surface was prevented by occlusion or exposure to a humidified atmosphere.
hairless SKH1 (Fig. 2D)/H11006 (Fig. 3A and C) and AQP3 null (−/−) mice. Thickness measurements on a series of sections showed no significant differences in the stratum corneum, epidermal, or dermal layers (Fig. 2E). The thickness of the innermost fat layer was reduced in the AQP3 null mice (p < 0.01), which may be related to the relative serum hypotriglyceridemia.

Transmission electron microscopy showed no apparent differences in the structures of the stratum corneum and keratinocytes in wild-type (Fig. 3, A and C) and AQP3 null (Fig. 3, B and D) mice. At the apex of the epidermis (Fig. 3, A and B), the stratum corneum contained five layers in both wild-type and AQP3 null mice, with each layer of a similar thickness. Keratohyalin granules seen in the superficial epidermal cells were of similar size in wild-type and AQP3 null mice. As expected, keratinocytes at the base of the epidermis (Fig. 3, C and D) were taller but showed no differences between genotypes. Despite the possible consequences of AQP3 deficiency on water movement, intercellular spaces were not dilated in AQP3 null mice from the base to the surface of the epidermis.

Osmotic water and glycerol permeability were measured to investigate the functionality of plasma membrane AQP3 in epidermal keratinocytes. Osmotic water permeability was measured using a well established surface conductance method (32). Surface electrical conductance is approximately linearly related to percentage water content of the outer stratum corneum (33). Skin conductance increased substantially during the first month in wild-type mice, to a much greater extent than in AQP3 null mice.

Glycerol permeability was measured from the uptake of radiolabeled [3H]glycerol in suspensions of freshly isolated keratinocytes. Measurements in keratinocytes from wild-type mice indicated approximately linear [3H]glycerol uptake for 10 min (not shown). Fig. 4B shows that the average [3H]glycerol uptake at 90 s (after subtraction of bound [3H]glycerol uptake by 0 time measurement) was significantly reduced in keratinocytes from AQP3 null mice. The −2-fold reduction in glycerol permeability represents a lower limit to the difference in epidermal glycerol permeability in wild-type versus AQP3 null mice because of possible effects of the protease treatment (needed to release keratinocytes) on AQP3 function. These results establish the functionality of AQP3 as a water/glycerol transporter in mouse epidermis.

Stratum corneum water content was measured using a well established surface conductance method (32). Surface electrical conductance is approximately linearly related to percentage water content of the outer stratum corneum (33). Skin conductance was measured using a well established surface conductance method (32). Surface electrical conductance is approximately linearly related to percentage water content of the outer stratum corneum (33). Skin conductance was measured in mice of different ages to determine when the defect in stratum corneum hydration is first manifest. As summarized in Fig. 5B, skin conductance was low and similar at 1 and 2 days after birth but was significantly lower by day 3 in AQP3 null mice compared with wild-type mice. Skin conductance increased substantially during the first month in wild-type mice, to a much greater extent than in AQP3 null mice.

To test the hypothesis that decreased stratum corneum hydration in AQP3 null mice is due to impaired replacement of surface evaporative losses by water transport across epidermal keratinocytes, conductance measurements were performed after subjecting mice to different external humidity conditions and after skin surface occlusion. Exposure to 10% humidity increases evaporative water loss, whereas exposure to 90% humidity or surface occlusion prevents evaporative water loss. The prediction is that the defective stratum corneum hydration in AQP3 null mice would be corrected by preventing evaporation but exaggerated by exposure to a 10% humidity atmosphere. Fig. 5C summarizes conductance measurements. Exposure to 90% humidity or occlusion for 24 h resulted in increased stratum corneum hydration in wild-type mice, but contrary to expectations, impaired hydration in AQP3 null mice was not corrected. Also, contrary to expectations, skin conductance of the wild-type and AQP3 null mice became similar after exposure to 10% humidity. Fig. 5D shows the full time courses of skin conductance after changing external humidity. In both cases the new steady-state conductance was achieved in 2–4 h but with a small undershoot for exposure to 10% humidity.

To investigate whether the impaired hydration occurs in deeper layers of the stratum corneum of AQP3 null mice, conductance measurements were made after layers of the stratum corneum were removed progressively by cellophane tape (tape stripping). Fig. 6A shows that the accumulated total protein was approximately linear with the number of tape stripplings.
and that the amount of protein removed was similar in wild-type and AQP3 null mice. Fig. 6B shows the correlation between skin conductance and total accumulated protein for a series of wild-type and AQP3 null mice subjected to serial tape stripping. Hydration increased progressively as deeper layers of the stratum corneum were exposed by tape stripping, which supports the notion of a water gradient from deep to superficial stratum corneum (12, 34). Skin conductance was lower in AQP3 null mice, but the steepness of the water gradient was increased. These results are consistent with the notion that a water gradient is established from the well hydrated epidermal keratinocytes through the relatively water-poor and watertight stratum corneum.

The results shown in Figs. 5 and 6 provide evidence against the hypothesis that impaired hydration in the stratum corneum of AQP3 null mice results from decreased epidermal water permeability. We tested whether the stratum corneum of AQP3 null mice has an intrinsic defect in its ability to be hydrated and its “water holding capacity,” as found in diseases associated with dry skin. An established sorption-desorption test was used in which skin conductance is measured before and at different times after a 10-s exposure of the skin to distilled water (32). Fig. 6C shows that skin conductance increased immediately after water exposure and then recovered over a few min. The initial increase in skin conductance, which was significantly greater in wild-type mice, has been taken as a measure of the ability of the stratum corneum to become hydrated. More importantly, the recovery after hydration has been taken as a measure of the water holding capacity of the stratum corneum. The area under the recovery curve (after subtraction of prehydration conductance) has been used as a single parameter describing water holding capacity (10). The recovery area parameter was remarkably reduced in AQP3 null mice (8.2 ± 1.7 × 10⁴ versus 4.5 ± 1.9 × 10⁴ μS/s, p < 0.005) (see “Discussion”).

**DISCUSSION**

The goal of this study was to determine whether AQP3 plays a role in skin physiology. As discussed in the Introduction, previous findings of AQP3 expression and regulation in epidermal keratinocytes provided indirect evidence for a role of AQP3 in skin function. To facilitate the study of stratum corneum hydration, the AQP3 null genotype was transferred to a SKH1 hairless background. The hairless AQP3 null mice manifested a urinary concentrating defect with polyuria and reduced urinary osmolality, as found previously for AQP3 null mice in the CD1 background (29). AQP3 null CD1 mice develop progressive renal failure with dilatation of the kidneys and urinary bladder by 8 weeks of life (35) but have unimpaired function of the airways (36), another major site of AQP3 expression. The hair-
less AQP3 null mice had normal perinatal survival, growth, and serum chemistries, except for a mild elevation in blood urea nitrogen and a decrease in serum triglyceride concentration. We do not believe that these mild alterations in blood chemistry cause the abnormalities in stratum corneum hydration in AQP3 null mice. The decreased stratum corneum hydration was found in AQP3 null mice at 4 weeks of age before changes in blood urea nitrogen were detected, and AQP1 null mice have an even greater degree of serum hypotriglyceridemia (37) but do not manifest altered stratum corneum hydration.

Adequate stratum corneum hydration is important in maintaining skin plasticity and barrier integrity. The stratum corneum gains water from underlying viable layers of epidermis and dermis to maintain its proper hydration status in the relatively dry external environment. There are several possible mechanisms by which AQP3 deletion might affect stratum corneum hydration. Reduced epidermal water permeability might impair water transport into the stratum corneum, which would lead to decreased stratum corneum water content when surface evaporation occurs but not when the skin surface is occluded or exposed to a humidified environment. Reduced permeability of the epidermal cell layer to glycerol or other small solutes might produce alternations in the composition and/or structure of the stratum corneum that alter its ability to hold water even when surface evaporation is blocked. High aquaporin-dependent water permeability in the epidermal cell layer might prevent water gradients within the viable keratinocytes, preserving their normal biosynthetic functions. However, it seems unlikely that water gradients are present in the layer of viable keratinocytes because water permeability of the stratum corneum is orders of magnitude lower than in the keratinocyte layer (38).

Transport measurements showed significantly reduced water and glycerol permeability in AQP3 null mice. These results are consistent with localization of AQP3 in the plasma membrane of keratinocytes, as shown by immunofluorescence and immunogold electron microscopy, and with the absence of the compensatory expression of another aquaporin in epidermis of AQP3 null mice, as shown by RT-PCR. The finding of functional expression of AQP3 in mouse epidermis agrees with the conclusion of a recent study showing that apparent water permeability in human epidermis was inhibited by mercurials and low pH (14). Utilizing a spatial filtering approach to measure osmotic water permeability in freshly isolated, intact epidermal sheets, we found that AQP3 deletion produced a 4-fold reduction in water permeability. This approach preserves the normal epidermal anatomy, and unlike keratinocyte isolation procedures, the isolation of epidermal sheets does not require digestion with proteases. In other studies we have found up to 10-fold reductions in water permeability after aquaporin deletion, for example in erythrocytes (35), the proximal tubule (19), and the thin descending limb of Henle (39) of AQP1 null mice.

**FIG. 4.** Water and glycerol permeability of the epidermis. A, osmotic water permeability of epidermal sheets in which the epidermal keratinocytes were subjected to osmotic gradients. Left, representative time courses of transmitted light intensity (related to epidermal cell volume) in response to changes of perfusate osmolality between 300 and 600 mosm. Single exponential functions fitted to the data are shown as thin smooth curves. Right, reciprocal exponential time constants (τ) fitted to time courses of increasing transmitted light intensity in separate measurement shown along with means ± S.E. (*, p < 0.01). B, [3H]glycerol uptake (at 90 s) measured in freshly isolated suspensions of keratinocytes (⁎, p < 0.01). See “Experimental Procedures” for methodological details.

**FIG. 5.** Decreased stratum corneum hydration in AQP3 null mice. A, skin surface conductance (proportional to stratum corneum water content) in indicated areas of hairless wild-type and AQP3 null mice (means ± S.E., 10–14 mice/group). *, p < 0.001. B, age dependence of skin conductance (means ± S.E., 25 mice/group). C, skin conductance (back skin) measured after a 24-h exposure to atmosphere (room temperature) at relative humidity of 10, 40, or 90%; "occluded" indicates an occlusion dressing (means ± S.E., 5 mice/group). *, p < 0.01. D, time course of skin conductance after exposure (at time 0) to a 90% (left) or 10% (right) humidified atmosphere.
and in the lungs of AQP1 and AQP5 null mice (20, 21). The relatively small reduction in epidermal water permeability in AQP3 null mice may be related to the relatively weak intrinsic water transporting activity of AQP3 compared with other aquaporins (40). In addition, unstirred layer effects in the multilayered epidermal sheet would decrease apparent osmotic water permeability and blunt the effects of AQP3 deletion. Nevertheless, the results shown here prove that AQP3 is functional as a plasma membrane water/glycerol transporter in the epidermis, rather than the intrinsic water permeability of the epidermal cell layer. Further studies are needed to determine whether water and/or glycerol transport by AQP3 is essential for normal hydration of the stratum corneum.

In summary, the impaired stratum corneum hydration in AQP3 null mice provides the first functional evidence for the involvement of AQP3 in skin physiology. Although the exact mechanism for impaired stratum corneum water holding remains to be established, we propose that pharmacological modulation of AQP3 expression or function may alter epidermal moisture content and water loss. Controlled modulation of skin moisture content and barrier function may thus be useful in the treatment of skin disorders associated with abnormally wet, dry, or permeable skin.

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