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Research Article

Iontophoretic Delivery of a Series of Tripeptides Across the Skin *in Vitro*

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The iontophoresis of eight tripeptides, of the general structure alanine–X–alanine, has been measured across hairless mouse skin *in vitro*. The peptides were blocked (a) at the carboxyl terminus using the mixed anhydride reaction with t-butylamine and (b) at the amino terminus by acetylation with ^{14}C-acetic anhydride. The nature of the central residue (X) was varied by selecting one of five neutral amino acids, two negatively chargeable moieties (aspartic and glutamic acids), and a positively chargeable species (histidine). Constant current iontophoresis at 0.36 mA/cm^2, using Ag/AgCl electrodes, was performed for 24 hr in diffusion cells, which allowed both anode and cathode to be situated on the same (epidermal) side of a single piece of skin. Due to a combination of osmotic and electroosmotic forces, the anodal iontophoretic flux of neutral peptides was significantly greater than passive transport. Steady-state fluxes were not achieved, however, suggesting that time-dependent changes in the properties of the skin barrier may be occurring. Limited, further experiments confirmed that, on a 24-hr time scale, these changes were not fully reversible. The cathodal delivery of anionic permeants was well controlled at a steady and highly enhanced rate by the current flow. This behavior closely paralleled earlier work using simple negatively charged amino acids and N-acetylated amino acid derivatives. It appears that the normalized iontophoretic flux of these anionic species is independent of lipophilicity but may be inversely related to molecular weight. The positively charged peptide, Ac–Ala–His–Ala–NH(Bu^t), showed greater anodal iontophoretic enhancement when delivered from a donor solution at pH 4.0 than from a solution at pH 7.4. This was consistent with (a) the corresponding behavior of histidine alone and (b) the existence of a pK_a for these compounds at ~6. Steady-state delivery was not achieved, although the levels of enhancement, especially at pH 4, were the largest observed. A preliminary investigation of tripeptide stability to either (i) electrolysis in the donor compartment or (ii) cutaneous metabolism revealed very little degradation under the conditions of the experiment. Overall, this research supports the principle of enhanced peptide delivery across the skin by iontophoresis and indicates a number of areas (e.g., mechanism and extent of current-induced changes in skin barrier function, molecular size dependence, pathways of current flow) on which further work should be focused.

KEY WORDS: iontophoresis; transdermal drug delivery; peptide delivery; percutaneous penetration enhancement; skin barrier function.

INTRODUCTION

The success of nitroglycerin transdermal dosage forms has driven attempts to exploit the potential of drug delivery across the skin (1). However, despite the advantages of the transdermal route, there are major obstacles to successful percutaneous drug administration (2). The greatest is the fact that the skin is an excellent barrier to the inward passive transport of topically applied drugs; consequently, achieving an adequate input rate of the therapeutic agent from a reasonably sized delivery system is frequently impossible (3).

Recognition of the skin's formidable barrier function has focused attention upon methods by which the membrane's resistance may be safely and reversibly undermined. A large body of literature now exists on a range of chemical penetration enhancers (2,4), vehicle components which enable increased drug flux through mechanisms distinct from a simple thermodynamic effect (i.e., increasing the chemical potential of the drug in the formulation). Current interest refocuses on iontophoresis (the facilitation of drug delivery by a transmembrane electrical potential) (5,6). While the current-enhanced membrane permeation of charged species has been known for over 100 years, adequate characterization of the phenomenon with respect to the skin remains at an early

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stage (6). Nevertheless, the large enhancement possible, especially for compounds that have little or no passive permeation, and the (theoretical) control provided by the level of current flow offer the promise of great flexibility and broad applicability.

One area of intense activity involves the evaluation of iontophoresis for the transdermal enhancement of peptides and small proteins (7,8). Indeed, there have been reports that molecules as large as insulin can be successfully delivered in this way (9–11). The "new drugs" of the biotechnology industry are an important target for effective delivery strategies; given that chronic injections are undesirable, and that the oral route is precluded (because of drug degradation), alternative input modalities are needed. In the preceding paper, we described the iontophoretic flux enhancement of amino acids and amino acid derivatives across hairless mouse skin *in vitro* (12). Here, we extend that work to the examination of a series of tripeptides of the general formula Ac–Ala–X–Ala–NH(Bu^t), in which the carboxy and amino termini are blocked and the central residue (X) is varied to provide a range of penetrants of different lipophilicity and charge. The experiments are designed, therefore, to complement the initial research, which examined the "building blocks" of protein molecules, and to extend the systematic elucidation of structure–permeation enhancement relationships in iontophoretic drug delivery.

MATERIALS AND METHODS

Model Peptide Permeants

The penetrants studied in this work were a series of eight tripeptides of the general formula Ala–X–Ala (see Table I). The amino terminus of each peptide was acetylated with acetic anhydride; the carboxy terminus was blocked using the mixed anhydride reaction with *t*-butylamine. Consequently, the central amino acid residue (X) was the principal determinant of the charge and relative lipophilicity of the permeant. The synthesis, radiolabeling, and purification of the peptides, and the determination of their octanol/aqueous buffer (pH 7.3) distribution coefficients, were recently described (13).

Table I. Structure and Properties of Tripeptides Studied: CH_3–CO–Ala–(X)–Ala–NH–$C(CH_3)_3$

(X)	Molecular weight (da)	log D^a
Neutral peptides		
Gly	314	−0.60
Ala	328	−0.51
His[b]	394	−0.48
Pro	354	−0.39
Phe	404	1.01
Trp	443	1.26
Anionic peptides[b]		
Asp	372	−3.20
Glu	386	−2.77

[a] Octanol/pH 7.3 TES-buffer (2 m*M* TES, 150 m*M* NaCl) distribution coefficient (13).

[b] Ac–Ala–His–Ala–NH(Bu^t) is predominantly uncharged, and Ac–Ala–Asp–Ala–NH(Bu^t) and Ac–Ala–Glu–Ala–NH(Bu^t) are predominantly negatively charged, at pH 7.3.

Iontophoretic Flux Determination

The basic experimental design paralleled that followed in our earlier study using amino acids and amino acid derivatives (12).

Diffusion Cells. Glass cells (Laboratory Glass Apparatus, Berkeley, CA) reported by Glikfeld *et al.* (14) were employed. The three-chambered donor compartment permitted positioning of both anode and cathode on the same (epidermal) side of a single piece of skin. The electrodes were AgCl-coated Ag wires (15).

Donor Solutions. Neutral and positively charged peptides were iontophoresed from the anode compartment; negatively charged peptides were delivered from the cathode. The electrolyte, in which the peptides were dissolved and which surrounded the "nondriving" electrode, was pH 7.4 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (25 m*M*), buffered saline (133 m*M*). The peptide solutions were prepared by evaporating to dryness (under a stream of N_2) an ethanolic solution containing 1–2 μCi of freshly purified, ^{14}C-labeled tripeptide (5 mCi/mmol), which was then reconstituted in about 6 cm^3 of aqueous buffer.

Receptor Solution. The receiver compartment (volume, 7 cm^3) of the diffusion cell was perfused at 2.7 cm^3/hr with pH 7.4 HEPES-buffered saline. Samples were collected, typically, every 3 hr.

Skin. Full-thickness skin, excised and used immediately postsacrifice, from female hairless mice [aged 9–12 weeks, CRL:SKHl(hr/hr) BR, Charles River, Wilmington, MA].

Procedures. The cells were assembled and the dermal side of the skin was perfused for 1 hr at 10 cm^3/hr. Then 1 cm^3 of peptide solution was added to the "driving" electrode chamber of the donor compartment and 1 cm^3 of electrolyte was introduced into the other electrode chamber. The power supply was set to provide a constant current between the electrodes of 0.36 mA/cm^2 over a 24-hr period. Samples of the receptor phase perfusate were collected appropriately and were analyzed for permeated peptide by liquid scintillation counting. The transport of peptide, as a function of time, was expressed in terms of a normalized flux, defined as the amount penetrating per unit time per unit area divided by the initial concentration of drug in the donor phase. For each peptide, parallel, "passive" experiments were also performed in which all conditions were identical except that no current flowed. Also, for the histidine analogue [Ac–Ala–His–Ala–NH(Bu^t)], experiments (both with and without current) were carried out using donor solutions at pH 7.4 and at pH 4.0 (the latter achieved by titrating the HEPES-buffered saline with hydrochloric acid). In all cases six replicates of flux measurements were performed.

Metabolism and Degradation of Peptides

During iontophoresis, peptide breakdown via electrolysis in the donor chamber, or through cutaneous metabolism, can be envisaged. Experiments were therefore undertaken, using thin-layer chromatography (TLC), to determine whether (and to what extent) these reactions were occurring.

The donor phase was examined prior to current passage (t = 0 hr) and after 24 hr of iontophoresis (t = 24 hr). Small aliquots (50 μl) were applied directly to a glass two-phase silica gel TLC plate (250-μm thickness, LKS DF, linear K, Whatman, Maidstone, Kent, UK). These plates were chosen because of their demonstrated usefulness for chromatography in the presence of high salt concentrations. The stationary phase of the plate consists of two components: the origin is a preadsorbent, diatomaceous earth layer; the remainder of the plate is silica gel. The donor phase solution was loaded onto the center of the preadsorbent layer and dried under N_2. Chromatography of the radioactivity was then performed using the appropriate development solvent (Table II). The inorganic salts in the applied sample are strongly bound to the preadsorbent material, whereas the peptide and other organic material (HEPES, peptide fragments, etc.) is chromatographed up into the silica gel layer. At the end of the chromatography, the plate was dried and the silica gel was divided into segments and counted for ^{14}C-radioactivity by liquid scintillation counting. The R_f values observed were compared with that of the pure peptide and the percentage remaining intact was determined.

TLC analysis of the receptor phase was less straightforward because of the (relatively) low levels of radioactivity involved. Typically, the 8 cm^3 of receptor phase collected during a 3-hr period contained only 1000 cpm. As the maximum loading of a lane on the TLC plate was 200 μl, it was impossible to achieve direct quantification of each sample collected. Consequently, it was necessary to combine all the perfusate (acquired over 24 hr) from three diffusion cells and then concentrate the cumulative radioactivity. The procedure involved was as follows. The receptor phases were placed in a 500-cm^3, silanized, round-bottomed flask and frozen by immersion in liquid nitrogen. The resulting material was then freeze-dried overnight at −60°C, and 100 mTorr, in a Unitrap II freeze-drier (Virtis, Gardiner, NY). Ethanol (5 cm^3) was added to the fully lyophilized sample to redissolve the radiolabelled peptide (and other organic fragments) but with minimal dissolution of the crystalline inorganic salts. The ethanolic solution was centrifuged; the supernatant was removed, filtered, and evaporated, under a stream of N_2, to less than 500 μl. Aliquots of this solution were then applied directly to the TLC plates and chromatography was performed as before using the appropriate development solvent (Table II). Once again, the R_f values were compared to previously measured standards to determine the percentage of intact tripeptide.

RESULTS

Anodic iontophoresis of the neutral tripeptides caused significant enhancement over the passive flux. Figure 1, for example, compares the iontophoretic transport of the Ac–Ala–Gly–Ala–NH(Bu^t) with the passive flow. For all of the uncharged peptides, the iontophoretic flux increased with time and failed to reach a steady state. This behavior paralleled closely that seen previously for neutral permeants both in our laboratory (12) and elsewhere (16,17).

To compare the relative iontophoretic enhancement of the uncharged peptides, the normalized flux values after 12 hr of current flow were arbitrarily chosen. This also allowed comparison with the corresponding data for the zwitterionic amino acids (12). Normalized flux was preferred over enhancement ratio (flux with current/passive flux) for this comparison because the donor phase concentrations of the permeants in the two studies were so different. In addition, for some peptides, the passive flux was at or below the level of detection, precluding, therefore, the determination of an enhancement ratio. In Fig. 2, the normalized fluxes of the neutral peptides, and of the zwitterionic amino acids from our earlier study (12), are plotted as a function of log D [where D is the octanol/pH 7.3 *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer (150 m*M* NaCl) distribution coefficient]. There is a weak inverse correlation between normalized flux and log D.

In Fig. 3, the effect of cathodal iontophoresis on the percutaneous flux of a negatively charged peptide is illustrated for Ac–Ala–Glu–Ala–NH(Bu^t). Within 3 hr, an elevated and sustained delivery rate was achieved. No appreciable passive flux was measurable. The results for Ac–Ala–Asp–Ala–NH(Bu^t) were comparable, and a steady-state permeability coefficient of 10.2 (±1.6) × 10^{-4} cm/hr was

Table II. Thin-Layer Chromatography and Stability of Tripeptides [Ac–Ala–X–Ala–NH(Bu^t)]

Tripeptide (–X–)	Mobile phase[a]	R_f value	Percentage of total radioactivity recovered as intact peptide[b]		
			Donor (t = 0 hr)	Donor (t = 24 hr)	Receptor (t = 24 hr)
Gly	3:1:0	0.85	98.0	98.0	88.0
Ala	4.5:1:0	0.75	98.2	97.4	87.4
His	4.5:1:0	0.4	97.9	96.9	85.0
His[c]	4.5:1:0	0.4	97.9[c]	95.2[c]	91.1[c]
Pro	4.5:1:0	0.4	96.2	95.1	78.8
Phe	9:1:0	0.7	98.0	97.9	84.2
Trp	9:1:0	0.6	96.9	97.6	81.0
Asp	15:5:0.3	0.4	99.1	96.9	91.4
Glu	15:5:0.3	0.4	94.1	93.2	86.2

[a] Chloroform:methanol:acetic acid.
[b] SD of all values typically ≤5% (n = 2).
[c] Values obtained for histidine experiments in which the peptide donor phase pH was 4.0.

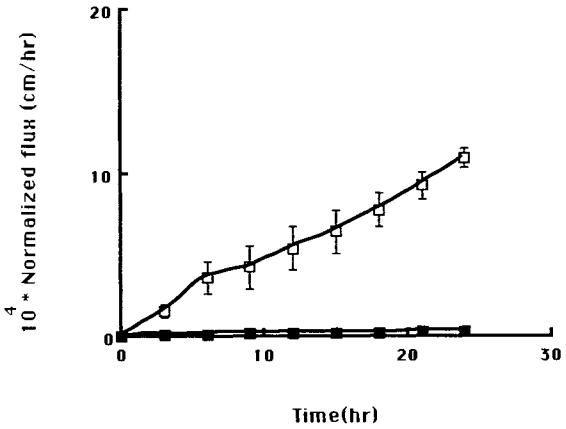

Fig. 1. Normalized flux of the Ac–Ala–Gly–Ala–NH(Bu^t), as a function of time, across hairless mouse skin *in vitro* (a) passively (filled squares) and (b) with anodal iontophoresis (open squares). Each data point is the mean (±SD) of six determinations.

observed in this case. When the data acquired here were combined with the iontophoretic behavior of (a) negatively charged amino acids and (b) negatively charged N-acetylated amino acids from our recent work (12), it was found that the normalized flux values were not significantly correlated with log *D*. A possible dependence upon molecular weight (Fig. 4), however, can be observed.

The iontophoretic enhancement of the positively chargeable peptide of this study [Ac–Ala–His–Ala–NH(Bu^t)] was again considerable (see Fig. 5a). As expected, the flux was greater when the peptide was delivered from a donor solution at pH 4 [where the histidine residue is most likely to carry a charge of +1 (18)] than from a vehicle at pH 7.4. The results mirror rather closely the comparable experiments for histidine itself (Fig. 5b). Unlike the negatively charged peptides, however, Ac–Ala–His–Ala–NH(Bu^t) does not attain a steady-state enhanced permeation rate; instead, the flux continues to increase with time of current passage.

The latter phenomenon might be interpreted in terms of current-induced changes in skin barrier properties. To ex-

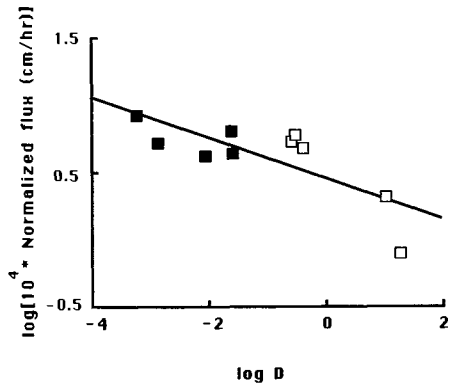

Fig. 2. Normalized flux, after 12 hr of anodal iontophoresis, for zwitterionic amino acids (filled squares) (12) and neutral tripeptides (open squares) plotted as a function of octanol/aqueous electrolyte distribution coefficient (*D*). Each data point is the mean (±SD) of six determinations. Linear regression through the results yields log [10^4 · normalized flux (cm/hr)] = 0.45 − 0.15 (log *D*), with r^2 = 0.59.

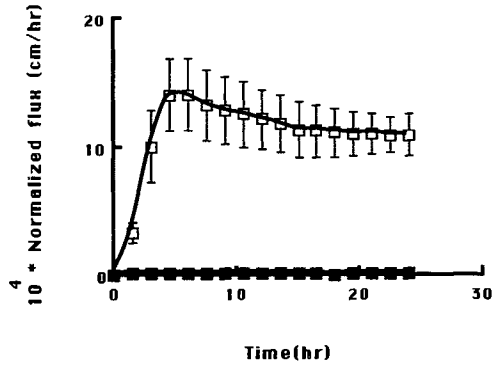

Fig. 3. Normalized flux of Ac–Ala–Glu–Ala–NH(Bu^t), as a function of time, across hairless mouse skin *in vitro* (a) passively (filled squares) and (b) with cathodal iontophoresis (open squares). Each data point is the mean (±SD) of five determinations.

amine this possibility, experiments involving interrupted current delivery were performed. Initially, the neutral peptide Ac–Ala–Gly–Ala–NH(Bu^t) was iontophoresed for 24 hr as before; the current was then terminated and the subsequent flux of the peptide was monitored for a further 18 hr. The results (Fig. 6) show that permeant transport did not return to the no-current (control) baseline level. Indeed, the flux appears to plateau at a significantly elevated level. We then modified the protocol and measured the effect of current interruption on the negatively charged Ac–Ala–Asp–Ala–NH(Bu^t). In this case, current flowed for only 9 hr before termination; this shorter duration reduced the exposure of the skin to current passage and shortened the time that the skin remained sandwiched between aqueous donor and receiver solutions [conditions that can lead to severe barrier compromization (19)]. Figure 7 indicates that the 9-hr period of iontophoresis again led to the attainment of steady-

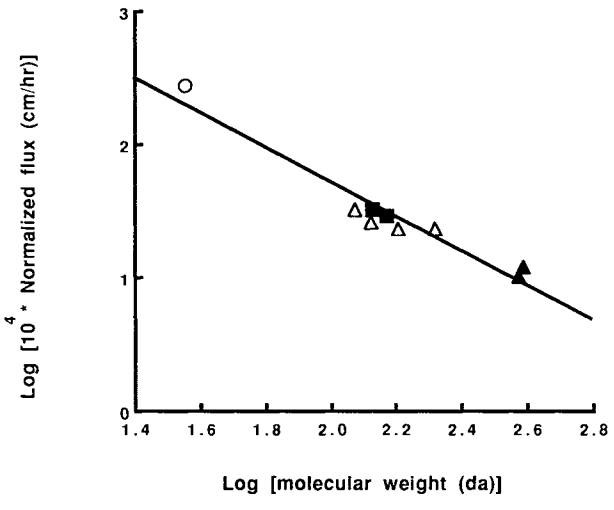

Fig. 4. Normalized (steady-state) flux, during cathodal iontophoresis, for anionic amino acids (filled squares) (12), negatively charged N-acetylated amino acid derivatives (open triangles) (12), anionic tripeptides (filled triangles), and chloride ion (open circle) (18) plotted as a function of molecular weight (MW, da). Each data point is the mean (±SD) of six determinations. Linear regression through the results yields log[10^4 · normalized flux(cm/hr)] = 4.30 − 1.30 (log MW), with r^2 = 0.94.

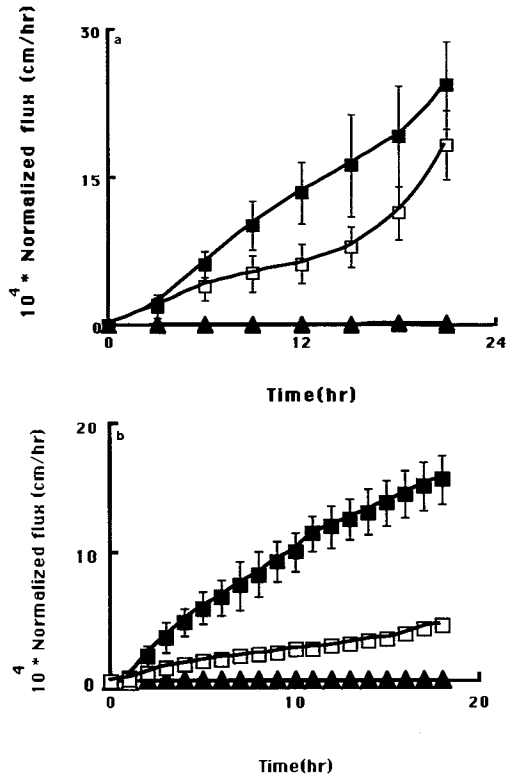

Fig. 5. (a) Normalized flux of Ac–Ala–His–Ala–NH(Bu^t), as a function of time, across hairless mouse skin *in vitro* (i) with anodal iontophoresis from a donor solution at pH 4 (filled squares), (ii) with anodal iontophoresis from a donor solution at pH 7.4 (open squares), and (iii) passively from donor solutions at either pH 4 or pH 7.4 (filled triangles). Each data point is the mean (±SD) of six determinations. (b) Corresponding normalized flux data for histidine (12).

state peptide delivery. Termination of current flow caused a rapid and considerable reduction in flux, but not (by 24 hr) to baseline levels.

DISCUSSION

The enhanced transport of neutral permeants by iontophoresis (Figs. 1 and 2) has been reported in several studies (16,17,20–22). The explanation for the phenomenon is generally given in terms of osmotic and electroosmotic arguments (6). In the experiments utilizing the neutral peptides, current in the iontophoretic circuit is carried primarily by Na^+ and Cl^- ions (6,16). The inferred permselectivity of the skin (to anions) is supported by the fact that the transport number of Na^+ across the membrane is approximately twice that of Cl^- (6,16). In the experimental configuration used in our work, therefore, the preferential use of Na^+ to carry the current leads to a net increase in the total number of ions in the cathode chamber of the donor compartment of the diffusion cell and a concurrent decrease in the anode chamber. There is, in consequence, an osmotic flow of water from the anode to the cathode chamber and a convective movement of dissolved solutes (i.e., the peptide). The electrochemical sequence of events is as follows: Na^+ is repelled from the anode and enters the skin, leaving a charge imbalance in the solution surrounding the positive electrode. This is resolved

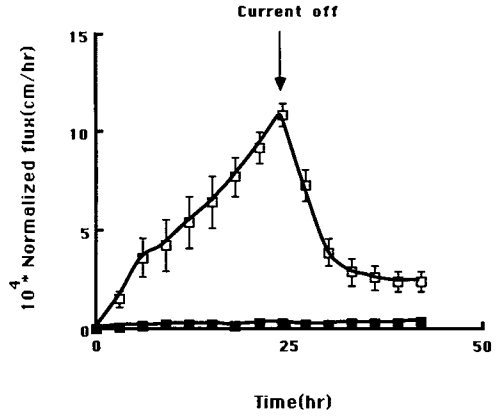

Fig. 6. Normalized flux of Ac–Ala–Gly–Ala–NH(Bu^t) across hairless mouse skin *in vitro*. Anodal iontophoresis was terminated at t = 24 hr. Each data point is the mean (±SD) of six determinations.

by Cl^-, which is, of course, attracted to the anode, combining with Ag° to form solid AgCl, and releasing an electron to the electrical circuit:

$$Ag°(s) + Cl^-(aq) \rightarrow AgCl(s) + e^-$$

In the cathodal compartment, the negative electrode attracts Na^+ through the skin into the receptor phase. Again, a charge imbalance is created, which is then resolved by dissociation of AgCl(s) from the electrode using an electron from the circuit:

$$AgCl(s) + e^- \rightarrow Ag°(s) + Cl^-(aq)$$

Thus, the net transfer of one $Na^+(aq)$ and one $Cl^-(aq)$ from anode chamber to cathode chamber is effected. While the reverse movement of Cl^- balances, to a certain extent, the above process, the permselectivity of the skin to Na^+ (by a factor of about 2:1) dictates the *net* flow of ions as stated (and the net osmotic flow of solvent in the same direction). Superimposed on this osmotic effect is an electroosmotic force, which has its roots in irreversible thermodynamics (6). Simplistically, when a system is subject to a force, it responds (or recovers) via a flow. For example, a concentra-

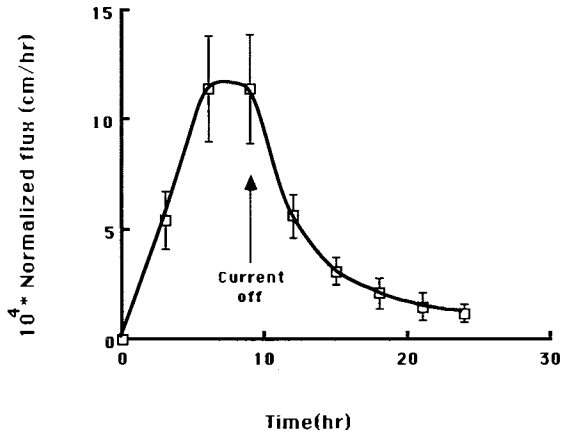

Fig. 7. Normalized flux of Ac–Ala–Asp–Ala–NH(Bu^t) across hairless mouse skin *in vitro*. Cathodal iontophoresis was terminated at t = 9 hr. Each data point is the mean (±SD) of six determinations.

tion gradient represents a nonequilibrium situation that imposes a force (i.e., the thermodynamic need to equalize solute activities) which leads to a diffusive flux. In ideal circumstances, the flows induced by simultaneously applied forces are additive. Hence, ions will flow in response to both a potential gradient and a concentration gradient according to the Nernst–Planck equation (23). Furthermore, the flow of one solute in a system affects the flow of a second. Therefore, the movement of ions in response to a field imposes a force on the passive flow of an uncharged solute also present in the solution. In the context of our experiments with neutral peptides, the movement of Na^+ and Cl^- provides an additional force (so-called electroosmosis) over and above that caused by the peptide concentration gradient. Again, because of the skin's permselectivity, the net effect is in the direction of anionic movement, and this explains why the peptides were associated with the positive electrode solution.

Although it has been shown by others (16,24) (under conditions similar to those used here) that the flows of Na^+ and Cl^- reach, and are maintained at, steady-state levels, the flux of neutral peptide (Fig. 1) continues to increase as the time of iontophoresis is prolonged. Possible explanations for this phenomenon were postulated in our preceding paper describing amino acid iontophoresis (12), where comparable behavior was observed. The results in Fig. 2 do not appear to support the hypothesis that iontophoresis is dramatically altering the so-called "lipid pathways" across the stratum corneum. It should be noted that, despite the huge range of penetrant log *D* (more than 4 orders of magnitude), normalized flux changes by only slightly more than a factor of 10 (Fig. 2). The enhancement effects become slightly attenuated as the permeant lipophilicity increases.

In Figs. 6 and 7, we note that termination of current flow does not result in either an immediate or a complete return to passive transport levels. An alteration in skin barrier function due to current flow is therefore implicated. However, it should be recognized that iontophoresis leads to elevated permeant concentrations in the skin and that an extended period of desorption from the membrane is to be expected (25). Nevertheless, one would ultimately anticipate (if no irreversible barrier changes took place) a return to the control (no-current) flux.

The results for the two negatively charged peptides (exemplified by Fig. 3) show that the passage of current quickly establishes and sustains a steady-state delivery rate of peptide across the skin. The findings are in good general agreement with our earlier observations using smaller negatively charged species (12). Furthermore, the small, but clear, "tailing-off" of the flux with increasing time is consistent with theoretical predictions based upon the flows of Cl^- and Na^+ ions into and out of the cathodal chamber during the experiment (12). No dependence of normalized anion flux on log *D* could be demonstrated (although, admittedly, a much smaller range of lipophilicity has so far been examined); however, a possible inverse dependence on molecular weight may be suggested (Fig. 4). If it is true that the paths of iontophoretic current flow are "pores" through the stratum corneum, as has been suggested in the literature (e.g., 6,7,26–28), then it is reasonable to expect that a size dependency will be operative. While Fig. 4 does not prove such a dependency, it is consistent with the possibility. Furthermore, the iontophoretic flux of chloride ions appears to be consistent with the molecular weight trend which we have observed. In Fig. 4, we have included a data point for Cl^-, which has been deduced from the published work of Burnette and Ongpipattanakul (16). The normalized flux was calculated for the current used in our experiments by linear extrapolation of the previously reported dependence of Cl^- flux upon current (16). However, one should recognize that further work with larger permeants is clearly needed in order to validate the true existence of this perceived trend.

The behavior of Ac–Ala–His–Ala–NH(Bu^t) (Fig. 5a) and of histidine itself (Fig. 5b), while qualitatively comprehensible, is more difficult to analyze at a quantitative level. Simplistically, at least, the enhanced anodic transport at pH 4, as compared to that at pH 7.4, meets expectations associated with charge; namely, at pH 4, Ac–Ala–His–Ala–NH(Bu^t) and His carry predominantly a net charge of +1, while at pH 7.4 they are (again, predominantly) neutral species (18). Further, at pH 7.4, these positively chargeable molecules behave in a manner quite consistent with their neutral counterparts (i.e., their iontophoretic flux increases with increasing duration of current passage). At pH 4, considerable enhancement of the transport of the cationic molecules is measured. The fluxes do not attain steady state but increase progressively with time of iontophoresis. As we demonstrated in the preceding paper (12) using the penetration of lysine at pH 7.4, the continually increasing flux profile is predictable on the basis of Na^+ depletion in the donor chamber and a concomitant increase in the transference number of the cationic permeant. However, such a quantitative analysis is not possible for Ac–Ala–His–Ala–NH(Bu^t) and His at pH 4 because experimental measurements of Na^+ and Cl^- mobilities are not available under these conditions. In addition, the situation is further complicated by the H^+ activity gradient across the membrane. Another unknown is the degree to which Ac–Ala–His–Ala–NH(Bu^t) and His (when positively charged) bind to the fixed negative charges within the skin (6). Clearly, anodal iontophoresis of cationic drug species requires systematic investigation, under carefully controlled conditions of pH and ionic strength, to be completely understood.

Finally, the data in Table II indicate that a relatively small amount of peptide degradation occurred during the course of the iontophoretic experiments. Significant electrolysis in the donor phase was not measurable, and there was only a small degree of breakdown detected in the accumulated receptor phases. It remains to be seen whether these findings are borne out either under more stringent *in vitro* conditions or *in vivo*.

In summary, this study has shown that iontophoresis can be used to enhance the transdermal delivery of tripeptides. Significant enhancement can be achieved for neutral, anionic, and cationic species. Generally speaking, the augmentation of flux is independent of the permeant lipophilicity; on the other hand, the enhanced flux profiles are very sensitive to penetrant charge and size. The research reported leaves open a number of questions (including the importance of skin metabolism in peptide delivery and the identification of ionic transport pathways across the skin) that form the focus of ongoing research.

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