Iontophoretic Delivery of Ropinirole Hydrochloride: Effect of Current Density and Vehicle Formulation

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Purpose. The objectives of this work were 1) to establish the feasibility of the transdermal iontophoretic delivery of ropinirole hydrochloride; 2) to investigate the possibility of delivering therapeutic doses of this drug; and 3) to determine the key factors that control ropinirole electrotransport.

Methods. A series of *in vitro* transdermal iontophoretic experiments were instituted to study the effects of drug concentration, co-ion concentration, intensity of current, and application time on ropinirole flux. The convective contribution to ropinirole electrotransport was evaluated by following the transport of the electroosmotic marker mannitol.

Results. Ropinirole flux decreased dramatically in the presence of competing ions. This effect was observed even when the molar fraction of the two competing cations was kept constant. Anodal flux of mannitol decreased with drug concentration, indicating a possible alteration of the skin permselectivity. In the absence of competing co-ions, ropinirole transport number reached a maximum value (8–13%). In these conditions, the main factor controlling drug delivery was the intensity of current applied.

Conclusions. Transdermal iontophoresis allowed the delivery of therapeutic doses of ropinirole. The dose administered and the input rate were controlled by the judicious choice of the key delivery factors here described.

KEY WORDS: ropinirole; iontophoresis; Parkinson's disease; transdermal delivery.

INTRODUCTION

Parkinson's disease is a neurological, chronic, and progressive disorder that affects the extrapyramidal system of the brain. Ropinirole hydrochloride (4-[2-dipropylamino)ethyl]-1,3-dihydro-2-H-indol-2-one hydrochloride) (RHCl) is a new dopamine nonergoline agonist recently introduced into Parkinson's disease therapy (1–5). It is generally well tolerated, and it can be used alone or in combination with levodopa. Ropinirole HCl treatment starts with a titration period during which the dose is adjusted for each patient in a stepwise procedure (2–5). Most of the adverse effects, such as nausea or dyskinesia, occur during this titration period. Usually, an initial daily dose of 0.75 mg (0.25 mg every 8 h) is maintained for a week. Each subsequent week, the dose is increased in 0.75 mg/day until a satisfactory response is observed. For most patients, this is achieved at a dose ranging from 3 to 9 mg/day. In addition, the dose is also carefully adapted to the different stages of the disease.

Iontophoresis enhances the transdermal transport of charged and neutral molecules across the skin by application of a low electric field. The total iontophoretic transport is the result of two mechanisms (6,7), the first of which is electromigration, or the direct interaction between the charged molecules and the electric field (6). Therefore, electrorepulsion only concerns charged molecules. The second mechanism of transport is the convective flow (electroosmosis) that results from the permselective properties of the skin (7). At pH 7.4, the skin is negatively charged and behaves as a cation-permselective membrane. As a consequence, there is a net flow of solvent moving with the cations, that is, from anode to cathode. The iontophoretic delivery of the cationic RHCl from the anode will also be enhanced by this electroomostic contribution.

The small doses required and the physicochemic characteristics (Fig. 1) of ropinirole make it a suitable candidate for iontophoresis. Furthermore, an iontophoretic device could easily provide the different inputs required during the titration period and at different stages of the disease.

This work explores the use of iontophoresis for the systemic delivery of RHCl. A series of *in vitro* experiments investigates the possibility of delivering a therapeutic dose of RHCl as well as the key factors that control the iontophoretic flux. In particular, we studied the role of competing ions, drug concentration, and current intensity. The role of electroosmosis in ropinirole delivery also was addressed briefly.

MATERIAL AND METHODS

Materials

Ropinirole hydrochloride was generously provided by GlaxoSmithKline (Beecham, UK). HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanosulfonic acid), NaCl, mannitol, Ag (99.9% purity), and AgCl (99%) were purchased from Sigma (Sigma-Aldrich-Química, Madrid). [1-^{14}C] labeled mannitol was purchased from NEN (NEN Life Science Products, Paris, France).

Skin

Full-thickness skin was excised from 2- to 5-day-old piglets and frozen at –20° until use.

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The pieces of skin were allowed to thaw overnight. Then, they were clamped between the two halves of standard side-by-side diffusion cells (0.78 cm^2). The stratum corneum side always faced the donor, anodal chamber of the cell. A power supply (either an APH 1000M Kepco Inc., Adler Instruments, S.L. Madrid, or a custom-built, computer-controlled apparatus from Professional Design and Development Services, Berkeley, CA) was used to deliver a constant direct current for 8 h via Ag/AgCl electrodes (8). Preliminary tests estab-

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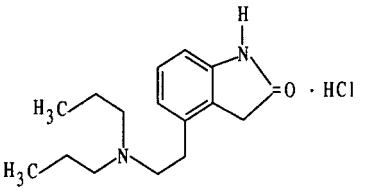


Fig. 1. Structure and physicochemic characteristics of Ropinirole HCl.: M.W: 296.84; pKa: 9.68 and 12.43; log $P_{\text{oct/phosphate buffer pH 7.4}}$ = 3.32.

lished the stability of RHCl under current application (0.39 mA for 8 h).

The receptor solution (cathodal chamber) was 3 mL of a 154 mM NaCl solution buffered to pH 7.4 with 25 mM HEPES. Both donor and receptor were magnetically stirred. Every hour, the whole receiver solution was sampled and replaced with fresh buffer.

In a first group of experiments, the effect of RHCl concentration in a 154 mM NaCl donor solution was investigated. Three experiments were performed using 2.5, 25, and 250 mM of ropinirole. The current applied was 0.32 mA.

In a second series of experiments, both NaCl and RHCl concentrations were varied in such a way that the molar ratio [RH^+:Na^+] was kept constant. Three experiments were performed at 0.32 mA using the following three combinations of [RHCl:NaCl]: [2.5:1.56]; [25:15.6]; and [250:154].

Finally, the iontophoresis of RHCl in the absence of competing ions in the donor solution was performed. Three concentrations of drug: 2.5, 25, and 250 mM were assayed at three levels of current intensity: 0.08, 0.16, and 0.32 mA. In these conditions, only the chloride from the salt form of the drug is available for the electrochemical reaction at the anode. Therefore, the donor solutions at 2.5 mM (at all current intensities) and 25 mM (at 0.32 mA) were replaced as necessary to ensure a sufficient supply of chloride to the anode. A passive control was done with a 250 mM RHCl donor solution and the same receptor solution described above. In this case, the receptor samples were taken only every 2 h.

Ropinirole Assay

Ropinirole was assayed by high-performance liquid chromatography (Merck–Hitachi Lichrograph series: AS-4000 autoinjector, L-6200 pump, D-6000 interface, L-4500 diode array detector) under isocratic conditions. A mobile phase consisting of 80:20 (v:v) acetonitrile:ammonium acetate buffer (0.05 M; pH 7) was pumped (1.0 mL · min^{-1}) through a C_8 reverse-phase Kromasil® column (Waters, 5 μm, 250 × 4.6 mm id) thermostated at 35°C. Ropinirole was quantified via its UV absorbance at 254 nm.

Electroosmotic Flow Measurements

The direction and extent of electroosmosis during the first group of experiments described above was determined using mannitol. In these experiments, the anodal chamber always faced the epidermal side of the skin and contained a solution of 2.5, 25, or 250 mM ropinirole hydrochloride in 154 mM NaCl. The cathodal side contained a 154 mM NaCl and 25 mM HEPES, pH 7.4 buffer. 1 mM cold mannitol together with ~0.8 μCi · mL^{-1} of the [1-^{14}C] labelled compound (45–60 mCi · $mmol^{-1}$) were added to the RHCl anodal solution to measure the anode-to-cathode convective flow. A 0.32 mA constant current was applied for 6 h. In another set of experiments, 1 mM of mannitol was added to the HEPES-saline cathodal buffer and this solution "spiked" with ~0.8 μCi · mL^{-1} ^{14}C mannitol. Every hour, 2 mL of the corresponding receptor solution was sampled and replaced with fresh buffer. The amount of ^{14}C mannitol in the samples was measured after addition of 5 mL of Ultima Gold XR (Packard Instrument S.A.; Rungis, France) in a liquid scintillation counter (LS 6500 Beckman Instruments France S.A., Gagny, France).

Conductivity Measurements

The specific conductivity (κ; $ohm^{-1} \cdot cm^{-1}$) of distilled water solutions containing 2.5, 25, 125, and 250 mM RHCl and 1.56, 15.6, 77, 120, and 154 mM of NaCl were determined at 25°C. A Wheatstone bridge (Databridge 451, Hungtingdon, UK; cell constant = 0.91) and a circulating water bath (Digiterm S-542, J.P. Selecta S.A. Spain) were used for the measurements.

The molar conductivities (Λ_m; $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$) of the RHCl and NaCl solutions were estimated from the measured specific conductance (9,10) using the following expression:

$$\Lambda_m = 1000 \times \frac{\kappa}{C_s}$$

in which κ is the specific conductance ($ohm^{-1} \cdot cm^{-1}$) and C_S the solute concentration (mol · dm^{-3}).

The representation of molar conductivity as a function of the square root of the concentration allowed the molar conductivity at infinite dilution (Λ°_m; $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$) to be estimated for each salt. The molar conductivity of the ropinirole cation was deduced via the Kohlrausch law of independent migration of ions (9,10). Then, the RH^+ mobility was estimated by dividing its molar ionic conductivity by the Faraday constant.

Statistics

A minimum of five replicates was made for all experiments. The results are expressed in RHCl and presented as the mean ± standard deviation. One-way and two-way ANOVA followed by the Student–Newman–Keuls test were used to compare the results. Simple linear and multiple regressions were also perfomed as described in the text. The level of statistical significance was fixed at $P \leq 0.05$.

RESULTS AND DISCUSSION

The objective of this work was to establish the feasibility of therapeutically administering RHCl by iontophoresis. Therefore, an extensive series of experiments that explored the effect of competing ions, current intensity, and drug concentration were performed. First, it should be said that the passive delivery of ropinirole from a 250 mM donor solution resulted in a flux at 8 h of 3.43 ± 0.7 nmol · $h^{-1} \cdot cm^{-2}$; cumulative transport in this time was 8 h ~4 μg/cm^2. All iontophoretic protocols performed resulted in significantly increased delivery.

Table I shows the 8-h ropinirole cumulative delivery together with the fluxes measured at 2, 4, 6, and 8 h of ionto-

Table I. RHC Fluxes (nmoles · h^{-1} · cm^{-2}), Cumulative Delivery after 8 H (mg) and Transport Number (%) Measured for the Iontophoretic Experiments Performed in the Presence of Competing Co-Ions (Mean ± SD; n ≥ 5)

[RHCl]/[NaCl]	2.5/154	25/154	250/154	25/15.6	2.5/1.56
$J_{2\,h}$	27.6 ± 12.5	82.0 ± 62.2	49.5 ± 35.6	149.5 ± 70.6	480.4 ± 127.6
$J_{4\,h}$	24.4 ± 12.1	191.9 ± 120.2	127.5 ± 76.5	333.6 ± 110.2	1396.5 ± 238.6
$J_{6\,h}$	35.4 ± 19.5	326.6 ± 143.8	335.9 ± 152.2	894.1 ± 460.4	1419.3 ± 255.5
$J_{8\,h}$	43.3 ± 9.8	319.3 ± 140.0	469.7 ± 26.7	1046.3 ± 441.4	1452.9 ± 93.0
Accum. (mg)	0.06 ± 9.10^{-3}	0.37 ± 0.2	0.43 ± 0.18	1.09 ± 0.4	2.12 ± 0.3
$T_{RH+8\,h}$ (%)	0.28 ± 0.08	2.08 ± 0.9	3.06 ± 0.1	6.83 ± 2.9	9.49 ± 0.6

phoresis corresponding to the series of experiments where the role of competing co-ions (Na^+) and drug concentration were studied. First, Na^+ concentration was kept constant (154 mM) whereas RHCl concentration was varied from 2.5 to 250 mM. Ropinirole iontophoretic delivery significantly increased ($P < 0.05$) when the 2.5 mM donor solution was replaced with the 25 mM whereas the use of a 250 mM solution failed to significantly increase the flux. The concentration of a drug has a different impact on its own iontophoretic flux depending upon the vehicle composition (11–13). A proportionality between flux and concentration is usually observed when competing co-ions are present in the donor solution (14–16). Although, it has been reported for several cations that their iontophoretic fluxes do not proportionally increase with their concentration (17–20). Their possible interaction with the skin, which would result in a progressive neutralization of the negative charges of the membrane, has been invoked as an explanation. That is, the low flux measured at a high drug concentration would be caused by a progressively reduced electroosmotic contribution. Accordingly, we decided to test this hypothesis for ropinirole. The flux of mannitol, a well-known marker of electroosmosis (21), was followed in the presence of increasing concentrations of ropinirole. Table II shows the anodal and cathodal fluxes of mannitol measured at six hours of iontophoresis. The anodal transport of mannitol did not change when RHCl content was increased from 2.5 to 25 mM, but clearly decreased for the 250 mM drug solution ($P < 0.05$). Consequently, a reduction in the electroosmotic contribution could explain, at least partially, the unexpectedly low flux measured for the 250 mM donor. The possibility that RH^+ self-aggregates or forms micelles was not supported by the conductivity measurements performed (see below). In addition, the cathodal flux of mannitol can only be modified by the drug (present in the anodal chamber) via a modified permselectivity of the membrane. Other two factors (22,23) that may decrease electroosmosis are the progressively higher ionic strengths of the donor solutions and their lower pH when RHCL was a present at higher concentration (i.e., the pH of the 2.5 mM and 250 mM RHCl donors was 5.5 an 4.8, respectively).

Table II. Anodal and Cathodal Mannitol Fluxes (nmoles · h^{-1} · cm^{-2}) Measured at 6 H of Iontophoresis in the Presence of RHCl (Mean ± SD; n ≥ 5)

[RHCl] (mM)	Anodal flux	Cathodal flux	Electroosmotic contribution to RHCl flux (%)[a]
2.5	2.24 ± 0.71	0.07 ± 0.02	13
25	2.42 ± 0.74	—	20
250	0.43 ± 0.25	0.33 ± 0.52	23

[a] The electroosmotic flux of a solute is the product of the solvent flow and the solute concentration in the solvent. The solvent flow is deduced from the mannitol experiments.

In another set of experiments, both RHCl and NaCl molarity were changed in such a way that the ratio $[R^+]/[Na^+]$ was kept constant (~1.6). It was found that (Table I, Fig. 2) ropinirole flux decreased with Na^+ concentration (the three conditions resulted in statistically different results, $P < 0.05$). This reflects the combined role of both concentration and mobility as determinants of the transport number. Furthermore, this could indicate that the mobilities of both ions are not mutually independent or that they perhaps change as a function of the vehicle.

We examined then whether two iontophoresis models predicted the iontophoretic behavior of ropinirole. In the first approach, the iontophoretic flux of an ion can be related to its transport number. The transport number of an specific ion "i" is the ratio among the charge carried by the ion "i" and the total charge transferred in the system (24). In the experiments described above, ropinirole ions compete with the sodium (co)-ions present in the anodal chamber as well as with the chloride (counter)-ions arriving from the cathodal side. The flux of ropinirole is then, given by the equation:

$$J_R = \frac{t_R I}{F Z_R} \tag{1}$$

Where J_R , t_R, and z_R are the flux, transport number and valence of RH^+ ion, I is the current intensity; and F the Faraday constant. The transport numbers of ropinirole determined in these experiments are shown in Tables I and III and Figure 2.

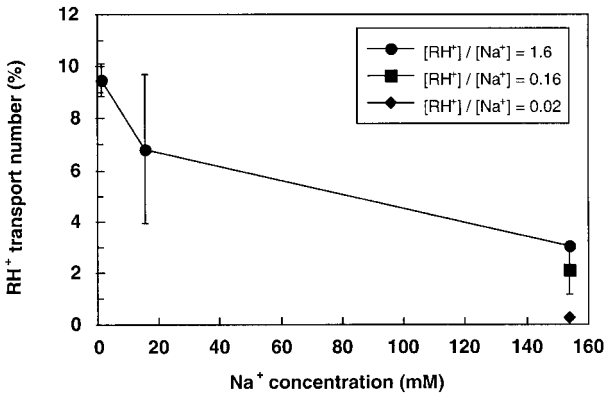


Fig. 2. Influence of Na^+ concentration on RH^+ transport number for the different donor vehicles assayed.

Table III. RHCl Fluxes (nmoles · h^{-1} · cm^{-2}), Cumulative Delivery after 8 Hours (mg) and Transport Number (8h; %) Measured for the Iontophoretic Experiments Performed in the Absence of Competing Co-Ions (Mean ± SD; n ≥ 5)

[RHCl] (mM)	250			25			2.5		
I (mA)	0.32	0.16	0.08	0.32	0.16	0.08	0.32	0.16	0.08
$J_{2\,h}$	332.0 ± 263.0	267.9 ± 245.1	63.1 ± 15.6	265.2 ± 120.2	42.3 ± 18.6	25.2 ± 18.2	96.5 ± 72.6	60.3 ± 43.3	33.1 ± 16.0
$J_{4\,h}$	780.0 ± 641.7	302.0 ± 84.6	220.5 ± 44.5	666.3 ± 250.2	248.6 ± 110.5	112.5 ± 83.1	436.0 ± 288.3	294.3 ± 140.0	213.7 ± 73.2
$J_{6\,h}$	1414.0 ± 640.7	447.7 ± 130.3	374.0 ± 65.9	1100.3 ± 212	515.1 ± 286.0	292.5 ± 94.7	895.4 ± 252.2	546.6 ± 257.8	261.3 ± 21.2
$J_{8\,h}$	1873.5 ± 153.7	628.4 ± 113.0	524.7 ± 59.9	1564.0 ± 256.3	574.6 ± 193.5	281.3 ± 188.0	1367.04 ± 351.2	575.9 ± 146.1	366.9 ± 33.8
Accum. (mg)	2.20 ± 0.39	0.85 ± 0.46	0.46 ± 0.07	1.80 ± 0.23	0.62 ± 0.20	0.31 ± 0.15	1.32 ± 0.30	0.62 ± 0.03	0.39 ± 0.05
$T_{RH+8\,h}$ (%)	12.23 ± 1.1	8.21 ± 1.5	13.92 ± 1.5	10.22 ± 1.7	6.8 ± 1.2	7.34 ± 4.9	9.69 ± 2.8	7.50 ± 1.9	9.58 ± 0.9

Phipps and Gyory (24) have derived an expression for the transport number of a drug in a binary cation situation and assuming (a) a homogeneous non-ionic membrane; (b) neither interaction nor association among the ions in solution; and (c) both ionic charges and mobilities are independent. An inverse linear relationship between the transport number of a cationic drug and the molar fraction of the competing cation is also predicted:

$$t_R = \frac{c_R u_R z_R}{\sum c_i u_i z_i} \tag{2}$$

$$t_R = \frac{\left[\dfrac{t^0_R}{1 - t^0_R}\right]}{\left[\dfrac{t^0_R}{1 - t^0_R}\right] + BZ_c X_c \left[\dfrac{1}{1 - t^0_{Na}}\right] + 1} \tag{3}$$

Where c_i; z_i, and u_i correspond to the membrane concentration; valence and mobilities of the ion "i". t°_R and t°_{Na} are the transport numbers of ropinirole and sodium ions in the single cation situation. B is the proportionality constant that relates the cation concentration ratio in the skin to their ratio in the donor solution. Zc and Xc are the valence ratio and mole fraction ratio of the two cations (24).

Substitution of t°_R from Table III and t°_{Na} = 0.6 (7,25) in Equation 3 leads to the following expression:

$$\frac{1}{t_R} = 9.5 + 21.37\, B\, X_c \tag{4}$$

This equation does not, however, agree with the results for RHCl because it predicts a constant drug flux for the set of experiments performed at a fixed molar fraction and is not the case (Table I, Fig. 2).

Ropinirole electrotransport cannot be predicted solely from its molar fraction in the range of experimental conditions studied here. Thus, RHCl differs from other drugs such as lidocaine (11,24,25) and hydromorphone (13) for which linear relationships between transport number and co-ion molar fraction have been demonstrated. The more complicated behavior of RHCl indicates that a useful predictive model should also take into account (a) the contribution of electroosmosis; (b) a more complex dependence of transport number on mobility and concentration; and (c) that ion mobilities may not be constant in our experiments (for example, RH^+ and Na^+ mobilities inside the skin could be modified by an altered skin permselectivity).

In fact, these data suggest that increasing the (co)-ion concentration "x-fold" results in a different outcome depending on the ion considered; otherwise, the experiments performed at a constant molar fraction should result in similar fluxes. Increasing the concentration of both ions by 10 times decreased the RH^+ transport number. It seems, then, that the effect of concentration on transport number depends on the mobility of the ion concerned. Perhaps, therefore, the concentration and mobility terms in Equation 2 cannot be considered as independent parameters for the derivation of Equations 3 and 4.

A final multiple regression was performed simply introducing both cation concentrations as independent predictors (r^2 = 0.8031 and $F_{(2,22)}$ = 44.86; $P < 0.01$):

$$J_{8h} = 1314.5 + 1.29\, C_R - 7.6\, C_{Na} \tag{5}$$

This empirical equation lacks any fundamental or mechanistic insight; it does, however, provide information on the relative importance of the co-ion concentrations: That is, changes in Na^+ concentration "weigh" nearly 6 times more than changes in R^+ ($P <$;0.048 and $P < 0.0001$ for the terms in RH^+ and Na^+ concentrations, respectively).

A second approach to predict iontophoretic fluxes by Roberts *et al.* is based on the specific conductance (26,27). However, we found no relationship bewteen the specific conductance of the vehicles used and drug flux (data not shown). This is somehow expected given that the specific conductance of a RHCl solution (a) results from both Cl^- and RH^+ contributions and (b) it is a concentration-dependent parameter.

We tried then to estimate the conductance and mobility of the isolated RH^+ cation via the molar conductance at infinite dilution (9,10). Table IV shows the values of specific conductance measured for various concentrations of RHCl

Table IV. Specific Conductance (κ; $mohm^{-1}$ · cm^{-1}) and Molar Conductivity (Λ_m; ohm^{-1} · cm^2 · mol^{-1}) Measured for Various RHCl and NaCl Aqueous Solutions

	[mM]	κ ($mohm^{-1}$ · cm^{-1})	Λ_m (ohm^{-1} · cm^2 · mol^{-1})
RHCl	2.5	0.23	93.20
	25	2.02	80.88
	125	6.63	53.06
	250	9.97	39.91
NaCl	1.56	0.20	129.65
	15.6	1.89	121.47
	77	7.84	101.00
	120	11.23	93.62
	154	13.78	89.48

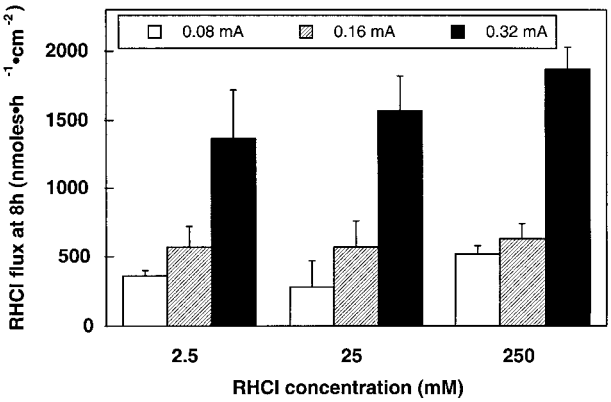
and NaCl in distilled water, as well as their molar conductivities. The molar conductance of both NaCl and RHCl is a linear function of the square root of concentration. This confirms that there is no micellization taking place at the highest drug concentration. The molar conductivity at infinite dilution, Λ^0_m is obtained from the Y-axis intercept. For NaCl, Λ^0_{NaCl} was 134 $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$, a value close to 126-128 $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$ in the literature (9,10,28). Λ^0_{RHCl} was 99.36 $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$. According to the Kohlrausch's law, the difference between Λ^0_{NaCl} and Λ^0_{RHCl} (34.65 $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$) corresponds to the difference between the molar ionic conductivities at infinite dilution (λ^0_+) of Na^+ and RH^+. From the tabulated value of the ionic conductivity at infinite dilution for Na^+ (50.11 $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$), a molar ionic conductivity of 15.46 $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$ can be estimated for RH^+. A ionic mobility of $1.6 \cdot 10^{-4}$ $cm^2 \cdot s^{-1} \cdot V^{-1}$ was then estimated[+] (9) for RH^+. It follows, then, that the mobility ratio is ~3 for the pair Na^+/RH^+ and ~5 for the pair Cl^-/RH^+.

Obviously, more research is required to better understand the intriguing nature of the transport number and the physicochemic parameters that condition its value in the very different situations in which transdermal iontophoresis can be performed. Finally, it will also be interesting to test if the molar ionic conductance could be of any predictive value for iontophoretic fluxes.

Setting aside the discussion of mechanistic issues, it is clear that ropinirole flux is optimized in the absence of competing co-ions. Therefore, we examined the factors controlling RHCl delivery under these conditions using a 3 × 3 factorial experimental design. The predictors considered were current intensity (0.08, 0.16, and 0.32 mA) and RHCl donor concentration (2.5, 25, and 250 mM). The results of these experiments are shown in Table III. Two two-way ANOVAs and the corresponding post-hoc tests were performed on both the accumulated ropinirole delivered in 8 h and on the 8-h flux. These two ANOVAs yielded the same results; therefore, only the results for the fluxes are shown in Figure 3. On the whole, the current intensity was the most significant factor, the three levels of intensity of current applied resulting in three statistically significant different deliveries. On the other hand, whereas the 250 mM donor led to a higher RHCl transport than the 2.5 and 25 mM vehicles, no differences were observed between the 2.5 and 25 mM solutions. There was no interaction between the two predictors considered.

According to Equation 1, iontophoretic flux depends linearly on current intensity, and the transport number of ropinirole can be estimated from the slope (Fig. 4). We deduce that Ropinirole transports 7.8, 10.3, and 13.3% of the total charge in the experiments involving 2.5, 25, and 250 mM drug, respectively. This modest variation agrees with the two-way ANOVA analysis, which also states the secondary role of drug concentration as a determinant of flux. The influence of current density is clearly predicted by Equation 1 and has often been observed. It seems, then, that manipulating current density is the easiest way to modulate iontophoretic drug delivery.

The small effect of ropinirole concentration has been theoretically predicted (12) and experimentally observed (11–13). According to Kasting *et al.* (12), and in the absence of competing co-ions in the donor solution, the fluxes become dependent only on the diffusivity ratio of the counter-ion



Intensity (mA)	[RHCl] (mM)
0.08 vs 0.16 $p < 0.05$	2.5 vs 25 Not Significant.
0.08 vs 0.32 $p < 0.05$	2.5 vs 250 $p < 0.05$
0.16 vs 0.32 $p < 0.05$	25 vs 250 $p < 0.05$

Fig. 3. Effect of intensity and drug concentration on RHCl 8 h iontophoretic flux (nmoles $\cdot h^{-1} \cdot cm^{-2}$) for the experiments performed in the absence of competing co-ions. Below are shown the results for the two-way ANOVA performed for this variable and the two predictors considered.

(chloride on the receptor side) and the drug. Similar results have been described for hydromorphone and lidocaine (11,13,24). Unfortunately, the competition from endogenous counter-ions, such as chloride, cannot be eliminated in the *in vivo* situation, and it follows that a maximum transport number will be identified for each particular drug. For most drugs, the maximal transport number reported falls around 10–15%. This value probably depends on the physicochemic properties of the specific ion, although, a complete structure-activity investigation on this matter has yet to be performed. From a practical point of view, this is quite convenient as it allows drug delivery to be optimized at less than maximum drug

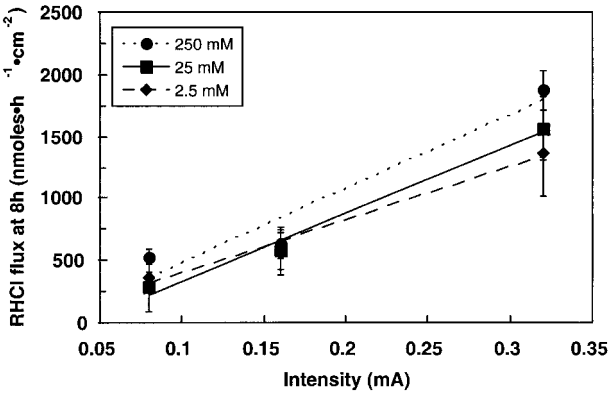


Fig. 4. Relationship between RHCl electrotransport and intensity of current for the three drug concentrations studied in the absence of competing ions. The transport number of Ropinirole can be estimated from the slopes.

concentrations. However, there are some restrictions to this approach for cationic drugs. For example, a 2.5 mM RHCl solution does not provide enough Cl^- for the anodal electrochemistry. Consequently, either a higher concentration of the drug hydrochloride or another chloride salt (of a preferably immobile cation) is required for practical purposes.

The pH of the vehicle also plays an important role in iontophoresis (21,22). The pH of the unbuffered ropinirole vehicles was 5.5, 5.3, and 4.8 for the 2.5, 25, and 250 mM solutions, respectively. Given (a) the permselective properties of this skin model (29) and (b) that the receptor pH was always 7.4, we know that the skin remained cation-permselective in these experiments. It is complicated to predict the behaviour of the convective term, which we might expect to slightly increase with current (30) and with ropinirole concentration (11), but to decrease with increasing ionic strength and acidity (22,23) and as a result of interaction between the drug and the skin thereby modifying the membrane's net charge.

Another matter of interest is the time to reach the steady-state iontophoretic flux. After 4 h of iontophoresis, the fluxes were 30–58% of J_{8h} for the experiments without NaCl, and 10–60% for those performed in the presence of various sodium ion concentrations (Tables I and III). At 6 h, the corresponding percentages were 60–100% and 70–100%, respectively. We could not consistently identify the experimental conditions that allowed the fastest approach to steady state. Certainly, in other studies (11), a faster attainment of iontophoretic steady-state flux has been reported. Differences could be due to the type and thickness of skin used, and to the presence of endogeneous co- and counter-ions inside the skin and within the body. The time to steady state may depend, therefore, on how long it takes to deplete the ionic reservoir pre-existing inside the skin. It is plausible that depleting a full-thickness membrane (as the one used in our studies) takes longer than dermatomed skin. Phipps and Gyory (24) described the continuous (8-h) endogenous delivery of sodium and potassium during iontophoresis through dermatomed pig skin. The presence of endogenous co-ions introduced an apparent lag-time in drug flux and curvature in the cumulative drug delivered vs. time plot (24). The relevance of this lag-time to the *in vivo* situation has not been completely characterized.

Finally, it is essential to examine the possibility of administering therapeutic doses of ropinirole. As mentioned before, a daily dose of 3–9 mg orally is required for most patients. Taking into account the fact that RHCL oral bioavailability is about 50% due to a significant first-pass effect (3) the dose required transdermally should be about one-half that administered orally. In Tables I and III, we show the accumulated amount of RHCl delivered in 8 h for each of the protocoles studied. In five cases, 1–2 mg of ropinirole were transported and, in many others, 0.4–1 mg was delivered. Given the range of intensities used in these experiments we can conclude that the delivery of therapeutic doses of ropinirole is entirely feasible by the judicious combination of the key factors described. By carefully selecting the area of the delivery system and of the current intensity and profile adopted, it should be possible to appropriately control the total dose and dose delivery rate while minimizing the potential for significant skin irritation. For example, a current of 0.32 mA delivered over 4 cm^2 would result in a current density of only 0.08 $mA \cdot cm^{-2}$, a level which, according to literature (6), is unlikely to provoke reasonable skin irritation in humans.

CONCLUSIONS

Transdermal iontophoresis allows therapeutic doses of ropinirole hydrochloride to be delivered. The dose administered and the input rate can be modulated by manipulation of the current intensity and the vehicle composition. Maximum iontophoretic flux of ropinirole flux is obtained in the absence of competing co-ions and is characterized by a transport efficiency of 8-13%. Under these conditions, the main factor controlling delivery is the intensity of current applied. Ropinirole flux decreases in a complicated manner in the presence of competing (co)-ions: transport decreases with increasing concentrations of Na^+ in the formulation; delivery is not proportional to the mole fraction of the drug as predicted theoretically (and observed for other model ions); ropinirole at high concentration probably inhibits its electrotransport possibly due to an effect on electroosmosis.

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REFERENCES

1. I. F. Tulloch. Pharmacologic profile of Ropinirole. *Neurology* **49**: S58–S62 (1997).
2. M. J. Vidalilhet, A. M. Bonnet, S. Belal, B. Dubois, C. Marle, and Y. Agid. Ropinirole without levodopa in Parkinson's disease. *Lancet* **336**:316–317 (1990).
3. A. J. Matheson and C. M. Spencer. Ropinirole. A review of its use in the management of Parkinson's disease. *Drugs* **60**: 115–137 (2000).
4. C. H. Adler , K. D. Sethi, R.A. Hauser, T. L. Davis, J. P. Hammarstad, J. Bertoni, R. L. Taylor, J. Sanchez–Ramos, and C. F. O'Brien. Ropinirole for the treatment of early Parkinson's disease. *Neurology* **49**:393–399 (1997).
5. A. E. Scharg, D. J. Brooks, E. Brunt, D. Fuell, A. Korczyn, W. Poewe, N. P. Quinn, O. Rascol, and F. Stocchi. The safety of Ropinirole, a selective nonergoline dopamine agonist, in patients with Parkinson's disease. *Neuropharmacology* **21**:169–175 (1998).
6. B. H. Sage. Iontophoresis. In E. W. Smith, H. I. Maibach, (eds), *Percutaneous Penetration Enhancers*. CRC Press, Boca Raton, Florida 1995 pp 351–368.
7. R. R. Burnette and B. Ongipattanakul. Characterization of the permselective properties of excised human skin during iontophoresis. *J. Pharm. Sci.* **76**:765–773 (1987).
8. P. G. Green, R. S. Hinz, C. Cullander, G. Yamane, and R. H. Guy. Iontophoretic delivery of aminoacids and aminoacid derivatives across the skin in vitro. *Pharm. Res.* **8**:1113–1120 (1991).
9. P. W. Atkins. *Physical Chemistry*. Oxford University Press, 1978.
10. W. J. Moore. *Physical Chemistry*. Prentice–Hall Inc, London, 1972.
11. D. Marro, M. B. Delgado-Charro, Y. N. Kalia, and R. H. Guy. Iontophoretic transport mechanisms: Effect of background electrolyte and competing ions. *Proceed. Intl Symp. Control. Release Bioact. Mater*. **26**:94–95 (1999).
12. G. B. Kasting and J. C. Keister. Application of electrodiffusion

theory for a homogeneous membrane to ion iontophoretic transport trough skin. *J. Control. Release* **8**:195–210 (1989).
13. R. V. Padmanabhan, J. B. Phipps, G. A. Lattin, and R. J. Sawchuk. In vitro and in vivo evaluation of transdermal iontophoretic delivery of hydromorphone. *J. Control. Release* **11**:123–135 (1990).
14. L. Wearly, J. C. Liu, and Y. W. Chien. Iontophoresis facilitated transdermal delivery of verapamil II. Factors affecting the skin permeability. *J. Control. Release* **9**:231–242 (1989).
15. S. Thysman, C. Tasset, and V. Préat. Transdermal iontophoresis of fentanyl: delivery and mechanistic analysis. *Int. J. Pharm.* **101**: 105–113 (1994).
16. L. L. Miller and G. A. Smith. Iontophoretic transport of acetate and carboxylate ions through hairless mouse skin: cation exchange membrane model. *Int. J. Pharm.* **49**:15–22 (1989).
17. M. B. Delgado–Charro and R. H. Guy. Iontophoretic delivery of nafarelin across the skin. *Int. J. Pharm.* **117**:165–172 (1995).
18. A. J. Hoogstraate, V. Srinivasan, S. M. Sims, and W. I. Higuchi. Iontophoretic enhancement of peptides: Behavior of leuprolide versus model permeants. *J. Control. Release* **31**:41–47 (1994).
19. J. Hirvonen and R. H. Guy. Iontophoretic delivery across the skin: electroosmosis and its modulation by drug substances. *J. Control. Release* **14**:1258–1262 (1997).
20. J. Hirvonen, Y. N. Kalia, and R. H. Guy. Transdermal delivery of peptides by iontophoresis. *Nat. Biotech.* **14**:1710–1713 (1996).
21. A. Kim, P. G. Green, G. Rao, and R. H. Guy. Convective solvent flow across the skin during iontophoresis. *Pharm. Res.* **10**:1315–1320 (1993).
22. P. Santi and R. H. Guy. Reverse iontophoresis—Parameters determining electroosmotic flow: I. pH and ionic strength. *J. Control. Release* **38**:159–165 (1996).
23. M. P. Pikal and S. Shah. Transport mechanisms in iontophoresis II. Electroosmotic flow and transference number measurements for hairless mouse skin. *Pharm. Res.* **7**:213–223 (1990).
24. J. B. Phipps and J. R. Gyory. Transdermal ion migration. *Adv. Drug Deliv. Rev.* **9**:137–176 (1992).
25. D. Marro, M. B. Delgado–Charro, Y. N. Kalia, and R. H. Guy. Optimizing iontophoretic drug delivery: Identification and distribution of the charge-carrying species. *Proceed. Intl* Symp. *Control. Release Bioact. Mater.* **27**:938–939 (2000).
26. N. H. Yoshida and M. S. Roberts. Prediction of cathodal iontophoretic transport of various anions across excised skin form different vehicles using conductivity measurements. *J. Pharm. Pharmacol.* **47**:883–890 (1995).
27. N. H. Yoshida and M. S. Roberts. Role of conductivity in iontophoresis. Part 2. Anodal iontophoretic transport of phenylethylamine and sodium across excised human skin. *J. Pharm. Sci.* **35**: 350–344 (1994).
28. R. C. Weast. *Handbook of Chemistry and Physics,* 55th Edition. CRC Press, Cleveland, Ohio 1974.
29. A. Luzardo–Alvarez, M. Rodriguez–Fernandez, J. Blanco-Mendez, R. H. Guy, and M. B. Delgado-Charro. Iontophoretic permselectivity of mammalian skin: characterization of hairless mouse and porcine membrane models. *Pharm. Res.* **15**:984–987 (1998).
30. M. B. Delgado–Charro and R. H. Guy. Characterization of convective solvent flow during iontophoresis. *Pharm. Res*. **11**:929–935 (1994).