Role of Appendages in Skin Resistance and Iontophoretic Peptide Flux: Human Versus Snake Skin

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Purpose. 1. The assessment of the role of hair follicles and sweat glands in skin resistance and percutaneous iontophoretic flux of 9-desglycinamide, 8-arginine vasopressin (DGAVP) by comparing two skin species: human stratum corneum which contained hair follicles, sweat and sebaceous glands, and shed snake skin which lacked all appendages. 2. The effect of 1-dodecylazacycloheptan-2-one (dodecyl-Azone, a lipid perturbing agent) on the iontophoretic DGAVP flux.

Methods. Iontophoresis *in vitro* was performed in a transport cell (0.79 cm^2 area available for percutaneous transport) by 8-hours application of a pulsed constant current of 100 Hz, 50% duty cycle and 0.26 $mA.cm^{-2}$ current density delivered by a pair of Ag/AgCl electrodes, of which the anode was facing the anatomical surface of the skin samples.

Results. The initial resistances of human stratum corneum and shed snake skin samples were of the same order of magnitude (20–24 $k\Omega.cm^2$) and both skin species showed a comparable resistance-decrease profile during 8-hours iontophoresis, indicating that the resistances were mainly determined by the stratum corneum and not greatly influenced by the appendageal structures. The initial resistances of the skin samples pretreated with dodecyl-azone were less than 50% of the values of untreated samples. Because dodecyl-azone is known to perturb the ordering of the intercellular lipids, the effect of azone on the resistance confirms that the resistance mainly resides within the intercellular lipids of the stratum corneum. No correlation was found between the iontophoretic DGAVP-flux and the conductance of human skin. For shed snake skin, however, a good correlation was found, indicating that the iontophoretic permeability of human skin *in vitro* for a peptide such as DGAVP is, unlike shed snake skin, not related to its overall permeability to ions. While the initial resistances of both human and snake skin were in the same order of magnitude and showed the same declining profile during iontophoresis, the steady state iontophoretic DGAVP flux across human stratum corneum was approximately 140 times larger than through shed snake skin. These findings suggest that small ions follow pathways common to both skin types, presumably the intercellular route, while the peptide on the other hand is transported differently: across snake skin presumably along intercellular pathways only, but across human stratum corneum along additional pathways (most likely of appendageal origin) as well. This interpretation is supported by the observations made of the effects of dodecyl-azone on DGAVP-iontophoresis. Pretreatment with dodecyl-azone did not significantly change steady state fluxes and lag times of DGAVP-iontophoresis across human stratum corneum, but resulted in a significant 3-fold lag time decrease and a 3-fold flux increase of DGAVP-iontophoresis across snake skin.

Conclusions. The results of these *in vitro* studies emphasize the importance of the appendageal pathway for iontophoretic peptide transport across human stratum corneum.

KEY WORDS: skin resistance and impedance; skin appendages; human and snake skin; iontophoresis; peptide delivery; azone.

INTRODUCTION

The delivery of peptides by percutaneous iontophoresis is an elegant way out of the problems inherent to peroral peptide delivery, including insufficient absorption due to degradation of the peptide in the gastro-intestinal tract. First of all, since peptides usually carry a charge around physiological pH, they can in principle be transported across the skin by iontophoresis; as a result much higher bioavailabilities may be achieved than by e.g. passive percutaneous absorption, even in the presence of skin penetration enhancers (1). Furthermore, there are many examples of peptides, for which pulsatile rather than constant delivery would ideally be required (2–4). To achieve this type of modulated peptide delivery, iontophoresis in principle may offer excellent opportunities.

However, in order to achieve such a high degree of control over percutaneous peptide flux across skin, we need to know more about the relationships between the electrical properties of the skin and the fluxes and pathways of iontophoretic peptide transport across it. It would be ideal, if iontophoretic drug delivery would be no longer controlled by the intrinsic permeability of the skin, which is determined by the composition of the stratum corneum and the density of appendages, but would only depend on externally controlled parameters such as current density. However, one of the problems associated with passive diffusion and also with transdermal iontophoresis is the variability of diffusion and iontophoretic transport rates across human skin (5–7). This variability may be related to the variability of the human skin resistance. In the present study this hypothesis was tested using two skin species, shed snake skin and human abdominal stratum corneum, applying 9-desglycinamide, 8 arginine vasopressin (DGAVP) as a model peptide. DGAVP is a neuropeptide drug which has been shown to improve memory processes in men (8).

Shed snake skin consists of an inner alpha and an outer beta keratin layer and a middle layer, the so called mesos layer, which is the main barrier to water exchange. The low permeability to water and water-soluble tracers of the mesos layer has been related to the presence of lipids (9,10). The mesos region resembles the stratum corneum of human skin in that its consists of cornified epidermal cells surrounded by intercellular spaces filled with multiple lipid lamellar sheets (11). However, shed snake skin differs from human stratum corneum in that it is devoid of appendageal structures (12,13). This in contrast to human abdominal skin, where appendageal structures such as hair follicles, sweat and sebaceous glands penetrate the stratum corneum (14).

The contribution of appendageal transport to iontophoresis of DGAVP across stratum corneum *in vitro* was investigated by comparing two skin species, human stratum corneum and shed snake skin, and by modulating the skin permeability in both species with 1-dodecylazacycloheptan-

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2-one (dodecyl-azone), which is known to increase the disordering of stratum corneum lipids and hence promotes intercellular transport (15–18).

MATERIALS AND METHODS

Materials

9-Desglycinamide, 8-arginine vasopressin dicitrate (DGAVP; Mw 1412) was kindly supplied by Organon (Org 5667, Organon International, Oss, The Netherlands). It was dissolved in a concentration of 2 mg/ml in 0.15 M phosphate buffered saline (PBS) of pH 6.0, which was composed of 0.14 M NaCl and 0.01 M phosphate buffer. At a pH value of 6.0 about 60% of the DGAVP molecules in solution carried a net single positive charge (5).

1-Dodecylazacycloheptan-2-one (dodecyl-azone) was synthesized in the Department of Organic Chemistry (Leiden University, Leiden, The Netherlands). The preparation and analysis of dodecyl-azone has been described elsewhere (16). Propylene glycol was obtained from J.T. Baker Chemicals B.V. (Deventer, The Netherlands). All chemicals used were of analytical grade; all aqueous solutions were based on freshly prepared bidistilled water.

Human Stratum Corneum and Shed Snake Skin

Human stratum corneum was prepared from human abdomen skin of a 35 years old female donor within 24 years after surgical removal. The subcutaneous fat was removed and the skin was dermatomed to a thickness of 300 μm. The skin was incubated with its dermal side down on Whattman paper soaked in a solution of 0.2% (w/w) trypsin in 0.15 M phosphate buffered saline (PBS) of pH 7.4 for 24 hours at 4°C and subsequently for 1 hour at 37°C, after which the stratum corneum was peeled off from the underlying viable epidermis. Further trypsin activity was blocked by bathing the stratum corneum in an 0.2% (w/w) anti-trypsin solution (type II-S from soybean, Sigma Chemicals, The Netherlands) in PBS pH 7.4. The stratum corneum was washed several times in bidestilled water, then dried in a dessicator above silicagel and under N_2-gas to inhibit oxidation.

Shed snake skin was obtained from a 12-year old female snake, species Vipera Russelii, which was a generous gift from Dr. J. Roberts (GO-JO Industries, Akron, Ohio U.S.A.). The skin was stored before use under the same conditions as human stratum corneum.

Although the viable epidermis was degraded by trypsin during human stratum corneum isolation, most hair follicles remained intact and attached to the stratum corneum when this layer was peeled off. Removal of the stratum corneum from the underlying epidermis was carried out very carefully to prevent loss of hair follicles. Stratum corneum samples which had lost some hair follicles during the isolation procedure, showed holes of roughly 0.1 mm at these sites. Such samples were characterized by an initial resistance ≤ 6 $k\Omega.cm^2$ (measured with a current density of 13 $\mu A.cm^{-2}$) and were considered damaged and excluded from the experiments. Figure 1 shows the presence of intact hair follicles protruding from the basal side of human stratum corneum, whereas appendageal structures are absent in shed snake skin.

Fig. 1. Dermal side of human stratum corneum sample used *in vitro*. In the centre hair follicles are visible on the basal surface of the human stratum corneum sample (abdomen). The scale bar indicates 1 mm.

Skin Hydration

Before hydration the sheets of human stratum corneum and shed snake skin (ventral side) were punched into disks of 1.6 cm diameter. The desiccated stratum corneum samples were hydrated before they were mounted in transport cells. The stratum corneum samples were equilibrated to water vapor over a 0.15 M PBS solution of pH 6.0 for 24 hours at 4°C and subsequently submerged into a 0.15 PBS solution of pH 6.0 for 2 hours at room temperature. The weight of the sample was measured before and after hydration and the difference between wet (g_w) and dry (g_d) weight calculated for each stratum corneum sample. The water weight content was expressed as the difference between wet and dry weight, divided by the wet weight: $(g_w - g_d)/g_w$. After hydration, the water weight contents of human stratum corneum and shed snake skin were 69 ± 4 and 62 ± 7%, respectively.

Skin Pretreatment with Dodecyl-Azone

1-Dodecylazacycloheptan-2-one (dodecyl-azone) was dissolved at a concentration of 0.15 M in propylene glycol. The hydrated skin samples were spread out on aluminum foil for support. The anatomical surfaces of the skin samples were covered with the azone solution (120 μl azone/cm^2). The samples were stored for 24 hours in a closed glass tray at room temperature, after which the residual liquid was removed by a paper tissue.

Transport Cells

The skin samples were mounted between the two compartments of transport cells, leaving a skin area of 0.79 cm^2 for transport (5). The 3-ml acceptor compartments were con-

nected to a flow-through system driven by a peristaltic pump (Ismatec, IPS-8, Zürich, Switzerland) equipped with Tygon tubes (Ismatec; 1 mm inner diameter), which continuously supplied fresh 0.15 M PBS pH 6.0 acceptor fluid and which was tuned to deliver 1-ml fractions every hour into a fraction collector. The media in the 1.5 ml donor compartments were circulated at a flow rate of 15 ml/hr using a similar peristaltic pump as discussed before. The total volume of the donor chamber plus attached tubing amounted to 3.0 ml. The donor compartment was filled with a solution of 1.4 mM DGAVP in 0.15 M PBS (pH 6.0). The entire set-up was situated in an incubator at a temperature of 32°C. During the iontophoresis protocol used, the pH of the donor compartment was found to remain constant (5).

Experimental Protocol

Three different series of experiments were performed:
(1) Firstly, a series of experiments was carried out to measure the passive diffusion of DGAVP across human and snake skin samples, which were not pretreated with dodecyl-azone. The skin samples (n = 6) were not exposed to any current. Passive DGAVP diffusion was measured for 18 hours.
(2) Another series of experiments (n = 4–6) was performed to measure the flux of DGAVP during 8 hours of iontophoresis across human and snake skin. Simultaneously, the voltage waves across the skin resulting from the current pulses were high-frequency sampled at intervals of 10 min and stored by the computer to be analyzed afterwards. The skin samples were not pretreated with dodecyl-azone.
(3) In a third series (n = 4–6), the iontophoretic DGAVP flux and the voltage across the skin were measured for 8 hours using skin samples pretreated with dodecyl-azone.

Current Delivery and Voltage Measurement

The transport cells were connected to a computer-controlled pulsed current source and high-frequency voltage sampling system by means of two separate sets of electrodes: one for current-delivery ("driving electrodes") and one for voltage-measurement ("measuring electrodes"), respectively. Both types of electrodes consisted of a pure silver core (≥99.9% purity), which was electrolytically coated with a layer of AgCl in 4 N HCl. During iontophoresis, the driving electrodes delivered a pulsed constant current of 100 Hz, 0.26 $mA.cm^{-2}$ current density and 50% duty cycle, while the anode was in the donor-compartment and the cathode in the acceptor-compartment. The voltage-measuring electrodes were placed at a distance of 2 mm from the skin surface. The voltage drop across the skin samples was high-frequency sampled (100 kHz) at intervals of 10 min and the data stored by the computer. The voltage wave V(t) across the skin resulting from a current pulse was accurately ($R^2 \geq 0.99$) fitted to a bi-exponential equation (equation 1), corresponding to an equivalent electrical circuit of both human stratum corneum and shed snake skin consisting of two R || C-circuits coupled in series: $R_a \parallel C_a$ and $R_b \parallel C_b$.

$$V(t) = V_a \exp\left(-\frac{t}{\tau_a}\right) + V_b \exp\left(-\frac{t}{\tau_b}\right) + S\,V_\infty, \qquad (1)$$

in which the sum of V_a and V_b equals the total voltage drop across the skin, τ_a and τ_b are the relaxation times and $V\infty$ is a residual potential. The total skin resistance (R), which is the sum of its component resistances R_a and R_b was calculated from the values of V_a and V_b, obtained by the bi-exponential fitting-procedure, as follows:

$$R = \frac{V_a + V_b}{I} \qquad (2)$$

in which I is the current amplitude.

Analysis of Percutaneous Peptide Penetration

Every hour 1-ml acceptor fractions were collected and the amount of percutaneously permeated peptide in each fraction was quantified by a radioimmunoassay (20). Steady state fluxes (J_i) were calculated from a plot of the cumulative permeated amount of peptide versus time. By extrapolation of the steady state portion of the plot to the time axis, an intercept was obtained, which represents the lag time (t_l). Enhancement factors were calculated as the quotient of the steady state fluxes measured during application of current (J_i) and those observed with passive diffusion (J_d): $E = J_i/J_d$ (21). A one-tailed students t-test was performed to assess the significancy of flux-measurements. Significance was specified at the 5% level. The Spearman's rank correlation test was applied to establish whether a rank correlation exists between skin conductance and the iontophoretic DGAVP-flux.

RESULTS

Resistances and DGAVP-Fluxes Across Skin Samples in the Absence of Azone

The initial resistances of human stratum corneum and shed snake skin were 27.6 ± 5.4 and 25.1 ± 9.8 $k\Omega.cm^2$, respectively (Figures 2 and 3) and these values are in the same order of magnitude. During application of the 0.26 $mA.cm^{-2}$ current, the resistances of both human stratum

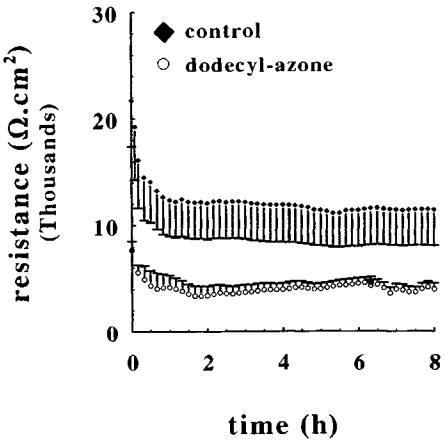

Fig. 2. Effect of dodecyl-azone on the resistance of human stratum corneum during iontophoresis. (◆) Human stratum corneum samples, which were not pretreated (◆, controls) or pretreated (○) with dodecyl-azone. Data represent Mean ± S.D. of n = 4 samples (○) and n = 6 samples (◆).

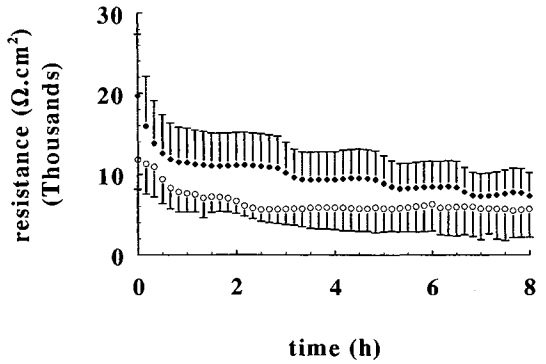

Fig. 3. Effect of dodecyl-azone on the resistance of shed snake skin during iontophoresis. Shed snake skin samples, which were not pretreated (♦, controls) or pretreated (○) with dodecyl-azone. Data represent Mean ± S.D. of n = 6 samples.

corneum and shed snake skin decreased (Figures 2 and 3). The time-course of the resistance-decrease of human and snake skin was very similar. The decrease was most rapid during the first hour of current-application. After 1 hour, the resistances of human stratum corneum and shed snake skin were reduced to 57 ± 16% and 58 ± 22% of their mean initial values, respectively. Thereafter, the decrease continued more slowly. The final resistance after 8 hours of iontophoresis was 53 ± 16% of the initial value for human stratum corneum and 37 ± 15% of the initial value for shed snake skin (Figures 2 and 3).

Figures 4A and 4B show the amounts of DGAVP transported by iontophoresis across human stratum corneum and shed snake skin, respectively (see diamonds for no-azone data). A steady state iontophoretic DGAVP-flux was achieved using human skin as well as shed snake skin, but was reached more quickly through human stratum corneum than through shed snake skin. The lag time across human stratum corneum was 0.7 ± 0.3 hr, which is about 3 times smaller than that for shed snake skin (2.5 ± 1.1 hr; Table I). Application of iontophoresis decreased the lag times of DGAVP-transport across both human stratum corneum and shed snake skin with respect to passive diffusion by approximately 2 times (Table I).

The most striking difference between the two skin species was that the steady state iontophoretic DGAVP-flux across human stratum was 141.4 ± 52.4 $ng.cm^{-2}.hr^{-1}$, which is two orders of magnitude larger than the steady state iontophoretic flux across shed snake skin, (1.0 ± 1.0 $ng.cm^{-2}.hr^{-1}$; Table I). The enhancement factors obtained with iontophoresis across human stratum corneum and snake skin with respect to passive diffusion were about 33 and 10, respectively (Table I).

In Figures 5 and 6 the iontophoretic DGAVP-fluxes are plotted versus the mean conductances (= 1/resistances) of human stratum corneum and shed snake skin over the same time interval. These data were subjected to a Spearman's rank correlation test. For human skin, no satisfactory correlation was found between peptide flux and skin conductance at the 5% level (n = 28; r_s = 0.332), whereas for shed snake skin a good rank correlation between peptide flux and skin

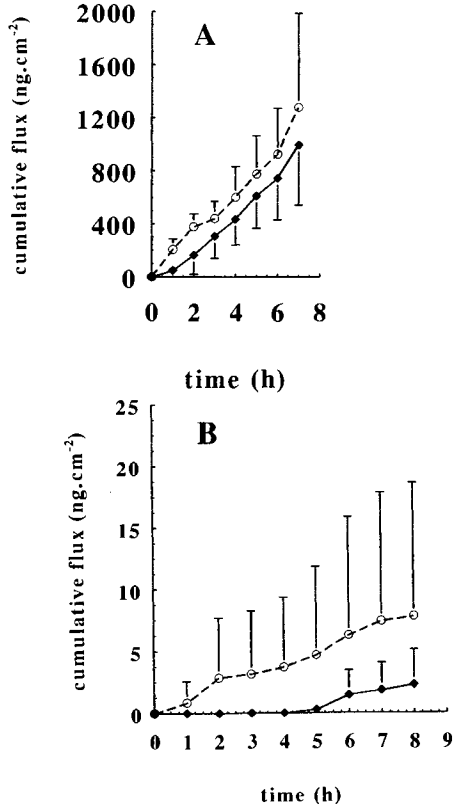

Fig. 4. Iontophoretic DGAVP-transport across human stratum corneum and shed snake skin. (A) DGAVP-iontophoresis across (♦) untreated human stratum corneum samples and (○) samples pretreated with dodecyl-azone. (B) DGAVP-iontophoresis across (♦) untreated shed snake skin samples and (○) samples pretreated with dodecyl-azone. Data represent Mean ± S.D. on n = 4–6 samples.

conductance was found at the 0.1% level (n = 32; r_s = 0.999).

Resistances and DGAVP-fluxes Across Azone-Pretreated Skin Samples

The mean initial resistances of human stratum corneum and shed snake skin, which had been pretreated with dodecyl-azone, were 9.8 ± 1.1 and 15.0 ± 4.7 $k\Omega.cm^2$, respectively (Figures 2 and 3). These values are between 30 and 50% of the mean resistance values of skin samples, which were not pretreated with azone (Figures 2 and 3). During application of the 0.26 $mA.cm^{-2}$ current, a decrease of the resistance of azone-treated skin samples was observed, which was most rapid within the first hour of current-application (Figures 2 and 3). After 1 hour the resistance values of human stratum corneum and shed snake skin were 55 ± 15% and 65 ± 20% of their mean initial values, respectively; subsequently both of them remained rather constant.

In Figures 4A and 4B the iontophoretic DGAVP-fluxes across dodecyl-azone pretreated human and snake skin samples are plotted (see open circles). Treatment with azone did not change the lag time of DGAVP iontophoresis across human stratum corneum, but resulted in a 3-fold decrease of the lag time of DGAVP iontophoresis through shed snake skin (Table I).

Table I. Lag Times and Steady State Fluxes for Passive and Iontophoretic DGAVP Transport Across Untreated and Dodecyl-azone Pretreated Human and Snake Skin[a]

flux/lag time	J_p ($ng.cm^{-2}.hr^{-1}$)	J_i ($ng.cm^{-2}.hr^{-1}$)	t_p (hr)	t_i (hr)
Skin type				
Human	0.4 ± 0.3	141.4[b] ± 52.4	1.4 ± 0.4	0.7[b] ± 0.3
Snake	0.10 ± 0.07	1.0[c] ± 1.0	4.3 ± 0.5	2.5[c] ± 1.1
Human/Azone	—	161.4 ± 76.3	—	0.7 ± 1.2
Snake/Azone	—	2.9[d] ± 1.5	—	0.7[d] ± 0.4

[a] Human stratum corneum or shed snake skin samples, which were not pretreated (human or snake) or pretreated with dodecyl-azone (human/Azone or snake/Azone). Flux during passive diffusion (J_p) or iontophoresis (J_i); lag time of passive diffusion (t_p) or inotophoresis (t_i). Data represent Mean ± S.D. of n = 4-6 samples. Passive diffusion through skin samples pretreated with dodecyl-azone was not measured.
[b] Significantly different from passive diffusion across untreated human stratum corneum ($P < 0.05$).
[c] significantly different from passive diffusion across untreated shed snake skin ($P < 0.05$).
[d] significantly different from iontophoresis across untreated snake skin ($P < 0.05$).

The iontophoretic DGAVP-flux measured across azone pretreated human stratum corneum (162.4 ± 76.3 $ng.cm^{-2}.hr^{-1}$) was not significantly different from the iontophoretic flux across untreated human stratum corneum samples, whereas the iontophoretic flux across azone pretreated shed snake skin (2.9 ± 1.48 $ng.cm^{-2}.hr^{-1}$) was significantly different from the flux across untreated samples (Table I). The additional enhancement of the iontophoretic steady state flux by dodecyl-azone in case of shed snake skin contributed a factor 3.

DISCUSSION

Resistance of DGAVP-flux Across Human Stratum Corneum Versus Shed Snake Skin

The resistances of human stratum corneum and shed snake skin were in the same order of magnitude, which is surprising at first sight because human stratum corneum is penetrated by appendageal structures such as hair follicles, sweat and sebaceous glands. Hence, apparently small charge carriers such as Na^+ and Cl^- predominantly travel along pathways, which cross the stratum corneum and are situated away from the appendages. The contribution of appendageal shunts to the overall transport of small ions therefore appears to be low. This suggests that the intrinsic permeability of the appendages for small ions is not high enough to compensate for their relatively small surface area contribution compared to stratum corneum (21).

The resistances of both human stratum corneum and shed snake skin decrease rapidly during iontophoresis, indicating that the skin barrier for ions decreases in both species. Thermo-electrical analysis, X-ray diffraction and electron microscopic studies of human stratum corneum have shown, that the transport barrier is located within the intercellular lipids and that application of electrical currents of the same current density as used in this study probably results in a decrease in the longitudinal lamellar and lateral ordering of

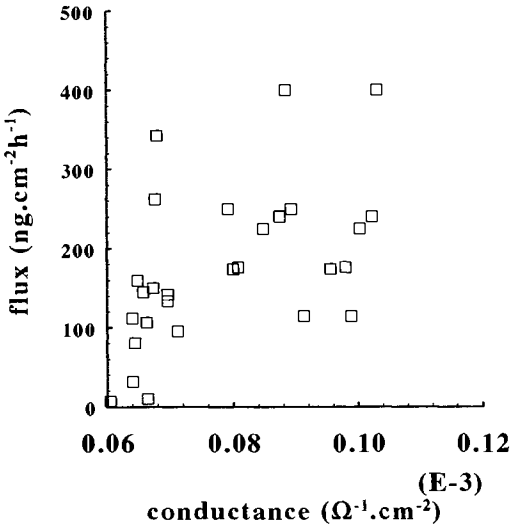

Fig. 5. Iontophoretic DGAVP flux versus human stratum corneum conductance. The iontophoretic DGAVP flux conductance values of human stratum corneum samples, which were not pretreated with dodecyl-azone, were plotted versus the iontophoretic DGAVP-flux within the same time interval.

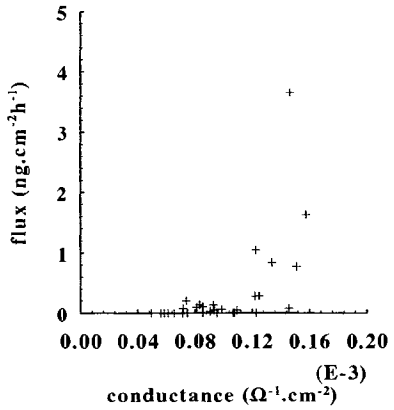

Fig. 6. Iontophoretic DGAVP flux versus shed snake skin conductance. Data represent the conductance values of one shed snake skin sample, which was not pretreated with dodecyl-azone, and the iontophoretic DGAVP-flux within the same time interval.

the intercellular lipids (22–27). This lipid perturbing effect of the electrical field most likely explains the observed resistance decline, and once more underscores the likelihood that small ions travel predominantly through the intercellular domains of stratum corneum, rather than through appendageal channels.

The iontophoretic flux of DGAVP across human stratum corneum is, however, about two orders of magnitude larger than across shed snake skin, despite the fact that the resistances of human stratum corneum and shed snake skin are in the same order of magnitude. This suggests that the iontophoretic transport of a relatively large peptide such as DGAVP across human stratum corneum and shed snake skin occurs along different sets of routes: In the case of snake skin, the peptide travels along pathways, which go across the stratum corneum, possibly the intercellular route; in the case of human skin, the bulk of the peptide most likely follows appendageal channels, with only a small contribution from pathways across the stratum corneum. This is supported by the fourfold difference between the observed lag times of iontophoretic DGAVP-transport across human and snake skin.

For human skin, no relationship was found between the skin conductances and the iontophoretic DGAVP-flux, whereas for shed snake skin a good rank correlation between these parameters was found, indicating that the iontophoretic permeability for DGAVP of human skin (in contrast to shed snake skin) is not directly related to its conductance. The variability in the conductances of the different human samples can therefore not explain the variability in the fluxes. Because the conductance is almost completely determined by the lipids within the stratum corneum (22) and because this study has shown that the presence of appendages is of minor influence on the conductance, the variability within the DGAVP-fluxes across human stratum corneum might be rooted in the appendageal shunts.

Effects of Dodecyl-Azone on Skin Resistance and Iontophoretic DGAVP-Flux

The resistances of both human stratum corneum and shed snake skin were found to decrease as a result of the treatment with dodecyl-azone. Electron microscopic and X-ray diffraction studies have shown that the penetration enhancing effect of dodecyl-azone resulted from an increase of the lateral lipid disordering within the intercellular lipid bilayers of the stratum corneum (15–17). The decreasing effect of dodecyl-azone on the skin resistance in both species is yet another indication that the electrical barrier is mainly situated in the intercellular lipid domains, confirming observations in earlier studies (22–27).

The effect of dodecyl-azone on the steady state flux and lag time of DGAVP-iontophoresis across human stratum corneum was rather small. This suggests that the intercellular lipid pathway contributes very little to iontophoretic DGAVP transport across human stratum corneum. On the other hand, the effect of dodecyl-azone on the low-flux kinetics of DGAVP-iontophoresis across shed snake skin was much more pronounced than for human stratum corneum. The Azone pretreatment of shed skin resulted in a 3-fold decrease of the lag time of DGAVP-iontophoresis and a 3-fold increase of the steady state DGAVP flux. Hence, the Azone pretreatment of snake skin leads to an increased electrophoretic mobility of the peptide (26), which strongly suggests that iontophoresis across snake skin drives the peptide through intercellular pathways. This difference between human stratum corneum and shed snake skin with respect to the effect of dodecyl-azone on the transdermal iontophoretic flux of DGAVP indicates that the iontophoretic transport through human stratum corneum *in vitro* predominantly occurs through appendageal shunts. Surprisingly, pretreatment of human stratum corneum with dodecyl-azone has been found to significantly enhance steady state passive DGAVP diffusion by a factor 3-4 (16). This observation has suggested that during passive diffusion DGAVP follows intercellular routes, whereas according to the present results, during iontophoresis it predominantly follows the appendageal routes.

CONCLUSIONS

DGAVP is transported iontophoretically across shed snake skin despite the absence of hair follicles, sweat and sebaceous glands. The DGAVP fluxes during iontophoresis, however, were much larger across human stratum corneum than across shed snake skin and the lag times were much shorter. These differences might be explained if iontophoretic transport of the peptide across human stratum corneum primarily follows the appendageal routes. Support for appendageal transport across human stratum corneum is also provided by the observed effect of skin pretreatment with the lipid perturbing agent dodecyl-azone, which decreased the lag time and increased the steady state flux of DGAVP-iontophoresis across shed snake skin, whereas it had no influence on the flux and lag time in the case of human stratum corneum.

Interestingly, the initial resistances of human stratum corneum and shed snake skin were of the same order of magnitude, which indicates that the transport pathways for small ions run directly across the stratum corneum and are of non-appendageal origin, being most likely the intercellular lipid domains. This conclusion is also supported by the resistance decreasing effect of the lipid-perturbing agent dodecyl-azone. In contrast to shed snake skin, in human skin no relationship between conductance and iontophoretic DGAVP-flux was observed, suggesting that the variability in peptide permeability of the *in vitro* human skin model is not caused by a variability of the overall skin conductance, but by a variability in density or permeability of structures of appendageal origin.

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