Transdermal Iontophoresis of Rotigotine Across Human Stratum Corneum *in Vitro*: Influence of pH and NaCl Concentration

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Purpose. The aim of this study was to characterize the influence of pH and NaCl concentration on the transdermal iontophoretic transport of the dopamine receptor agonist rotigotine across human stratum corneum (HSC).

Methods. Rotigotine transport was studied *in vitro* in side by side diffusion cells according to the following protocol: 6 h of passive diffusion, 9 h of iontophoresis, and 5 h of passive diffusion. A current density of 0.5 mA cm^{-2} was used. The influence of donor phase pH (4, 5, and 6) and different concentrations of NaCl (0.07 and 0.14 M) on rotigotine iontophoretic flux were examined. The acceptor phase was phosphate-buffered saline (PBS) at pH 7.4 except in one series of experiments aimed to study the effects of rotigotine solubility on its iontophoretic transport. In this study, PBS at pH 6.2 was used. In separate studies, ^{14}C-mannitol was used as a marker to determine the role of electro-osmosis during iontophoresis.

Results. The estimated iontophoretic steady-state flux ($Flux_{ss}$) of rotigotine was influenced by the pH of the donor solution. At a drug donor concentration of 0.5 mg ml^{-1}, the iontophoretic flux was 30.0 ± 4.2 nmol cm^{-2} h^{-1} at pH 6 vs. 22.7 ± 5.5 nmol cm^{-2} h^{-1} at pH 5. However, when the donor concentration was increased to 1.4 mg ml^{-1}, no significant difference in iontophoretic rotigotine transport was observed between pH 5 and 6. Increase of NaCl concentration from 0.07 M to 0.14 M resulted in a decrease of the rotigotine $Flux_{ss}$ from 22.7 ± 5.5 nmol cm^{-2} h^{-1} to 14.1 ± 4.9 nmol cm^{-2} h^{-1}. The contribution of electro-osmosis was estimated less than 17%. Probably due to the lipophilic character of the drug, impeding the partitioning of rotigotine from HSC to the acceptor compartment, steady-state transport was not achieved during 9 h of iontophoresis.

Conclusions. Both pH and NaCl concentration of the donor phase are crucial on the iontophoretic transport of rotigotine. Electro-repulsion is the main mechanism of the iontophoretic transport of rotigotine.

KEY WORDS: dopamine receptor agonist; electro-osmosis; electro-repulsion; lipophilicity; Parkinson's disease.

INTRODUCTION

Although levodopa has been used for many years as the drug of choice in the treatment of Parkinson's disease, the occurrence of side effects like dyskinesia and motor response fluctuations (i.e., "on-off" phenomenon) has turned the interests into the development of direct dopamine receptor agonists. Several dopamine receptor agonists (i.e., R-apomorphine, lisuride, bromocriptine, and pergolide) have been developed for use as monotherapy or in combination with levodopa (1). Furthermore, several new dopamine receptor agonists are in development.

Rotigotine (also known as N-0923) is a new and potent dopamine receptor agonist with high selectivity for the D_2 dopamine receptor (2). In a clinical study with 9 Parkinson's disease (PD) patients, Calabrese *et al.* reported the effective reversal of parkinsonian symptoms as reflected in a reduction in MCRS scores by zero-order i.v. infusion rates of 0.5–5.6 μg/kg body weight per h (3). However, upon oral administration, rotigotine is extensively metabolized by glucuronidation in the gut wall and the liver (4–7). For this reason per-oral administration is not feasible. This underscores the need for an alternative method of delivery.

Transdermal delivery is a well-established method of drug administration whereby the hepatic first-pass effect is circumvented (8). Several studies into the transdermal delivery of rotigotine *in vitro* and *in vivo* have been carried out. The results were quite promising showing a significant increase in bioavailability in comparison to oral delivery and providing a continuous delivery pattern (9–11). *In vivo* studies in patients have been carried out by Hutton *et al* (9) and Metman *et al* (10). In these studies, transdermal administration of rotigotine was found to significantly reduce the levodopa dose requirement in an on-demand dosing regimen. However, monotherapy with rotigotine has not yet been achieved. Monotherapy of rotigotine requires a permeation rate, which is much higher than that can be achieved by passive diffusion.

Transdermal iontophoresis is a well-established approach to enhance drug transport across the skin by using an electric driving force. The method offers the possibility to enhance the transdermal transport of in particular polar electrically charged drugs. In addition to increasing drug transport, iontophoresis offers the possibility to deliver the drug in a programmed way (12). This is important in the treatment of Parkinson's disease in which, due to a narrow therapeutic window, accurate individualized dosing is crucial.

Several studies on the transdermal iontophoresis of dopamine receptor agonists have been reported. From an *in vitro* study across full thickness piglet skin, promising results on the iontophoretic delivery of ropinirole HCl have been reported (13). Moreover, the delivery of the direct dopamine receptor agonist R-apomorphine by transdermal iontophoresis has been the subject of a series of investigations *in vitro* (14–18) as well as *in vivo* (19,20) in patients with Parkinson's disease. Although all of those studies indicate that transdermal iontophoresis is a promising approach to the delivery of R-apomorphine, recent *in vivo* studies showed that therapeutic concentrations of R-apomorphine can only be achieved in a few patients, when iontophoresis is combined with chemical enhancers (20). Because the required therapeutic plasma levels for rotigotine are much lower than for R-apomorphine, rotigotine is a promising drug candidate for delivery by iontophoresis.

In this study, we examined rotigotine iontophoresis

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ABBREVIATIONS: DPM, disintegration per minute; $Flux_{ss}$, estimated steady-state flux; HSC, human stratum corneum; Jv, volume flow; J_{EO}, electro-osmosis flux; J_{EO} Max, theoretical value of the maximum electro-osmosis flux; J_{TOTAL}, total iontophoretic flux.

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across human stratum corneum (HSC) *in vitro*. Specifically the effects of rotigotine donor concentration, pH, and NaCl concentration on the iontophoretic transport of rotigotine were examined. The effect of rotigotine solubility in the acceptor phase was studied as well. In order to estimate the contribution of electro-osmosis to the iontophoretic transport of rotigotine, a series of mannitol transport studies was performed.

MATERIALS AND METHODS

Materials

Rotigotine (HCl salt) was kindly supplied by Schwarz Pharma (Monheim, Germany). Silver and silver chloride (purity >99.99%) were obtained from Aldrich (Borneum, Belgium). Ascorbic acid, sodium meta bisulphite, trypsin (Type III, from a bovine pancreas) and trypsin inhibitor (Type II-S, from soybean) were purchased from Sigma Chemicals (Zwijndrecht, The Netherlands). Dialysis membrane disks (cut-off value 5000 Da) were purchased from Diachema (München, Germany). HPLC grade acetonitrile for the mobile phase was obtained from Rathburn (Walkerburn, UK), and 1-octanol was obtained from Fluka Chemica AG (Buchs, Switzerland). ^{14}C-Mannitol (activity of 200 μCi ml^{-1}) was purchased from Amersham Biocience Europe GmbH (Roosendaal, The Netherlands). All other chemicals and solvents were of analytical grade. All solutions were prepared in Millipore water with a resistivity of more than 18 MΩ.

Preparation of Human Stratum Corneum

Within 24 h after surgical removal of the human skin (abdominal or breast), residual subcutaneous fat was removed. To avoid interference with contaminating subcutaneous fat, the skin surface was carefully wiped with a tissue paper soaked in 70% ethanol. The skin was dermatomed to a thickness of about 300 μm using a Padgett Electro Dermatome Model B (Kansas City, KS, USA). It was then incubated with the dermal side on Whatman paper soaked in a solution of 0.1% trypsin in 0.15 M phosphate-buffered saline (PBS) pH 7.4 (NaCl 8 mg ml^{-1}, Na_2HPO_4 2.86 mg ml^{-1}, KH_2PO_4 0.2 mg ml^{-1}, KCl 0.19 mg ml^{-1}) overnight at 4°C and subsequently for 1 h at 37°C. Then, the human stratum corneum (HSC) was peeled off from the underlying epidermis and dermis. Remaining trypsin activity was blocked by bathing the HSC in a 0.1% trypsin inhibitor solution in PBS pH 7.4. HSC was subsequently washed several times in water and stored in a silica gel containing desiccator in a N_2 environment to inhibit oxidation of HSC lipids.

Solubility Studies

Rotigotine was solubilized in 5 mM citrate buffer pH 4, 5, or 6, with and without NaCl presence. After adjusting the pH by using 0.05 M NaOH or 0.05 M HCl, each solution was shaken at 700 rpm (IKA-VIBRAX-VXR, Omnilabo International BV, Breda, The Netherlands) at room temperature for 48 h. The pH was then rechecked and adjusted whenever required. All saturated solutions were centrifuged at 2000 rpm for 30 min (GS 6R Centrifuge, Beckman, Palo Alto, CA, USA). Each supernatant was then filtered by using 0.2 μm porous membrane (Nylon Acrodisc, Pall Gelman Laboratory, Ann Arbor, MI, USA), after which the concentration of rotigotine was determined by HPLC.

Distribution Coefficient of Rotigotine and the Determination of log P and pK_a

The octanol/water distribution coefficient (log D) was measured at pH 7.4 and 6.2, which are the pH values used in the acceptor phase in the *in vitro* iontophoresis studies. Briefly, after 24 h of equilibration between the octanol and water phases (0.15 M of PBS pH 7.4 and 6.2), 5.0 ml of 0.02 mg ml^{-1} of rotigotine solution in buffer was mixed with 0.5 ml of octanol. Then the mixtures were shaken (GFL 1086, Salm en Kip BV, Breukelen, The Netherlands) at room temperature for 72 h. After equilibration, the mixtures were centrifuged for 15 min at a speed of 1800 rpm. Then, the water and octanol phases were separated manually. Rotigotine concentration was analyzed in both phases using HPLC. The octanol-water distribution coefficient was calculated according to Eq. 1.

$$\log D = \log \frac{C_{octanol}}{C_{water}} \quad (1)$$

in which $C_{octanol}$ and C_{water} refer to rotigotine concentration in the octanol and water phases, respectively. As the distribution coefficient at two different pH values (i.e., 7.4 and 6.2) has been measured, log P (octanol/water partition coefficient) as well as pK_a (the equilibrium dissociation constant) of rotigotine could be estimated using the equation below:

$$\log D = \log P - \log (1 + 10^{pK_a\text{-}pH}) \quad (2)$$

in which *pH* is the pH of the water phase.

In vitro Iontophoretic Studies

The *in vitro* iontophoresis studies were performed in side-by-side diffusion cells as described before (21). Briefly, a 9-channel computer controlled power supply was used to provide a constant current (Electronics Department, Gorlaeus Laboratories, Leiden University, The Netherlands). A silver plate electrode was used as anode and a silver/silver chloride electrode as cathode.

All diffusion experiments were carried out at a constant current density of 0.5 mA cm^{-2}, by using three-chamber continuous flow through diffusion cells at room temperature. HSC (Ø 18 mm) was hydrated for 2 h in PBS pH 7.4 *prior to* mounting in the cells. A sheet of HSC was placed between the anodal and acceptor side, and another sheet between acceptor and cathodal side, with the dermal sides of HSC facing the acceptor cell. At least three skin specimens were used for each experimental condition examined. Dialysis membrane (cut-off 5000 Da) was used as supporting membrane. Rotigotine was applied at the anodal side. The donor formulation was buffered with 5 mM citric acid. In the donor phase, ascorbic acid (6 mM) was added to prevent any possible oxidation of the drug. To maintain the solution osmolarity, an appropriate amount of D-mannitol was added to the donor solution. The cathodal side was filled with 0.15 M PBS, pH 7.4. The acceptor chamber was continuously perfused using a peristaltic pump with 0.15 M PBS of pH 7.4 or pH 6.2 (see below) containing sodium meta bisulphite (5 mM) as antioxidant. A flow rate of 6.5 ml h^{-1} was used. Each diffusion experiment

consisted of 6 h of passive diffusion, 9 h of iontophoresis followed by 5 h of passive diffusion. Every hour, samples were collected with an automatic fraction collector (ISCO Retriever IV, Beun De Ronde BV, Abcoude, The Netherlands). During the experiments, the anodal and cathodal compartments were magnetically stirred at 375 rpm. The composition of donor solutions for individual studies is outlined below. All other parameters of the procedure were identical.

Variation in pH and Rotigotine Concentration at the Anodal Side

The pH of the donor solution was either 4, 5, or 6 (citric buffer 5 mM: citric acid/Na-citrate = 0.62/0.63, 0.37/0.96, 0.12/1.30 g L^{-1} for pH 4, 5, and 6, respectively). The NaCl concentration in the donor compartment was 0.07 M. The concentration of rotigotine was either 0.5 or 1.4 mg ml^{-1} for each pH.

Variation in NaCl Concentration at the Anodal Side

NaCl concentration in the donor compartment was either 0.07 or 0.14 M at a pH of 5. The concentration of rotigotine was 0.5 mg ml^{-1}.

Influence of Using PBS pH 6.2 as Acceptor Compartment Medium

In this study, a PBS at pH 6.2 was used to perfuse the acceptor compartment. The rotigotine donor concentration was 1.4 mg ml^{-1} at a pH of 5.

Analytical Method

Samples collected during iontophoresis were injected directly into the HPLC system and analyzed by using a fluorescence detector (Jasco 821-FP, Gynkotek Separations, H.I. Ambacht, The Netherlands) at excitation and emission wavelengths of 270 and 305 nm, respectively. The attenuation and gain were set at 1 and 10, respectively. A Superspher 60, RP-select B, 75 mm-4 mm column (Merck KGaA, Darmstadt, Germany) was used. The mobile phase consisted of acetonitrile/0.1 M acetate buffer at pH 3.6 (40/60) v/v (flow rate of 0.7 ml min^{-1}). The calibration curves were linear ($r > 0.999$) in the concentration range of 0.02 to 1.2 μg ml^{-1}. The intra- and inter assay variation were less than 5% for all concentrations tested. The detection limit under these conditions was 12 ng ml^{-1}.

Electro-Osmosis Study

Rotigotine solution (1.4 mg ml^{-1}) and control solution (without rotigotine) containing 0.07 M of NaCl and 0.13 M of D-mannitol in 5 mM citric buffer at pH 6 were prepared. The solutions were spiked with 1 μCi ml^{-1} of ^{14}C-mannitol *prior to* filling into the donor compartments. The same iontophoresis protocol as described above was applied and the amount of radioactive mannitol transported was measured by liquid scintillation counting (1500 TRI-CARB Liquid Scintillation Analyzer Model A2010/01, Packard Bioscience, Groningen, The Netherlands). For this purpose, 1.4 ml of the sample was mixed with 3.5 ml of liquid scintillation cocktail (Ultima Gold XR, Packard Bioscience). For the donor and stock solutions, 50 μl of the solution was mixed with 1.4 ml PBS buffer, pH 7.4, before mixing with the scintillation cocktail. The number of disintegrations per minute (DPM) of each sample was counted for 10 min. Blank DPM (1.4 ml of PBS pH 7.4) was subtracted as a correction for the background activity. Volume flow (Jv) in μl cm^{-2} h^{-1} was determined by using Eq. 3.

$$Jv = \frac{DPM_{\text{acceptor}} \times \dfrac{Flow\ rate}{1.4}}{DPM_{\text{donor stock}} \times \dfrac{1}{0.05}} \times \frac{1000}{0.64} \tag{3}$$

in which DPM_{acceptor} and $DPM_{\text{donor stock}}$ are the DPM values for the acceptor solutions and the donor stock solutions, respectively.

Based on the volume flow values, the electro-osmosis flux of rotigotine can be estimated as follows:

$$J_{EO} = Jv \times C_D \tag{4}$$

in which J_{EO}, Jv, and C_D are the electro-osmosis flux, the volume flow, and the concentration of rotigotine donor solution, respectively.

By obtaining the electro-osmosis flux, the contribution of electro-osmosis to the total iontophoretic flux (*%Contribution*) can be calculated as follows:

$$\%Contribution = \frac{J_{EO}}{J_{TOTAL}} \times 100\% \tag{5}$$

in which J_{TOTAL} is the total iontophoretic flux of rotigotine.

Data Analysis

The flux and the cumulative amount of rotigotine transported were plotted as a function of time. From the cumulative flux plot, the estimated steady-state flux ($Flux_{ss}$) was calculated based on the diffusion lag-time method (22,23). The term *estimated* is given, as for most conditions, the real steady-state situation was not reached during the 9 h of iontophoresis. For this reason, the $Flux_{ss}$ was calculated from the slope of linear portion of the plot between 5 and 9 h of iontophoresis ($r > 0.999$). All results were expressed as mean values ± standard deviations. Statistical analysis was performed by using the one-way ANOVA followed by Newman-Keuls multiple comparison test (the influence of pH data), the two-way ANOVA test (rotigotine solubility data and the volume flux *vs.* time profile), and the unpaired Student's *t* test (the influence of NaCl concentration and the influence of the acceptor pH data). For all statistical analysis, the probability value of less than 0.05 was considered to be significant.

RESULTS

Solubility of Rotigotine

The values of rotigotine solubility at different pH value in the absence or presence of NaCl are presented in Table I. In the absence of NaCl, the solubility of rotigotine decreased from approximately 8.6 mg ml^{-1} to 7.9 mg ml^{-1} and 5.6 mg ml^{-1} at pH 4, pH 5, and pH 6, respectively ($p < 0.05$). The addition of 0.07 M NaCl decreased rotigotine solubility to around 2 mg ml^{-1} in all tested citric acid buffer solutions (pH 4, 5, and 6) ($p < 0.0001$).

Distribution Coefficient and the Determination of log P and pK_a of Rotigotine

The values of the octanol/water distribution coefficient of rotigotine (log D) at pH 7.4 and 6.2 were 3.41 ± 0.01 and

Table I. Solubility of Rotigotine in the Medium of pH of 4, 5, and 6 in the Absence or Presence of 0.07 M NaCl as Co-Ions

pH	No NaCl (mg ml^{-1})	0.07 M NaCl (mg ml^{-1})
4	8.56 ± 1.64	2.37 ± 0.11
5	7.88 ± 0.22	2.23 ± 0.12
6	5.57 ± 0.44	2.29 ± 0.06

The presence of NaCl significantly reduces rotigotine solubility in all pH values ($p < 0.0001$).

2.32 ± 0.02, respectively. The parameters of log P and pK_a of rotigotine were calculated as 4.03 and 7.90, respectively. The latter value is equal to the pK_a value provided by Schwarz Pharma (24).

Flux vs. Time Profile of Rotigotine Iontophoretic Transport

A representative example of a flux vs. time profile of rotigotine *prior to*, during, and after iontophoresis is shown in Fig. 1. As is shown in this figure, *prior to* iontophoresis, the flux is very low. By switching on the current after 6 h of passive diffusion, the fluxes gradually increase in time during the 9 h of iontophoresis. At 15 h the current was switched off, which resulted in a gradual decrease in flux. However at 5 h post-iontophoresis, the flux was still not equal to the value observed *prior to* iontophoresis. Interestingly when the higher concentration of rotigotine was used, namely 1.4 mg ml^{-1} (Fig. 1), the decrease in flux was delayed between 16 to 17 h.

Influence of pH

First, the influence of pH on the iontophoretic flux of rotigotine was studied at a rotigotine concentration of 0.5 mg ml^{-1} (Fig. 2). As shown in the figure, the rotigotine $Flux_{ss}$ was similar with values of 21.2 ± 6.8 nmol cm^{-2} h^{-1} at pH 4 vs. 22.7 ± 5.5 nmol cm^{-2} h^{-1} at pH 5. The highest $Flux_{ss}$ was achieved at pH 6, namely 30.0 ± 4.2 nmol cm^{-2} h^{-1} which is significantly

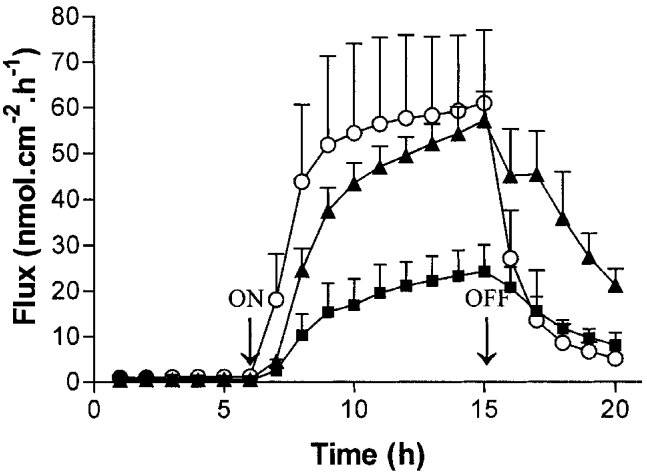

Fig. 1. Flux vs. time profiles for a 0.5 mg ml^{-1} rotigotine (closed square) and 1.4 mg ml^{-1} rotigotine (closed triangle) donor solution using PBS pH 7.4 as acceptor compartment medium and the flux vs. time profile of a 1.4 mg ml^{-1} rotigotine donor solution when PBS pH 6.2 was used as acceptor compartment medium (open circle). The anodal compartment is at pH 5 (citrate buffer) in the presence of 0.07 M NaCl. Data are presented as mean + SD (n = 5 to 6).

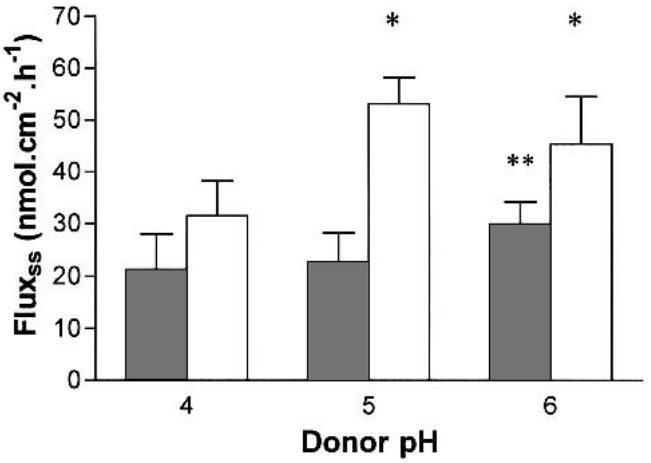

Fig. 2. Iontophoretic $Flux_{ss}$ of 0.5 mg ml^{-1} (filled bars) and 1.4 mg ml^{-1} (open bars) of rotigotine at pH 4, 5, and 6. Data are presented as mean + SD (n = 6): * indicates the significant different in $Flux_{ss}$ at pH 5 or 6 over pH 4 value (rotigotine concentration 1.4 mg ml^{-1}); ** indicates the significant difference in $Flux_{ss}$ at pH 6 over pH 4 and 5 values (rotigotine concentration 0.5 mg ml^{-1}).

different from the $Flux_{ss}$ at pH 4 and at pH 5 ($p < 0.05$). Similar experiments were also performed at a 1.4 mg ml^{-1} rotigotine concentration. Interestingly, at this concentration, no significant difference in $Flux_{ss}$ was observed between pH 5 and pH 6 ($p > 0.05$). The rotigotine iontophoretic $Flux_{ss}$ increased significantly from 31.5 ± 6.7 nmol cm^{-2} h^{-1} at pH 4 to 53.2 ± 5.0 nmol cm^{-2} h^{-1} at pH 5 ($p < 0.001$). A further increase in pH to 6 resulted in a rotigotine iontophoretic $Flux_{ss}$ of 45.4 ± 9.2 nmol cm^{-2} h^{-1} that was not significantly different from the $Flux_{ss}$ at pH 5 ($p > 0.05$).

Influence of NaCl Concentration

Because on theoretical grounds ion competition is one of the parameters affecting the iontophoretic flux, the effect of NaCl concentration on the rotigotine transport was studied. Increase in NaCl concentration in the donor compartment from 0.07 to 0.14 M resulted in a significant reduction in rotigotine $Flux_{ss}$ from around 23 to 14 nmol cm^{-2} h^{-1} ($p < 0.05$) (Table II).

Influence of Using PBS pH 6.2 as Acceptor Medium

In order to study the role of drug solubility in the acceptor medium, in one set of experiments the pH of the PBS solution was reduced to the value of 6.2. At the anodal side, a rotigotine concentration of 1.4 mg ml^{-1} at pH 5 was used. The flux profile is plotted in Fig. 1 and the calculated $Flux_{ss}$ is provided in Table II. The results show that a reduction in pH in the acceptor phase does not alter the iontophoretic $Flux_{ss}$ ($p > 0.05$), but had a dramatic effect on the flux profile. After switching the current on and off, the flux response was much faster than with an acceptor phase at pH 7.4. Furthermore, the delay of the flux decrease as observed with an acceptor phase at pH 7.4 at high rotigotine donor concentration was not observed with the acceptor phase at pH 6.2.

Electro-Osmosis During Iontophoresis of Rotigotine

Volume flow vs. time profile of iontophoresis rotigotine and buffer control solution is presented in Fig. 3A. In the

Table II. Iontophoretic Rotigotine $Flux_{ss}$ at Different NaCl Concentrations in the Donor Phase and Different pH Values in the Acceptor Phase

Conditions			Rotigotine $Flux_{ss}$ (nmol cm^{-2} h^{-1})		
Rotigotine conc. (mg ml^{-1})	NaCl conc. (M)	Acceptor pH	Mean	SD	n
0.5	0.07	7.4	22.7	± 5.5	6
0.5	0.14	7.4	14.1	± 4.9	6
1.4	0.07	7.4	53.2	± 5.0	6
1.4	0.07	6.2	58.9	± 17.0	5

Increase in NaCl concentration significantly reduces the $Flux_{ss}$ ($p < 0.05$), whereas pH of the acceptor phase did not change the $Flux_{ss}$ ($p > 0.05$).

absence of rotigotine after turning on the current, the volume flow gradually increased approaching an apparent steady-state condition (control experiment). After switching off the current, volume flow slowly decreased to a value that was higher relative to the value *prior to* iontophoresis. In the presence of rotigotine (1.4 mg ml^{-1}), the volume flow during current application was significantly lower compared to the control situation ($p < 0.0001$). After switching off the current, an elevated volume flow was observed relative to the value *prior to* iontophoresis. However, the post-iontophoresis volume flow in the presence and absence of rotigotine was not significantly different ($p > 0.05$).

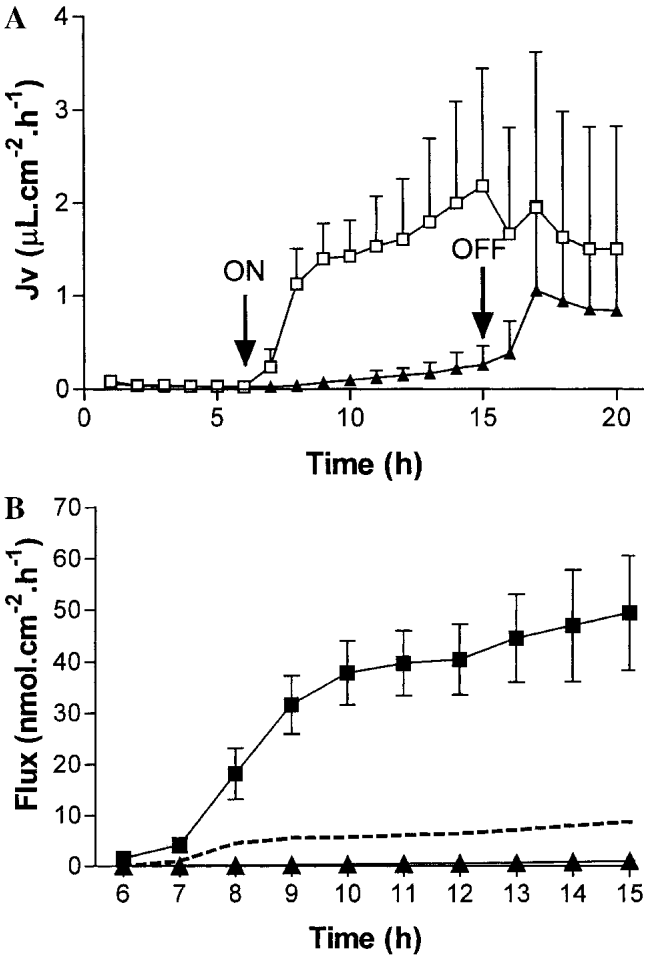

Fig. 3. (A) Volume flow vs. time profile of rotigotine (closed triangle) and control (open square) during iontophoresis with a pH of 6 in the anodal compartment. Data are presented as mean + SD (n = 4). (B) Time course profiles of the estimated electro-osmosis flux (closed triangle) in comparison to the total iontophoretic flux (closed square) of rotigotine during the 9 h of iontophoresis of 1.4 mg ml^{-1} of rotigotine at pH 6. Data are presented as mean ± SD (n = 4 to 6). The dashed curve refers to the theoretical values of the maximum electro-osmosis flux of rotigotine.

Time course profile of the electro-osmosis flux of rotigotine in comparison to the total iontophoretic flux is shown in Fig. 3B. The figure demonstrates that in the presence of rotigotine at the highest concentration, the electro-osmosis contribution to the total iontophoretic flux is very low and estimated less than 2%.

DISCUSSION

The solubility of rotigotine is an important determinant of the maximum drug concentration in the donor compartment. In the absence of NaCl, the solubility was approximately 8 (at pH 4 and 5) and 5.5 mg ml^{-1} (at pH 6). However, in the presence of NaCl, the solubility reduced to around 2 mg ml^{-1} for all donor solutions irrespective of the pH. This illustrates that NaCl has a strong salting out effect. To avoid rotigotine crystal formation during the experiment, it was decided to use 1.4 mg ml^{-1} rotigotine as the maximum donor concentration, which is approximately 70% of the maximum rotigotine solubility in the presence of NaCl.

As already indicated by the low solubility of rotigotine, the value of the partition coefficient confirms that rotigotine is indeed a very lipophilic compound with a log P value of 4.03. A reduction in the distribution coefficient is observed at pH 6.2 as a result of the much higher solubility of rotigotine at this pH value. Furthermore the calculated pK_a of rotigotine is 7.9. This value confirms that rotigotine is positively charged at the pH values chosen for the anodal compartments.

As shown in Fig. 1, the pH in the anodal compartment has an important influence on the iontophoretic rotigotine transport. At a concentration 0.5 mg ml^{-1} of rotigotine, an increase in pH from 4 to 6 enhanced the rotigotine transport. At a 1.4 mg ml^{-1} rotigotine concentration, a shift in pH from 4 to 5 also increased the iontophoretic flux, but an additional increase from pH 5 to 6 did not enhance the iontophoretic transport further.

There are four mechanisms of transport during iontophoresis: passive diffusion, convective flow, electro-osmosis, and electro-repulsion. For all conditions used in our studies, roti-

gotine passive flux *prior to* iontophoresis was always very low. This is probably due to the ionic nature of the drug at our experimental conditions. Due to this low transport, the passive flux contribution to the rotigotine flux during iontophoresis is negligible. The second factor, the convective flow, may in certain cases play an important role. In case of azidothymidine iontophoresis, the addition of NaCl increased the flux until the concentration of 0.1 M after which a flux plateau was achieved (25). Likewise with iontophoresis of verapamil, above the minimum NaCl concentration threshold, verapamil permeation rate is increased due to an increase in the convective flow (26). Convective flow also significantly contributes to the iontophoretic transport of R-apomorphine. As recently reported, a decrease in NaCl concentration from 8 to 2 g L^{-1} did not change the steady-state iontophoretic flux of R-apomorphine. The authors explained this by a counterbalance between on one hand an increase in electro-osmosis and electro-repulsion contributions and on the other hand a decrease in convective flow contribution (16). However, the situation is probably different with rotigotine. The reduction in NaCl concentration from 0.14 to 0.07 M increased the iontophoretic flux significantly. Furthermore it appears that the convective flow plays a less important role than electro-osmosis and electro-repulsion.

At both concentrations of rotigotine, 0.5 and 1.4 mg ml^{-1}, the flux at the anodal compartment pH of 4 is smaller than at higher pH values. This can be explained by at least two factors. i) At pH 4, the concentration H^+ ions is increased, which promotes the competition for charge transfer across HSC. As a result, a smaller fraction of the charge will be transported by the larger rotigotine ion at pH 4 resulting in a reduction of the rotigotine iontophoretic flux. ii) As the pI of HSC is around 4.8 (27), at pH 5 and 6, the skin is negatively charged, which results in an electro-osmotic flow directed from anode to cathode. This will elevate the anodal iontophoretic flux of rotigotine. At pH 4 the HSC is slightly positively charged, which results in a reversed direction of the electro-osmotic flow and consequently a reduction of the anodal iontophoretic rotigotine flux.

The change in iontophoretic rotigotine flux between pH 5 and 6 strongly depends on the rotigotine concentration in the donor compartment (Fig. 2). This can be explained by a difference in electro-osmotic flow. Due to the stronger negative charge of HSC at pH 6, the electro-osmotic transport is higher at this pH value resulting in a higher rotigotine flux. At a rotigotine concentration of 0.5 mg ml^{-1}, indeed a higher iontophoretic flux was observed at pH 6 compared to pH 5. However, this was not observed at the higher rotigotine concentration. As demonstrated by the mannitol transport study (Fig. 3), at high rotigotine concentration, the volume flow from anode to cathode is minimized. This is most probably due to the positively charged rotigotine that shields the negative charge of HSC thereby minimizing the electroosmotic transport of rotigotine. This has also been observed in a number of previous studies for other drugs (28).

When rotigotine was present at the highest concentration in the donor phase, the electro-osmosis contributes for less than 2% to the iontophoretic flux of rotigotine indicating a low contribution of electro-osmosis to the total iontophoretic flux. However, as discussed above, this might also be due to strong rotigotine binding that reduces the HSC perm-selectivity thereby also reducing the electro-osmosis. To confirm this hypothesis, we calculated the theoretical value of the maximum electro-osmosis flux (J_{EO} *Max*) of rotigotine assuming that the presence of rotigotine does not affect HSC perm-selectivity. Thus, in this case, the maximum volume flux is assumed can be reached even in the presence of rotigotine at the highest concentration. The theoretical J_{EO} *Max* is presented as a dashed curve in Fig. 3B. Even in this theoretical condition, the average contribution of electro-osmosis is estimated to be less than 17%. This indicates that indeed electro-osmosis contributes less to the iontophoresis. This also confirms that electro-repulsion is the main mechanism of transport during rotigotine iontophoresis, although electro-osmosis might significantly contribute to the iontophoretic transport at low rotigotine concentrations. This is in an excellent agreement with previous observations on the small and charged molecules (29,30).

Another characteristic feature of the iontophoretic flux profile of rotigotine is a slow increase in iontophoretic transport during the 9 h of iontophoresis. As shown in Figs. 1A and 1B, except when PBS pH 6.2 was used as acceptor phase, no steady-state flux was achieved. In order to explain this phenomenon, we propose that the rate of drug transport to the acceptor phase depends on two factors: the rate of drug influx to HSC and the rate of drug release from HSC to the acceptor compartment. The first factor is determined by the rate of drug mass transport from the donor to the HSC which is driven by the electro-repulsion and the electro-osmosis contribution as discussed above. The second factor is determined by the rate of drug partitioning from the HSC into the acceptor compartment. Comparing the two profiles in Fig. 1B, where the compositions in the anodal and cathodal compartment were identical with regard to drug concentration, pH, and NaCl concentration, the contribution of the first factor is very similar in the two experiments, as the only difference is the pH of the acceptor phase. However, the rate of partitioning from HSC to the acceptor phase, the second factor, is influenced by the difference in pH of the acceptor phase during the two experiments. At pH 7.4, almost 24% of rotigotine is neutral, whereas at pH 6.2 this percentage is only 2%. The distribution coefficient values, log D at pH 7.4 was higher than that at pH 6.2. As a result, rotigotine partition into the acceptor phase at pH 7.4 was much slower than at pH 6.2. As the solubility in the acceptor phase is the only key parameter that changes in these experiments, this low solubility is most likely the main determinant for the slow response in flux by switching the current on and off.

The low solubility of rotigotine into the acceptor phase probably also plays a key role in the delay in the decrease of the flux between 16 and 17 h (Fig.1). If during iontophoresis the rate of drug transported into HSC is higher than the rate of drug partitioning from HSC to the acceptor medium, rotigotine accumulates in HSC. After switching off the current, the accumulated rotigotine is released from the HSC to the acceptor phase, which can explain the observed reduction in flux decline. As demonstrated in Fig.1, at the concentration of 0.5 mg ml^{-1}, no delay in flux decline is observed, which is in agreement with this hypothesis.

In the various conditions studied, at 5 h after termination of the iontophoresis, the rotigotine flux was still higher than the passive diffusion *prior to* iontophoresis. Several studies indicate that this is due to either an increased hydration of the HSC or a perturbation in the HSC lipid structure by ionto-

phoresis (31,32). Moreover, Fig. 3A shows an increase in volume flux after iontophoresis in comparison with pre-iontophoresis conditions in the presence as well as in the absence of rotigotine. As mannitol is a quite hydrophilic compound, increase in the passive transport of mannitol after switching off the current was only possible by an increase of the transport through the polar pathway in HSC. The presence of water is the most reasonable explanation of this phenomenon.

In summary, iontophoretic transport of rotigotine at a drug donor concentration of 0.5 mg ml^{-1} was highest at pH 6. However, when increasing the donor concentration to 1.4 mg ml^{-1}, no significant difference in iontophoretic rotigotine transport was observed between pH 5 and 6. Due to ions competition, increase in NaCl concentration reduced the iontophoretic transport of rotigotine across HSC. The slow increment in flux and retardation of flux declination was due to a low solubility of rotigotine from HSC to the acceptor phase. Electro-repulsion is the main mechanism for rotigotine iontophoresis as the electro-osmosis contribution was estimated less than 17%.

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