Pharmaceutical Research, Vol. 11, No. 7, 1994

Research Article

Characterization of Convective Solvent Flow During Iontophoresis

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Received January 7 1994; accepted February 24, 1994

During iontophoresis under neutral pH conditions, there is a net convective flow of volume (electroosmosis) from anode to cathode leading to the enhanced transport of dissolved polar (but uncharged) solutes in the same direction. The objective of this study was to address the following unresolved questions with respect to electroosmotic transport: [1] Whether the efficiency of electroosmotic transport is solute size-dependent and, if so, how severe is this dependence? [2] Is electroosmosis linearly related to current density in the same way that the iontophoretic flux of charged species appears to be? [3] Are positively charged permeants able to influence their own electrotransport across the skin (by modifying the net charge on the membrane and altering, as a result, the permselectivity) and, if so, why and to what extent? Electroosmosis was assessed from the iontophoreically driven fluxes of mannitol, sucrose and lactose across hairless mouse skin *in vitro*. It was found that:- (a) The electroosmotic transport rate of mannitol is similar to that of the disaccharides, sucrose and lactose, when examined under identical conditions. The dependence of electroosmotic flux upon molecular size requires study of solutes having a wider range of MW than those considered here. (b) Electroosmotic flow from anode-to-cathode increases with applied current density; similarly, convective flow in the opposite direction diminishes with increasing current density. Apparently, there is correlation between the net movement of solvent and the total flux of ions across the skin. (c) The permselectivity of skin can be 'neutralized' by driving, iontophoretically, a cationic, lipophilic peptide (specifically the leutinizing hormone releasing hormone (LHRH) analog, Nafarelin) into the membrane. The apparently tight association of the peptide with the fixed, negatively-charged sites in the skin reduces significantly, in a concentration-dependent fashion, the anode-to-cathode electroosmotic flow across the barrier. Peptide lipophilicity appears to be necessary for this effect to be seen: the parent peptide, LHRH, does not exhibit this phenomenon.

KEY WORDS: iontophoresis; electroosmosis; convective solvent flow; transdermal peptide delivery; mannitol; LHRH analogs; Nafarelin

INTRODUCTION

The skin is a permselective membrane that, at physiological pH, supports a net negative charge (1). This characteristic means that a positively charged ion can more easily cross the membrane than a comparably-sized anion. Furthermore, the ion-exchange nature of the barrier confers an additional major mechanism of enhanced solute transport across the skin by iontophoresis. Increased flow of ions following the establishment of an electrical potential gradient across the skin can be expected to result because of electrostatic repulsion: cations will be driven from the anode, anions from the cathode. All things being equal, cationic flux will be greater than anionic flux due to the electrostatic potential barrier resulting from the net negative charge on the skin. However, it is well-known that polar, but neutral, compounds can also be delivered at elevated rates by iontophoresis. This electroosmotic effect predominates in the anode to cathode direction for the following reason: Because current is preferentially carried by cations (the skin having a net negative charge), more momentum is transferred to the solvent by these mobile species than by the counter-anions. Thus, there is a net convective flow of volume (electroosmosis) from anode to cathode and, consequently, there will be enhanced transport of dissolved polar (but uncharged) solutes in the same direction.

Numerous studies have investigated the characteristics of electroosmotic flow and the literature contains much exemplary work which has both quantified the phenomenon and explored its theoretical underpinnings (2,3,4). Nevertheless, several unresolved questions remain, and our overall objective here was to consider these issues. Specifically, in a recently published study (5), we reported two intriguing find-

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0724-8741/94/0700-0929$07.00/0

ings:- First, when mannitol was used as a neutral polar solute, its equivalent electroosmotic flux across the skin was only ~50% of that of tritiated water under identical conditions. The question arises, therefore, whether the efficiency of electroosmotic transport is solute size-dependent and, if so, how severe is this dependence? Relatedly, is electroosmosis linearly related to current density in the same way that the iontophoretic flux of charged species appears to be? Secondly, lowering the pH (from 7.4 to 4) of the electrolyte bathing the skin, across which electrotransport was measured, caused the permselectivity of the membrane to change completely. It appeared that the net negative charge could be effectively neutralized by the significantly higher concentration of hydronium ions. Earlier reports in the literature (6) have suggested that a positively charged peptide (namely, the leutinizing hormone releasing hormone (LHRH) analog, leuprolide) may also be able to "cancel out" the skin's negative charge and alter electroosmotic flow. Results that we had obtained with Nafarelin (another LHRH analog) (7) led us to suspect parallel behavior with this peptide. Thus, we asked the additional question whether positively charged permeants are able to influence their own electrotransport across the skin and, if so, why and to what extent?

MATERIALS AND METHODS

Chemicals: N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), D-mannitol, sucrose, lactose, leutinizing hormone releasing hormone (LHRH), histidine, arginine and tyrosine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Nafarelin was a gift from Syntex Research (Palo Alto, CA). ^{14}C-Mannitol (specific activity = 55mCi/mmol; purity 98.2%), ^{14}C-sucrose (530 mCi/mmol; purity 99%), and ^{14}C-lactose (54 mCi/mmol; purity 99.5%) were obtained from NEN Research Products (Wilmington, DE). All other chemicals were analytical grade and were used without further purification. Deionized water (resistivity 18 megohm-cm) was used to prepare all solutions.

Skin: The tissue used was full-thickness skin from 8–10 week old hairless mice (HRS/hr hr, Simonsen Laboratories, Gilroy, CA). The mice were sacrificed by CO_2 asphyxiation, and their skin removed and used immediately. Typically, one mouse provided sufficient skin for two diffusion cells (a 'test' and a control).

Iontophoretic apparatus: Silver-silver chloride electrodes, prepared as previously described (8), were used in all iontophoretic experiments. Constant current was delivered to the electrodes by a custom-built power supply (Professional Design and Development Services, Berkeley, CA) interfaced to a Macintosh IIfx computer (Apple Computers, Inc., Cupertino, CA) running Labview software (National Instruments Inc., Austin, TX). Vertical, flow-through diffusion cells, as previously described (9), in which a single piece of horizontally-mounted skin separated both anodal and cathodal chambers (which were physically and electrically isolated from one another) on the epidermal side of the skin from the receptor phase. The latter (volume = 6 mL) was continuously perfused using a peristaltic pump (Manostat, New York, NY) with an appropriate buffer at 3 mL/hr, and hourly samples were collected on a fraction collector (Isco, Inc., Lincoln, NE) before analysis. In the experiments involving peptides (see below), the electrodes were separated from the donor and receptor chambers by salt bridges, which consisted of 1 M NaCl gelled with 3% agarose.

Experiments: The following sets of experiments were performed:-

1. Following the procedures of Kim *et al.* (5), the electroosmotic transport of mannitol, sucrose and lactose was determined. The flow-through diffusion cells were set up with pH 7.4 buffer (25 mM HEPES containing 133 mM NaCl), degassed by vacuum filtration through a 0.45 μm Millipore filter, allowed to bathe both the epidermal and dermal sides of the skin for a 2-hour equilibration period. At the end of this time, either the anodal or cathodal buffer solution was replaced by a 1 mM solution of the appropriate sugar, spiked with the ^{14}C-labeled solute to yield a final radioactivity level of ~1 μCi/cell. A constant current of either 0.42 or 0.55 mA/cm^2 was then delivered to the Ag/AgCl electrodes for 12 hours, followed by a further 12-hour period of post-iontophoretic passive delivery. Hourly samples of the receiver solution were collected and were analyzed for ^{14}C-radiolabel: the 3 mL samples were mixed with 10 mL of scintillation fluid (Ready Gel, Beckman Instruments, Irvine, CA) and radioactivity was then determined in a liquid scintillation counter. The measured dpm were transformed into molar flux, taking into account the appropriate correction necessary for flow rate and receiver phase volume (10).
2. To examine the effect of current density on electroosmotic transport, the protocol described above was repeated using mannitol as the marker solute at four current densities: 0.14, 0.28, 0.42 and 0.55 mA/cm^2. All other aspects of the procedure were identical.
3. We have explored whether positively-charged permeants (which may be expected to be transported by electroosmosis to a progressively larger extent as their molecular size increases (2,4)) can influence their own electrotransport kinetics by interaction with the net negative charge on the skin. To achieve this aim, the electroosmotic flux of mannitol (from either the anodal or cathodal chambers) was measured when the anodal chamber contained either (a) Nafarelin at 0.3 or 1.0 mg/mL, (b), LHRH, at 1.0 mg/mL, or (c) a mixture of histidine, arginine and tyrosine, the positively chargeable residues in LHRH, at concentrations (0.7 mM) equivalent to their representation within the peptide dissolved at 1 mg/mL. Measurements with Nafarelin were made at 0.55 mA/cm^2. To ensure maximal stability of the peptides during the 12-hour iontophoretic period, the background electrolyte in these experiments consisted of 0.17 M phosphate buffer containing 77 mM NaCl. All other aspects of the protocol were the same as those outlined above for experimental series #1. Control experiments (that is, electroosmosis in the absence of peptides or amino acids) were performed under identical conditions (with the same buffer and using salt bridges).

General: The corresponding passive, no-current, controls were conducted in all aspects of the work. Experiments were performed at least in triplicate. Statistical comparisons between datasets used ANOVA and the Scheffe test, and unpaired t-tests, as appropriate.

RESULTS

In the first series of experiments, the electroosmotic fluxes of mannitol, sucrose and lactose were determined and compared to their passive controls. Figure 1 illustrates typical profiles, which were observed for all three solutes, for the anodal and cathodal delivery of sucrose at 0.55 mA/cm^2. Passive diffusion of mannitol resulted in levels of radioactivity in the receptor chamber that were not significantly different from background. Electrotransport from the anode was considerable during the 12 hours of iontophoresis. Following termination of current, the anodal flux decreased to a final post-iontophoretic plateau that was clearly greater than the no-current control. Cathodal electrotransport was slightly above background during iontophoresis; however, solute transport from the cathodal chamber *increased* measurably *after* current termination, ultimately achieving (at 24 hours) a final flux that was indistinguishable from that, at the same time point, from the anodal compartment. Table I summarizes the experimental data for the three solutes. The Table includes (a) the solute flux (for both anodal and cathodal delivery) at 12 hours when the current was terminated, (b) the corresponding fluxes at 24 hours (i.e., after a further period of 12 hours without current passage), and (c) the differences between the transport rates at 12 hours and at 24 hours. These latter, "corrected 12-hr" fluxes reflect the actual electroosmotic transport rates produced by the passage of current, adjusted for the impact of current flow on the passive permeability properties of the membrane (5).

The effect of current density on the electroosmotic transport of mannitol was examined in the second series of experiments. The results for anodal and cathodal delivery are summarized, respectively, in Figures 2a and 2b, which show, as a function of applied current density, (a) mannitol flux at 12 hours when the current was terminated, (b) mannitol flux at 24 hours (i.e., after a further 12-hour period without current), and (c) the "corrected 12-hr" fluxes (as defined above).

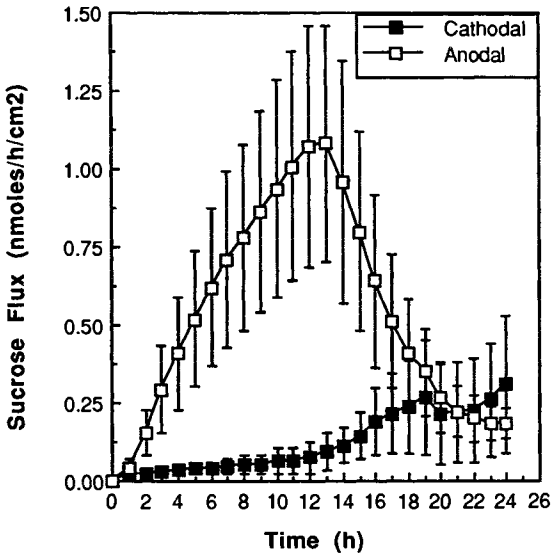

Figure 1: Molar flux of sucrose across hairless mouse skin (HMS) during, and after, 12 hours of iontophoresis at 0.55 mA/cm^2. Anodal (open squares) and cathodal (closed squares) transport profiles are shown. Each data point represents the mean (± SD) of at least 3 determinations.

The effect of positively-charged permeants on the electroosmotic transport of mannitol (the third series of experiments) is presented in Table II. Again, 12-hour, 24-hour and "corrected 12-hour" fluxes have been determined. Figure 3 shows the effect of two concentrations of Nafarelin on the anodal transport of mannitol, as a function of time during a 12-hour period of iontophoresis. Nafarelin clearly inhibits electroosmosis, with the effect being significantly greater at the higher concentration of peptide used. Indeed, even with the limited amount of data collected, there appears to be an inverse relationship between anodal electroosmotic transport of mannitol and the concentration of Nafarelin initially present in the anodal chamber (Figure 4). As expected, given the design of the iontophoretic diffusion cell employed, the presence of Nafarelin in the anodal chamber did not affect the electrotransport of mannitol from the cathode (Table II). Finally, the impact of another peptide, LHRH, and of the positively-chargeable amino acid residues from Nafarelin and LHRH, was examined. The results are summarized in Table II and Figure 5. Neither LHRH, nor the combination of amino acids significantly reduced the anodal electroosmosis of mannitol. It should be noted that, in all of the third series of experiments, a salt bridge was used to link the anodal chamber of the iontophoretic diffusion cell to an electrolyte solution in which the Ag/AgCl electrode was immersed. Although some leakage of NaCl from the salt bridge (where it was present at 1M) into the anodal chamber undoubtedly occurred, the changing ionic strength of this solution cannot account for the observed effect of Nafarelin on the electroosmotic transport of mannitol. Identical leakage would also have occurred in the 'control' experiments (i.e., those without Nafarelin, and those in the presence of either LHRH or the combination of amino acids), in which no effect on the electroosmosis of mannitol was found.

DISCUSSION

Figure 1 and Table I show that the electroosmotic transport behaviors of mannitol, sucrose and lactose are similar (and are consistent with our previous data for mannitol alone (5)). Anodal delivery was significantly higher than cathodal, in a manner expected from the permselective properties of the skin. Analysis of variance shows that the (absolute and "corrected") anodal fluxes of the three sugars, at the end of the 12-hour period of iontophoresis, were indistinguishable (regardless of whether the applied current density was 0.55 or 0.42 mA/cm^2). As we had observed for mannitol in an earlier study (5), the passive permeability of the skin is increased by the passage of current. The final, 24-hour, absolute fluxes show that this perturbation of barrier function is independent of the polarity of the electrode chamber used in the 12-hour iontophoretic period. The "corrected 12-hour" fluxes (iontophoretic flux at 12 hours *minus* passive flux 12 hours after termination of current) provide, therefore, a more relevant indication of true electroosmotic flow. Table I indicates that, while the "corrected" anodal fluxes are positive as expected, the "corrected" cathodal rates are, on average, negative. We confirm, therefore, that the net volume flow during iontophoresis at pH 7.4 is in the anode-to-

Table I. Electroosmotic Flux of Mannitol, Sucrose and Lactose.

Solute	Current Density (mA/cm^2)	Flux (nmol/cm^2/hr)[a] Anodal 12-hr[b]	Cathodal 12-hr[c]	Passive Anodal 24-hr[d]	Passive Cathodal 24-hr[e]	Corrected Anodal 12-hr[f]	Corrected Cathodal 12-hr[g]
Mannitol	0.55	1.17 ± 0.34	0.07 ± 0.05	0.36 ± 0.09	0.31 ± 0.10	0.81 ± 0.34	−0.24 ± 0.09
Sucrose	0.55	1.07 ± 0.39	0.08 ± 0.05	0.18 ± 0.05	0.32 ± 0.22	0.89 ± 0.03	−0.24 ± 0.23
Mannitol	0.42	1.06 ± 0.19	0.11 ± 0.01	0.26 ± 0.01	0.25 ± 0.06	0.80 ± 0.18	−0.15 ± 0.05
Lactose	0.42	1.39 ± 0.63	0.01 ± 0.02	0.20 ± 0.10	0.25 ± 0.11	1.18 ± 0.53	−0.23 ± 0.12

a Mean ± standard deviation of at least three separate determinations.
b Anodal flux at 12 hours of iontophoresis.
c Cathodal flux at 12 hours of iontophoresis.
d Passive flux 12 hours after termination of anodal iontophoresis.
e Passive flux 12 hours after termination of cathodal iontophoresis.
f Anodal iontophoretic flux at 12 hours minus passive flux 12 hours after termination of current.
g Cathodal iontophoretic flux at 12 hours minus passive flux 12 hours after termination of current.

cathode direction. From these data, however, we are not able to deduce whether the electrotransport of neutral solutes by electroosmosis is dependent upon molecular weight. Clearly, the results in Table I reveal no significant differences in the enhanced fluxes of mannitol, sucrose and lactose; i.e., increasing molecular weight by a factor of two (mannitol *versus* sucrose and lactose) had no impact on the efficiency of electroosmotic enhancement. Information in the literature suggests that we are probably unable to detect a difference because of the relatively small increment in size between mono-and di-saccharides; Kim *et al.* (5), for example, found that water moves more efficiently than mannitol, and other examples (from experiments in which much wider ranges of molecular size were considered) have been reviewed by Yoshida and Roberts (11).

Figures 2a and 2b show the effect of applied current density on the electroosmosis of mannitol from the anode and cathode, respectively. Analysis of variance reveals that there is a significant effect ($\alpha < 0.05$) of current density on electrotransport. Considering first the anodal data (Figure 2a), it can be seen that the "corrected 12-hour" flux is linearly correlated (correlation coefficient, r = 0.83) with applied current density. As one might expect, the electroosmotic flow is coupled with the increased flow of ions which takes place as the current density is raised. To what extent further increments in electroosmotic flow can be achieved by increasing the current density further is not known at this time. Whether such information is practically useful may be questioned, of course, given that current densities in excess of ~0.5 mA/cm^2 are typically regarded as inappropriate for

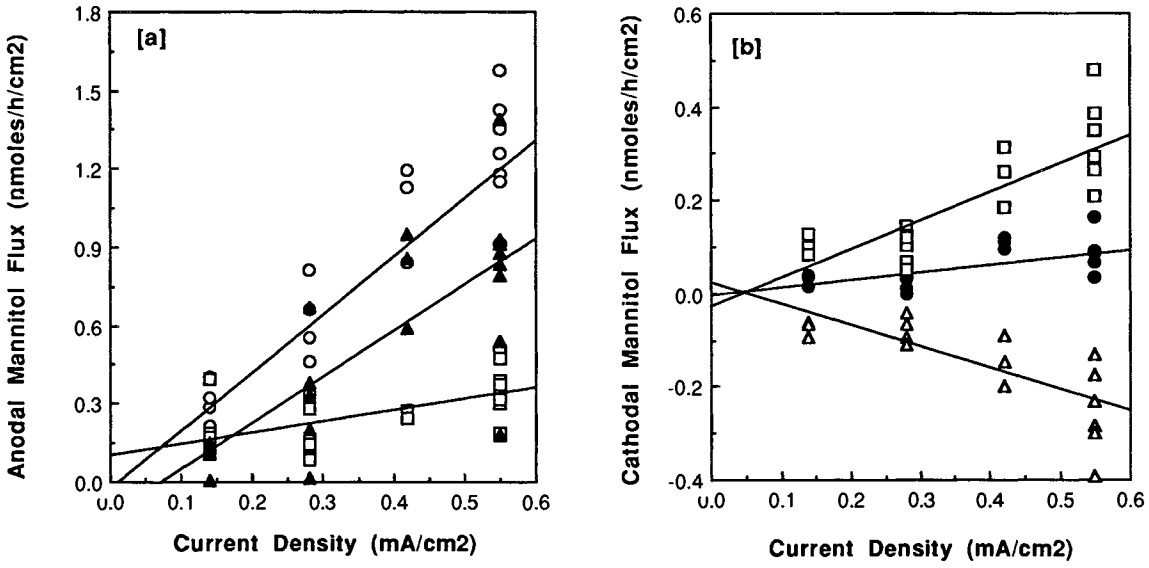

Figure 2: [a] Flux of mannitol as a function of current density applied during 12 hours of *anodal* iontophoresis: (i) absolute flux at the end of the 12-hour period of current flow (open circles); (ii) passive flux 12 hours after termination of iontophoresis (open squares); (iii) "corrected 12-hour flux", i.e., iontophoretic flux at 12 hours *minus* passive flux 12 hours after termination of current (closed triangles). Lines of least-squares linear regression are drawn through the data. [b] Flux of mannitol as a function of current density applied during 12 hours of *cathodal* iontophoresis: (i) absolute flux at the end of the 12-hour period of current flow (closed circles); (ii) passive flux 12 hours after termination of iontophoresis (open squares); (iii) "corrected 12-hour flux", i.e., iontophoretic flux at 12 hours *minus* passive flux 12 hours after termination of current (open triangles). Lines of least-squares linear regression are drawn through the data.

Table II. Modulation of the Electroosmotic Flux of Mannitol by the Presence of Nafarelin, LHRH and Positively-Chargeable Amino Acids.

Positively-Charged Species at Anode	Current Density (mA/cm^2)	Flux (nmol/cm^2/hr)[a]					
		Anodal 12-hr[b]	Cathodal 12-hr[c]	Anodal 24-hr[d]	Cathodal 24-hr[e]	Corrected Anodal 12-hr[f]	Corrected Cathodal 12-hr[g]
Control	0.55	2.39 ± 0.43	0.12 ± 0.05	0.49 ± 0.06	0.30 ± 0.01	1.90 ± 0.45	−0.18 ± 0.06
Nafarelin [0.3 mg/mL]	0.55	1.35 ± 0.50	0.06 ± 0.04	0.86 ± 0.15	0.54 ± 0.16	0.49 ± 0.38	−0.48 ± 0.12
Nafarelin [1.0 mg/mL]	0.55	0.40 ± 0.06	0.14 ± 0.04	1.73 ± 0.67	0.29 ± 0.08	−1.33 ± 0.67	−0.15 ± 0.10
LHRH [1.0 mg/mL]	0.55	1.55 ± 0.67	0.04 ± 0.02	0.28 ± 0.10	0.14 ± 0.03	1.27 ± 0.58	−0.10 ± 0.02
Amino acids [0.7 mM][h]	0.55	2.10 ± 0.61	ND[i]	0.48 ± 0.22	ND	1.62 ± 0.53	ND[i]

a Mean ± standard deviation of at least three separate determinations.
b Anodal flux at 12 hours of iontophoresis.
c Cathodal flux at 12 hours of iontophoresis.
d Passive flux 12 hours after termination of anodal iontophoresis.
e Passive flux 12 hours after termination of cathodal iontophoresis.
f Anodal iontophoretic flux at 12 hours minus passive flux 12 hours after termination of current.
g Cathodal iontophoretic flux at 12 hours minus passive flux 12 hours after termination of current.
h The amino acids employed were histidine, tyrosine and arginine.
i ND = not determined.

human, *in vivo* use (12). We also note from Figure 2a (and, for that matter, Figure 2b as well) that the post-iontophoretic flux increases with the magnitude of the current density applied during the 12-hour period of iontophoresis (analysis of variance, $\alpha < 0.05$ and 0.01 for anodal and cathodal delivery, respectively). Given that we have demonstrated, in this *in vitro* model, that iontophoresis perturbs the passive permeability barrier of the membrane, it is not too surprising that this effect becomes more pronounced as the level of applied current intensity increases. Consistent with the anodal data, the "corrected 12-hour" fluxes of mannitol from the cathode become increasingly negative with increasing applied current density ($r = -0.76$) (Figure 2b).

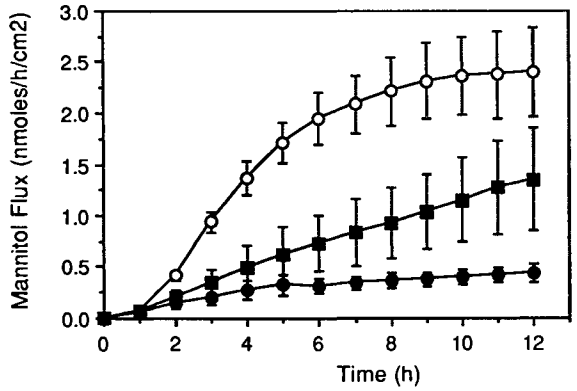

Figure 3: Molar flux of mannitol across HMS during 12 hours of anodal iontophoresis at 0.55 mA/cm^2. The transport profiles shown are: (i) "control" iontophoresis (open circles); (ii) iontophoresis in the presence of Nafarelin in the donor, anodal chamber at 0.3 mg/mL (closed squares); and (iii) iontophoresis in the presence of Nafarelin in the donor, anodal chamber at 1.0 mg/mL (closed circles). Each data point represents the mean (± SD) of at least 3 determinations.

The dramatic effect of the presence of Nafarelin, in the anodal chamber of the iontophoretic cell, on the electroosmotic transport of mannitol is shown in Figure 3 and Table II. Nafarelin clearly inhibits electroosmotic volume flow in a concentration-dependent fashion (see Figure 4). Nafarelin, at pH 7.4, is a positively charged decapeptide. Logically, therefore, if one intended to deliver Nafarelin transdermally using iontophoresis (13), one would associate the peptide with the anodal chamber. While Nafarelin, under these circumstances, may be expected to carry a fraction of the current flowing, the data available in the literature (4) suggests

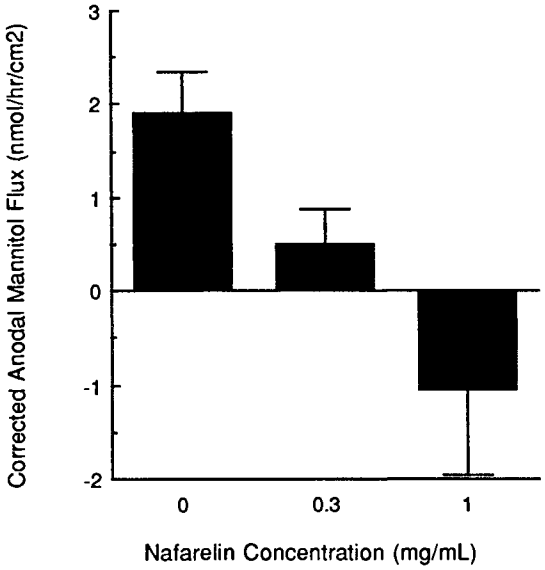

Figure 4: "Corrected" electroosmotic flux of mannitol across HMS, after 12 hours of iontophoresis at 0.55 mA/cm^2, as a function of initial Nafarelin concentration in the donor, anodal chamber. Each data point represents the mean (± SD) of at least 3 determinations.

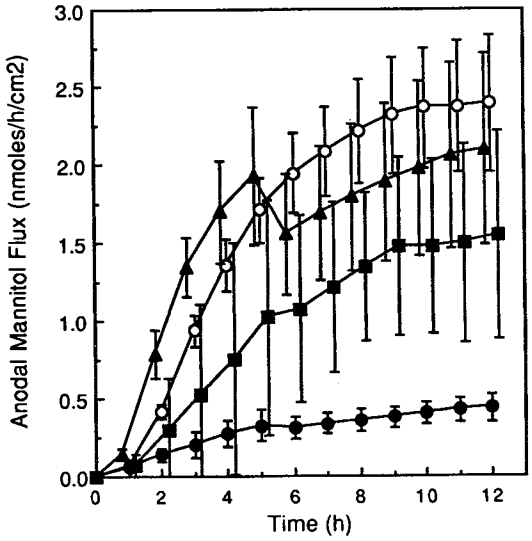

Figure 5: Molar flux of mannitol across HMS during 12 hours of anodal iontophoresis at 0.55 mA/cm^2. The transport profiles shown are: (i) "control" iontophoresis (open circles); (ii) iontophoresis in the presence of LHRH in the donor, anodal chamber at 1.0 mg/mL (closed squares); (iii) iontophoresis in the presence of Nafarelin in the donor, anodal chamber at 1.0 mg/mL (closed circles); and (iv) iontophoresis in the presence of the amino acids histidine, tyrosine, and arginine in the donor, anodal chamber at 0.7 mM (closed triangles). Each data point represents the mean (± SD) of at least 3 determinations. For the sake of clarity, the datapoints for the LHRH and amino acids experiments are off-set by +0.2hr and −0.2hr, respectively.

that electroosmosis is a predominant mechanism of transport for molecules of significant molecular size (i.e., moieties that may be characterized as larger than typical 'small' [MW < 300 daltons] drugs). Our interest in the effect of Nafarelin on electroosmotic flow was stimulated by the fact that the iontophoretic delivery of the peptide becomes less efficient with increasing initial concentration in the anodal donor chamber (14). To investigate the origin of this paradoxical finding, we decided, therefore, to determine the impact of the peptide upon electroosmotic volume flow.

The data in Table II (and Figures 3 and 4) indicate that the iontophoresis of Nafarelin into the skin causes a reversal of electroosmotic flow. The average "corrected 12-hour anodal flux" of mannitol in the absence of Nafarelin is +1.90 nmol/cm^2/hr; with 1 mg/mL peptide initially in the donor compartment, the flux falls (and reverses to the cathode-to-anode direction) to −1.33 nmol/cm^2/hr. At a lower initial concentration of Nafarelin, the effect is smaller but still significant (see Table II). As expected, the presence of Nafarelin (at either 0.3 or 1.0 mg/mL) in the anodal chamber causes no significant change in the "corrected 12-hour cathodal flux" of mannitol (Table II). However, with the higher concentration of peptide, the "corrected 12-hour" anodal flux of mannitol is significantly less (unpaired t-test, $p < 0.05$) than the cathodal flux. This implies the Nafarelin has entered the skin and has effectively neutralized the net negative charge on the membrane, which has lost, therefore, its permselectivity to cations. The later deduction is consistent with (a) the hypothesis that Nafarelin electrotransport is primarily mediated via electroosmosis (rather than a direct electrorepulsive effect), and (b) the observation that, as the applied concentration of peptide is increased, the net flux of the compound decreases (presumably because peptide driven into the skin neutralizes the negative charge on the membrane, leading to a decrease in electroosmotic flow, which results in diminished flux of the drug); consequently, as the amount of drug available to be driven into the skin is increased, the extent of permselectivity neutralization also increases, thereby progressively eliminating the anode-to-cathode electroosmotic flow, and peptide transport becomes smaller. A similar phenomenon has been reported for a different LHRH analog, namely leuprolide (6); in this *in vitro* study, peptide transport was studied at constant voltage. In addition, Lu *et al.* (15), using the same peptide, found, *in vivo*, in man, that iontophoretic delivery was less efficient at higher concentrations of the applied drug. Similarly, Miller *et al.* (16) reported that a 400-fold increase in the concentration of [DTrp^6, Pro^9-NHEt]LHRH resulted in less than an order of magnitude improvement in anodal iontophoretic delivery. It is likely that the explanation articulated above accounts for these other observations.

To assess the generality of the phenomenon reported here, we next examined whether the parent peptide, LHRH, inhibits electroosmotic flow in the same way. Figure 5 and the data in Table II reveal that, while the average "corrected 12-hour anodal" flux of mannitol in the presence of LHRH (1.27 nmol/cm^2/hr) is less than that in the control experiments without peptide (1.90 nmol/cm^2/hr), the difference is not significant. The D-naphthylalanine substitution for glycine at position 6 in LHRH, that results in the formation of Nafarelin, would appear to be crucial, therefore, to the manifestation of the electroosmosis-reversal effect observed. The chemical modification renders the peptide significantly more lipophilic than LHRH (17), of course, and arguably contributes to its apparent ability to associate itself tenaciously with the fixed negative sites in the current-conducting pathways through the skin. The same argument can be invoked to explain the previously published results for leuprolide (6,15), which has a (relatively lipophilic) leucine residue in place of the parent peptide's glycine at position 6 (18). The need for enhanced lipophilicity was further demonstrated in our iontophoretic experiments, in which the impact of the positively-chargeable residues in Nafarelin (introduced into the anodal chamber as the simple amino acids at the appropriate molar concentration to coincide with their presence in the peptide) on the electroosmosis of mannitol was examined. Clearly, the results in Figure 5 and Table II demonstrate that the simple amino acids themselves had absolutely no effect on mannitol flux.

In conclusion, the investigation described in this paper has demonstrated that: (a) Electroosmotic flow across mammalian skin at pH 7.4, in the presence of an applied potential gradient, is in the anode-to-cathode direction, consistent with a net negative charge on the membrane and permselectivity to cationic species (3,5). The transport rate of mannitol is similar to that of the disaccharides, sucrose and lactose, when examined under identical conditions. The dependence of electroosmotic flux upon molecular size requires study of solutes having a wider range of MW than those considered here. (b) Electroosmotic flow from anode-to-cathode increases with applied current density; similarly, convective

flow in the opposite direction diminishes with increasing current density. As anticipated, therefore, there is correlation between the net movement of solvent and the total flux of ions across the skin. (c) The permselectivity of skin can be 'neutralized' by driving, iontophoretically, a cationic lipophilic peptide (specifically, Nafarelin) into the membrane. The apparently tight association of the peptide with the fixed, negatively-charged sites in the skin reduces significantly, in a concentration-dependent fashion, the anode-to-cathode electroosmotic flow across the barrier. Peptide lipophilicity appears to be an important criterion for this behavior to be manifest: while the parent peptide, LHRH, does not exhibit this phenomenon, another relatively lipophilic analog, leuprolide, shows similar characteristics to Nafarelin (6,15). It remains to be seen whether the observed effect can be used to advantage in the design of iontophoretic delivery systems for peptide and other drugs.

ACKNOWLEDGMENTS

Supported by the U.S. National Institutes of Health (HD-27839) and by a Fulbright—Ministerio de Educacion y Ciencia Fellowship to MBDC. Nafarelin was a generous gift from Syntex Research, Palo Alto, CA. We thank our colleagues in the Skin Bioscience Group at UCSF for helpful discussion, and Dr. Aeri Kim at Pharmetrix, Inc. for her continued insight and input.

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