Pharmaceutical Research, Vol. 14, No. 9, 1997 *Research Paper*

Iontophoretic Delivery Across the Skin: Electroosmosis and Its Modulation by Drug Substances

Jouni Hirvonen[1,2] and Richard H. Guy[1,3,4]

Received January 28, 1997; accepted June 4, 1997

Purpose. The long-term objective of this research is to understand how the efficiency of iontophoresis depends upon the structural and physicochemical properties of the administered drug. Specifically, the ability of certain drug species to alter the permselective properties of the skin was examined.

Methods. Using conventional *in vitro* methodology, the inhibition of electroosmotic flow induced by the iontophoresis of five different β-blockers (of varying lipophilicity) was examined. The concomitant electrotransport of the most lipophilic species (propranolol) and the convective movement of solvent in the anode-to-cathode direction were measured. In addition, the possibility that electroosmosis might be augmented by the delivery of anionic drugs was also considered.

Results. Iontophoresis of lipophilic, cationic β-blockers caused a concentration-dependent inhibition of conventional electroosmosis. The most hydrophilic analogs elicited no effect. As a result of this charge neutralization phenomenon, the optimal concentration for propranolol iontophoresis was significantly less than the maximum achievable in aqueous solution. Only a very modest improvement in convective solvent flow was induced by the cathodal iontophoresis of anionic compounds.

Conclusions. The permselectivity of the skin can be altered by drugs which are positively charged and which possess a significant, adjacent hydrophobic surface. The latter seems able to "anchor" the molecule in the skin and the counter charge to the membrane's negative character ensures a tight association. Both lipophilicity and a positive charge are essential—without either, the phenomenon is not observed. The conformational flexibility of the drugs studied to-date, however, prevents unambiguous conclusions about the three-dimensional nature of the putative "binding site".

KEY WORDS: transdermal iontophoresis; electroosmosis; reverse iontophoresis; β-blockers; non-steroidal anti-inflammatory drugs; skin.

INTRODUCTION

The skin is a permselective membrane which, at ~pH 7, supports a net negative charge (1). When an electric field is imposed across the membrane, therefore, under normal conditions, there is a net flow of solvent (so-called electroosmosis) in the direction of counterion (i.e., cation) movement (2). As a result of this phenomenon, iontophoresis has been used to enhance the transdermal transport of not only ionic species, but also that of neutral, polar (and therefore, passively, poorly-permeable) substances (2,3). For example, the development of a noninvasive, reverse iontophoretic monitoring device for blood glucose relies upon the process of electroosmosis (4,5).

However, quite recently, it has been demonstrated unequivocally that the anodal iontophoresis of (in particular) certain lipophilic, cationic peptides can evoke a dramatic effect on the permselective properties of mammalian skin and hence on the extent (and, even, direction) of electroosmotic flow (6,7). The lipophilic leutenizing hormone releasing hormone (LHRH) analogs, Nafarelin and Leuprolide, were the first to be recognized specifically in this respect (6–9). It was found that these hydrophobic cations, when iontophoresed at, or near, neutral pH into mammalian skin, were apparently able to become strongly associated with the net negative charge on the membrane. This strong affinity, attributed to the close juxtaposition of a lipophilic "anchoring" feature (the D-naphthylalanine and leucine residues at positions 6 and 7 in Nafarelin, and the two leucines at the same positions in Leuprolide) to a positively charged group (i.e., arginine at position 8 in both LHRH analogs), was suggested to cause neutralization of the skin's net negative charge and, in consequence, an effective shut-down of electroosmotic flow in the direction anode-to-cathode (6–9). The inhibition of the concomitant transport of ^{14}C-radiolabeled mannitol (a marker for electroosmotic flow) was entirely consistent with the hypothesized action of the peptide analogs. Notably, (a) the parent peptide, LHRH, which lacks a full hydrophobic "anchor" (glycine, leucine at positions 6 and 7), did not change electroosmotic flow (6), and (b) the interruption of electroosmosis by Nafarelin also significantly impeded its own transport (the effect becoming more important as the concentration of the peptide in the 'driving' anodal chamber was increased) (8,9), strongly implicating a central role for electroosmosis in the mechanism of enhanced transdermal delivery of these larger peptides by iontophoresis (6–9).

To further probe this phenomenon, and to more precisely define the structural motif responsible for the inhibition of electroosmosis, we synthesized and investigated a series of oligo-tripeptides based upon the central residues (positions 6, 7 and 8) of LHRH and its lipophilic analogs (10). This study revealed that the central 'cores' of Nafarelin and Leuprolide were themselves sufficient to have an essentially identical effect on solvent flow, and that the complete peptide structures were not necessary. This finding provoked two questions which form the focus of the research described here:—(i) Are there other molecules (in particular, drugs) that are non-peptidic, which can induce the same phenomenon? (ii) Is it possible to provoke the opposite phenomenon; i.e., can additional negative charge be introduced into the skin to *increase* electroosmotic flow? (11). Clearly, the latter would be of considerable benefit with respect to the improved reverse iontophoretic extraction of glucose (4,5). To address the first question, the ability of a series of β-blockers (propranolol, timolol, metoprolol, nadalol and atenolol), spanning a broad range of lipophilicity, to inhibit electroosmosis when iontophoresed into the skin was examined. These molecules are all positively charged (+1) at pH 7.4 (12); the rationale for their selection was based upon the structure of propranolol (13) in which a lipophilic naphthyl moiety is

---

[1] Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, University of California, San Francisco, California 94143-0446.

[2] Current address: Department of Pharmaceutical Technology, University of Kuopio, Kuopio, Finland.

[3] Current address: Centre Interuniversitaire de Recherche et d'Enseignement, "Pharmapeptides", Campus Universitaire, F-74166 Archamps, France.

[4] To whom correspondence should be addressed. (e-mail: rhg@pharma1.cur-archamps.fr)

separated by a short 'spacer' from the positively charged secondary amine portion of the molecule, in a manner that calls to mind the previously implicated oligo-tripeptides (10). To complement the measurements of electroosmosis, the iontophoretic delivery of propranolol itself, as a function of the driving concentration, was carefully studied. To address the second question, the effect of cathodally iontophoresing three example non-steroidal anti-inflammatory drugs (ibuprofen, salicylic acid and piroxicam) on the outward electroosmotic flow of solvent was determined.

MATERIALS AND METHODS

Materials

N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) and sodium chloride were purchased from Fisher Chemical (New Brunswick, NJ). DL-propranolol (-HCl), S-timolol (-maleate), ±-metoprolol (-tartrate), nadolol, and atenolol were from Sigma Chemical Co. (St. Louis, MO), as were ibuprofen and salicylic acid. Piroxicam was a gift from Pfizer Inc. (Groton, CT). D-mannitol, 99+%, was from Aldrich Chemical Co. (Milwaukee, WI). ^{14}C-mannitol (specific activity = 55 mCi/mmol; purity 98.9%) and ^{3}H-propranolol (specific activity = 21.2 Ci/mmol; purity 96.7%) were obtained from NEN Research Products (Wilmington, DE). Silver wire (diameter 1.0 mm, purity 99.9%) and silver chloride (purity > 99%) for electrode preparation were from Aldrich Chemical Co. (Milwaukee, WI). Deionized water (resistivity ≥ 18 MOhm-cm) was used to prepare all solutions.

The skin membrane used was full-thickness tissue from 8–10 week old female hairless mice (HRS/hr hr, Simonsen Laboratories, Gilroy, CA). The mice were sacrificed by CO_2 asphyxiation, and the skin was removed and used immediately. Typically, one mouse provided sufficient skin for two diffusion cells. All animal procedures adhered to the "Principles of Laboratory Animal Care" (NIH publication #85–23, revised 1985) and were approved by the UCSF Committee on Animal Research.

Electrotransport Experiments

General

All transport experiments were performed in vertical flow-through diffusion cells (Laboratory Glass Apparatus Inc., Berkeley, CA) (14), in which a single piece of horizontally-mounted skin separated the anodal and cathodal chambers on the epidermal side of the membrane from the receptor phase which bathed the dermal surface. The current delivery electrodes were silver-silver chloride, prepared in the usual way (15). The power supply used to generate the constant current that was passed between the electrodes was the Phoresorscope® (Iomed Inc., Salt Lake City, UT). Voltage and current were monitored continuously during the experiments. For all measurements, the background electrolyte used in both the electrode chambers and the receptor phase was 25 mM HEPES-buffered normal saline at pH 7.4. Each individual set of measurements was performed in at least quadruplicate. Statistical comparisons between datasets used one-factor (repeat measures) ANOVA and two group paired t-tests.

Anodal Iontophoresis

The first part of the study examined whether electroosmosis was impaired in the normal anode-to-cathode direction by the anodal iontophoresis of various β-blockers of different lipophilicities. Anodal formulations (volume = 1 mL) were therefore prepared containing (a) background electrolyte, (b) a β-blocker at one of several concentrations in the range 0.1–50 mg/mL, and (c) 1 mM mannitol, "spiked" with 1 μCi of the ^{14}C-radiolabeled sugar. The cathodal chamber (1 mL) and the receptor compartment contained background electrolyte alone. Direct current (0.25 mA/cm^2) was delivered to the electrodes for 8 hours. Transport of mannitol across the skin was followed over this period, and for a further 8 hours post-iontophoretically, by collecting (Isco Retriever III fraction collector, Isco Inc., Lincoln, NE) hourly samples of the receptor solution (6 mL) which was perfused at 4 mL/hr using a peristaltic pump (Manostat®, New York, NY). The samples were mixed with 3 mL of scintillation cocktail (Ready Gel, Beckman Instruments, Irvine, CA) and analyzed by liquid scintillation counting (Beckman Instruments Inc., Irvine, CA).

In a subsequent series of experiments, using only the β-blocker propranolol, at initial anodal concentrations of 0.01 to 10 mg/mL, the identical procedures were performed except that, in addition to the ^{14}C-mannitol marker, 1 μCi of ^{3}H-labeled drug was also used so that electrotransport and electroosmosis could be followed simultaneously.

For all iontophoresis experiments, the measured dpm values were transformed into molar flux (nmol·cm^{-2}·h^{-1}) and cumulative transport, taking into account the appropriate correction necessary for flow rate and 'receptor' volume (16). Skin was digested at the end of each experiment (Soluene®, Packard, Downers Grove, IL) and the amount of radiolabel remaining in the tissue was determined. Each set of experiments included the appropriate passive (no current) and no-drug controls.

Reverse Iontophoresis (4,11,17)

To address whether electroosmosis might be enhanced by iontophoretic delivery of anionic drugs into the skin, three non-steroidal anti-inflammatory compounds (NSAIDs), ibuprofen, salicylic acid and piroxicam, were individually formulated as aqueous solutions at concentrations of either 0.1 or 1 mg/mL and placed into the cathodal chamber. The concentration range was limited by the aqueous solubilities of the NSAIDs. In this case, electroosmosis *towards* the cathode was followed by incorporating 5 mM mannitol ("spiked" at 0.4 μCi/mL with ^{14}C-sugar) into the receptor solution. During 4 hours of constant current iontophoresis at 0.25 mA/cm^2 (and for a subsequent 2-hour, no current period), the 'reverse' convective flow was monitored by completely removing the contents of the cathode chamber every 30 minutes for liquid scintillation analysis. During each sampling, the current was briefly interrupted and then restarted once fresh electrolyte had been reintroduced into the cathode compartment. Transport in the 'reverse' direction towards the anode was measured at the same time. Further, in another experiment, the effect of anodal propranolol iontophoresis (from solutions at either 1 or 10 mg/mL) on 'reverse' electroosmosis to the anode chamber was also determined. Again, each set of experiments included the appropriate passive (no current) and no-drug controls.

Table 1. Physicochemical Properties of the Model Drugs Studied

Drug	Molecular weight (da)	log P[a]	pK_a[b]	Charge at pH 7.4
β-blockers				
Propranolol	259	3.21	9.21	+1
Timolol	316	1.91	9.24	+1
Metoprolol	272	1.88	9.24	+1
Nadolol	309	0.93	9.39	+1
Atenolol	266	0.16	9.32	+1
NSAIDs[c]				
Ibuprofen	206	3.51	4.61	−1
Salicylic acid	138	1.50	3.00	−1
Piroxicam	331	0.26	6.30	−1

[a] P = octanol-water partition coefficient (12).
[b] pK_a values from ref. 12.
[c] NSAID = non-steroidal anti-inflammatory drug.

RESULTS

Anodal Iontophoresis

The physicochemical properties of the various drugs used in this work are summarized in Table 1. While all the β-blockers are positively charged at pH 7.4, and of similar molecular size (molecular weights ~300 daltons), they vary in their lipophilicity (as measured by their octanol-water partition coefficients) by about three orders of magnitude.

Figure 1 shows how the anodal iontophoresis of the different β-blockers (when formulated initially at a concentration of 1 mg/mL in the anodal compartment) impacts upon electroosmotic flow when measured in the classic anode-to-cathode direction. It is observed, in general, that the decrease in mannitol transport (i.e., the marker for convective flow) is correlated with β-blocker lipophilicity (Figure 2). The three most lipophilic β-blockers, propranolol, timolol and metoprolol, significantly ($p < 0.01$) decreased mannitol flux compared to the no-drug control. Table 2 presents the effects of the β-blockers on electroosmosis as a function of the different concentrations of the drugs used; a strong concentration dependence for the three most lipophilic species is seen. At the highest concentration used (50 mg/mL), propranolol inhibited mannitol transport by more than 7-fold compared to the control (no β-blocker) value. The two least hydrophobic β-blockers, nadolol and atenolol, did not significantly ($p > 0.05$) decrease electroosmosis at any concentration used.

In the experiments in which the concomitant electrotransport of propranolol (^3H) and mannitol (^{14}C) was followed as a function of initial propranolol concentration in the donor anodal chamber (Figure 3, upper panel), it was found that the flux of β-blocker first increased with increasing driving concentration, but then peaked and fell off with further augmentation of the initial concentration (Table 3). Mannitol flux followed the same pattern as before (Figure 3, lower panel). The propranolol fluxes were used to calculate the drug's effective iontophoretic permeability coefficient (i.e., drug flux measured at the end of 8 hours of current passage divided by the initial drug concentration in the donor, anodal compartment) as a function of concentration (Table 3). It is seen that while, at low concentrations, drug permeability is tremendously augmented relative to the passive value (determined at a propranolol concentration of 10 mg/mL), this enhancement rapidly falls off as the drug driving force

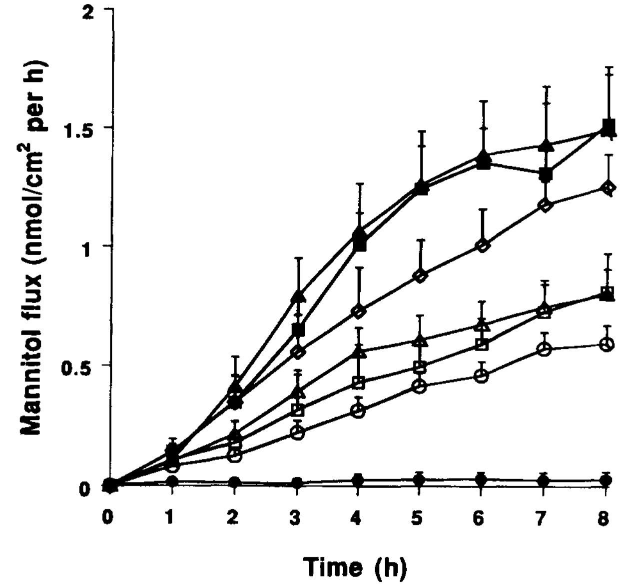

Fig. 1. Effect of β-blockers (at a concentration of 1 mg/mL) on the electroosmotic flux of mannitol during iontophoresis across hairless mouse skin *in vitro*. The effect of propranolol (○) is compared to those of timolol (□), metoprolol (△), nadalol (▲), and atenolol (◇). The passive (●) (no drug, no current) and DC control data (■) (no drug, with current) are also shown. Direct current of 0.25 mA/cm² was on for 8 hours, whereafter passive mannitol flux was monitored for a further 8 hours. Each data point represents the mean (±SD) of 4 experiments.

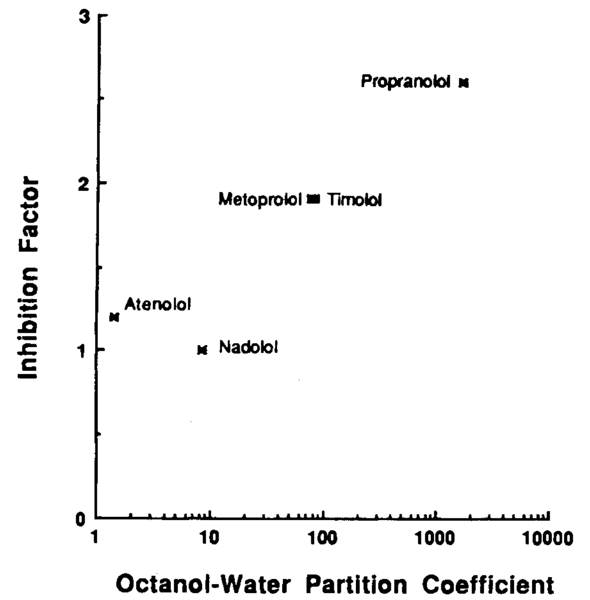

Fig. 2. Inhibition of electroosmosis as a function of β-blocker lipophilicity. The Inhibition Factor is defined as mannitol flux after 8 hours of iontophoresis in the absence of drug divided by the corresponding value when the β-blocker was present (at 1 mg/mL in the donor anodal chamber). Note that the data points for metoprolol and timolol essentially overlap. Linear regression through the data yields $r^2 = 0.88$.

Table 2. Effect of Five β-Blockers on the Electroosmotic Transport of Mannitol During Anodal Iontophoresis (Mean ± sd; n = 4)

Drug	[Drug] (mg/mL)	Mannitol flux[a] (nmol·cm^{-2}·hr^{-1})	Cumulative mannitol[b] transport (μg)	IF[c]
—	*Passive*[d]	0.03 ± 0.02	0.02 ± 0.02	—
—	*Iontophoresis control*[e]	1.52 ± 0.21	0.91 ± 0.11	—
Propranolol	0.1	1.36 ± 0.19	0.67 ± 0.24	1.4
	0.5	0.84 ± 0.14	0.55 ± 0.07	1.8
	1.0	0.59 ± 0.08	0.35 ± 0.05	2.6
	5.0	0.45 ± 0.06	0.27 ± 0.02	3.4
	10	0.24 ± 0.03	0.16 ± 0.02	6.3
	50	0.21 ± 0.05	0.16 ± 0.04	7.2
Timolol	0.5	1.18 ± 0.22	0.76 ± 0.22	1.3
	1.0	0.81 ± 0.16	0.46 ± 0.11	1.9
	10	0.65 ± 0.03	0.42 ± 0.04	2.3
	50	0.36 ± 0.11	0.22 ± 0.05	4.2
Metoprolol	0.5	1.24 ± 0.07	0.73 ± 0.07	1.2
	1.0	0.80 ± 0.11	0.51 ± 0.07	1.9
	10	0.67 ± 0.12	0.44 ± 0.11	2.3
Nadolol	1.0	1.50 ± 0.26	0.98 ± 0.16	1.0
	10	1.61 ± 0.20	1.00 ± 0.07	0.94
Atenolol	0.5	1.54 ± 0.12	0.95 ± 0.04	0.99
	1.0	1.25 ± 0.14	0.75 ± 0.07	1.2
	10	0.91 ± 0.14	0.55 ± 0.11	1.7

[a] Electrotransport rate achieved after 8 hours of constant current iontophoresis.

[b] Cumulative mannitol electrotransport after 8 hours of current passage.

[c] IF = Inhibition Factor: mannitol flux after 8 hours of iontophoresis in the absence of drug divided by the corresponding value when the β-blocker was present.

[d] No-current, no drug control.

[e] No drug, iontophoresis control.

is increased, entirely in line with the changes in electroosmosis deduced from the mannitol results.

Reverse Iontophoresis

As observed previously (11,17), under normal conditions (pH 7.4, no drug present), mannitol extraction to the cathode by electroosmosis is significantly elevated both with respect to passive transport and relative to anodal extraction (Table 4). The introduction of negatively charged drugs (NSAIDs) into the cathode chamber in the iontophoresis experiment did not significantly improve cathodal extraction except for salicylic acid at 1 mg/mL (the highest concentration achievable for any of the drug anions due to solubility limitations) (Table 4). When the most lipophilic β-blocker was placed in the *anode* chamber at either 1 or 10 mg/mL, the inhibition of electroosmosis into the receptor phase permitted a slightly enhanced extraction of mannitol towards the anode chamber (relative to the no-drug control) (Table 4).

DISCUSSION

The results obtained in this study are completely consistent with those from our earlier work (6,8–10) (and from other investigators (7,18)) in which the iontophoresis of lipophilic peptides has been shown to impact upon the permselectivity of the skin. The manner in which propranolol, the most lipophilic of the β-blockers considered, inhibits, in a concentration-dependent fashion (Figure 3), the normal convective solvent flow in the anode-to-cathode direction is completely similar to that seen for (e.g.) the lipophilic LHRH analog, Nafarelin (6,8,9). In addition, the sensitivity of the inhibition of electroosmosis to the lipophilicity of the iontophoresed β-blocker (that is, the effect decreases with decreasing lipophilicity (Figures 1 and 2)) parallels our recent observations with specifically designed oligo-tripeptides (10) which were used in an attempt to more precisely define the structural "motif" responsible for this phenomenon. It is now clear, we believe, (a) that the interruption of electroosmosis is not an effect caused uniquely by peptides, and (b) that the juxtaposition of a positive charge and a lipophilic surface is a key criterion to be satisfied for skin's permselectivity to be perturbed—a positive charge alone, in the absence of adjacent hydrophobicity, does not result in a decrease of convective flow; equally, a neutral, lipophilic species is similarly ineffective (10).

Previously, it has been hypothesized that the strong association of these lipophilic cations with the iontophoretic transport pathway involves the synergy of (i) "anchoring" the compound in the membrane via its lipophilic part, and (ii) the electrostatic interaction between its positive charge and the negative charge of the skin (6,8–10). Certainly, the results described here are

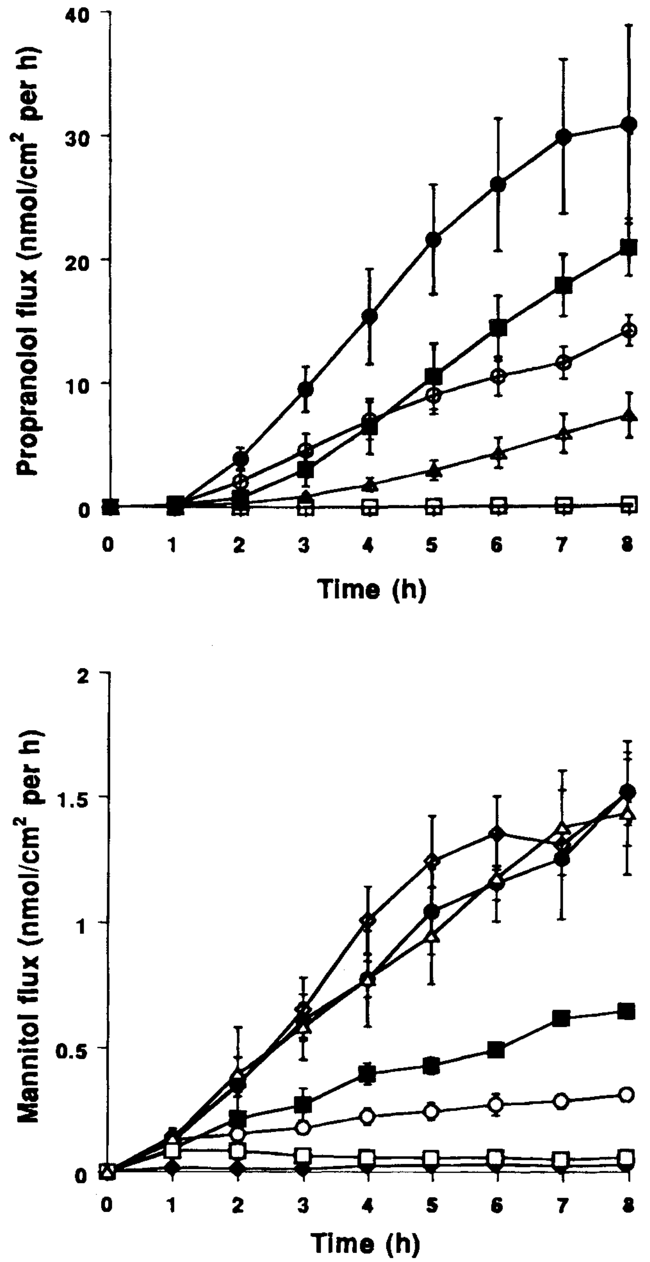

Fig. 3. *Upper panel*—The electrotransport of propranolol during iontophoresis across hairless mouse skin *in vitro*. Direct current of 0.25 mA/cm² was on for 8 hours, whereafter passive propranolol flux was monitored for a further 8 hours. Each data point represents the mean (±SD) of 4 experiments. The 'driving' concentrations of propranolol were 0.01 (△), 0.1 (●), 1 (■) and 10 (○) mg/mL. The passive (□) (no current) control data ([propranolol] = 10 mg/mL) are also shown. *Lower panel*—The concomitant electroosmotic flow (as measured by mannitol flux) during iontophoresis of propranolol at different concentrations (identified by the corresponding symbols as used in the *upper panel* of this figure) across hairless mouse skin *in vitro*. The passive, no-current, no-drug (◆) and no-current, with-drug ([propranolol] = 10 mg/mL) (□) control data are also shown, as are those with-current, but without drug (◇). Again, each data point represents the mean (±SD) of 4 experiments.

Table 3. Passive and Iontophoretic Transdermal Fluxes and Permeability Coefficients of Propranolol as a Function of Initial Donor Concentration (Mean ± sd; n = 4)

[Propranolol] (mg/mL)	Propranolol flux[a] ($\mathrm{nmol \cdot cm^{-2} \cdot hr^{-1}}$)	10^4·Permeability Coefficient (cm/hr)[b]	EF[c]
Passive[d]			
10	0.28 ± 0.08	0.08	—
Iontophoresis			
0.01	7.3 ± 1.8	2160	26
0.1	31 ± 8.2	911	110
1	21 ± 2.3	62	75
10	14 ± 2.6	4.2	51

[a] Electrotransport rate achieved after 8 hours of constant current iontophoresis.

[b] The transport rate achieved after 8 hours divided by the initial concentration of propranolol in the donor compartment.

[c] EF = Enhancement Factor: propranolol flux after 8 hours of iontophoresis divided by the corresponding no-current, control value.

[d] No-current control.

consistent with this idea, and with the fact that, if the positive charge is maintained, then increasing the drug's lipophilicity serves to progressively tighten the association. The optimal spatial arrangement of the lipophilic "anchor" and the positive charge necessary to elicit the skin 'neutralization' effect is not, at this time, deducible because the molecules studied to-date have all possessed sufficient conformational flexibility to render any answer ambiguous. However, further experimentation of the type described here using lipophilic cations of rigid structure are obviously envisaged.

The experiments, in which the concomitant electrotransport of propranolol and electroosmosis of solvent (as measured by mannitol flux) were determined, demonstrate the practical relevance of the phenomena investigated here. When the 'driv-

Table 4. Reverse Iontophoresis: Effect of Iontophoresing Either Negatively Charged NSAIDs from the Cathode, or the Positively Charged Propranolol from the Anode, on the 'Reverse' Electroosmotic Flow of Solvent (Mean ± sd; n = 4)

Drug (Electrode)	[Drug[b] (mg/mL)	Cumulative Mannitol Extracted (nmoles)[a] Anode (*EF*)[b]	Cumulative Mannitol Extracted (nmoles)[a] Cathode (*EF*)[b]
—	*Passive*[c]	1.22 ± 0.52	1.22 ± 0.52
—	*Iontophoresis control*[d]	4.35 ± 0.40	37.4 ± 6.13
Ibuprofen (Cathode)	0.1	3.80 ± 0.52	44.1 ± 5.39 (*1.2*)
Salicylic acid (Cathode)	0.1	4.49 ± 0.99	44.3 ± 6.35 (*1.2*)
	1	4.57 ± 0.01	63.1 ± 6.97 (*1.7*)
Piroxicam (Cathode)	0.1	4.22 ± 0.57	42.6 ± 4.68 (*1.1*)
Propranolol (Anode)	1	5.66 ± 2.07 (*1.3*)	44.4 ± 15.3
	10	7.26 ± 1.27 (*1.7*)	45.7 ± 14.4

[a] Cumulative mannitol electrotransport after 4 hours of current passage.

[b] EF = Enhancement Factor: cumulative mannitol electrotransport after 4 hours of current passage in the presence of drug divided by the corresponding value when the drug was absent.

[c] No-current, no drug control.

[d] No drug, iontophoresis control.

ing' β-blocker concentration was low (0.01 or 0.1 mg/mL), iontophoresis caused a very appreciable enhancement of both the flux and the effective permeability coefficient (Table 3); in addition, there was no significant change in mannitol transport compared to the no-drug control (Figure 3, lower panel). However, a further 10-fold increase in propranolol concentration caused a negative impact on drug flux. Apparently, at this point, sufficient β-blocker had been able to enter the transport pathways and cause significant neutralization of the net negative charge present. This presumably has two effects that combine to reduce propranolol electrotransport: (i) the lower charge on the membrane probably lowers the drug's transport number (i.e., the electrorepulsive contribution to its iontophoretic flux is compromised), and (ii) as we know, electroosmosis is significantly attenuated. Clearly, from the standpoint of optimizing drug delivery, and selecting the most appropriate drug concentration to include in an iontophoretic formulation, these results are of considerable significance.

Another important, practical question concerns the extent to which a drug reservoir forms in situations such as that seen here for propranolol. Unfortunately, because skin was digested following an 8-hour period of passive diffusion after the end of (the 8 hours of) iontophoresis, the residual amounts of drug were not dependent upon the applied concentration, nor were they significantly different from the amount found (1.05 (±0.23)% of the applied 'dose') after 16 hours of simply passive diffusion. Presumably, by this time, any stored drug had had the opportunity to diffuse out of the tissue. Obviously, it is important, in future work, to digest the skin immediately (preferably post-sectioning into specific layers) in order to quantify precisely the magnitude and location of the 'reservoir' formed.

Finally, it must be said that the 'reverse' iontophoresis part of this research was less productive than the recent systematic efforts that we have reported (11,17). Like that work, however, it was observed here that augmentation of the skin's net negative charge (and, as a consequence, increase in anode-to-cathode electroosmosis) is not easily realized (Table 4). For obvious reasons, it is difficult to push anions to any significant extent into a negatively charged membrane.

In conclusion, this study has contributed, we believe, to the evolving understanding of the relationships between permeant structure and physical chemistry and the efficiency of iontophoretic transport. Once again, it has been demonstrated that iontophoresis is more complicated than simple electrostatics, and that the interaction of certain types of compounds (including, in this case, some well-known drugs) with structures along iontophoretic transport pathways can provoke profound, and sometimes unexpected, behavior. In particular, the role of electroosmosis cannot be ignored—indeed, it must be considered seriously during the development of optimal iontophoretic formulations.

ACKNOWLEDGMENTS

We thank the US National Institutes of Health (HD-27839), Iomed Inc., The Cultural Fund, Finland, and The Academy of Finland for financial support.

REFERENCES

1. R. R. Burnette and B. Ongpipattanakul. *J. Pharm. Sci.* **76**:765–773 (1987).
2. M. J. Pikal. *Adv. Drug Deliv. Rev.* **9**:201–237 (1992).
3. N. H. Yoshida and M. S. Roberts. *Adv. Drug. Deliv. Rev.* **9**:239–264 (1992).
4. G. Rao, P. Glikfield, and R. H. Guy. *Pharm. Res.* **10**:1751–1755 (1993).
5. J. A. Tamada, N. J. V. Bohannon, and R. O. Potts. *Nature Medicine* **1**:1198–1201 (1995).
6. M. B. Delgado-Charro and R. H. Guy. *Pharm. Res.* **11**:929–935 (1994).
7. A. J. Hoogstraate, V. Srinivasan, S. M. Sims, and W. I. Higuchi. *J. Control. Rel.* **31**:41–47 (1994).
8. M. B. Delgado-Charro and R. H. Guy. *Int. J. Pharm.* **117**:165–172 (1995).
9. M. B. Delgado-Charro, A. M. Rodríguez-Bayón, and R. H. Guy. *J. Control. Rel.* **35**:35–40 (1995).
10. J. Hirvonen, Y. N. Kalia, and R. H. Guy. *Nature Biotech.* **14**:1710–1713 (1996).
11. P. Santi and R. H. Guy. *J. Control. Rel.* **42**:29–36 (1996).
12. R. D. Schoenwald and H. S. Huang. *J. Pharm. Sci.* **72**:1266–1272 (1983).
13. The Merck Index. 10 th Ed., Merck & Co., Inc., Rahway, NJ (1983).
14. P. Glikfeld, R. S. Hinz, C. Cullander, and R. H. Guy. *Pharm. Res.* **5**:443–446 (1988).
15. P. G. Green, R. S. Hinz, C. Cullander, G. Yamane, and R. H. Guy, *Pharm. Res.* **8**:1113–1120 (1991).
16. J. Sclafani, J. Nightingale, P. Liu, and T. Kurihara-Bergstrom. *Pharm. Res.* **10**:1521–1526 (1993).
17. P. Santi and R. H. Guy. *J. Control. Rel.* **38**:159–165 (1996).
18. D. T.-W. Lau, J. W. Sharkey, L. Petryk, F. A. Mancuso, Z. Yu, and F. L. S. Tse. *Pharm. Res.* **11**:1742–1746 (1994).