



Universiteit
Leiden
The Netherlands

Transdermal iontophoretic delivery of dopamine agonists: in vitro - in vivo correlation based on novel compartmental modeling

Nugroho, A.K.

Citation

Nugroho, A. K. (2005, May 11). *Transdermal iontophoretic delivery of dopamine agonists: in vitro - in vivo correlation based on novel compartmental modeling*. Retrieved from <https://hdl.handle.net/1887/2316>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/2316>

Note: To cite this publication please use the final published version (if applicable).

Chapter 8

PHARMACOKINETICS AND PHARMACODYNAMICS ANALYSIS OF TRANSDERMAL IONTOPHORESIS OF 5-OH DPAT IN RATS: *IN VITRO – IN VIVO CORRELATION*

Akhmad Kharis Nugroho^{1,2}, Stefan Romeijn¹, Raphaël Zwier³, Jan B de Vries⁴,
Durk Dijkstra⁴, Håkan Wikström⁴, Oscar Della-Pasqua⁵, Meindert Danhof⁵ and
Joke A. Bouwstra¹

¹. Division of Drug Delivery Technology Leiden/Amsterdam Center for Drug Research, Einsteinweg 55 2300 RA Leiden The Netherlands, ². Faculty of Pharmacy Gadjah Mada University Sekip Utara Yogyakarta 55281 Indonesia, ³. Fine and Mechanical Department Leiden University Einsteinweg 55 2300 RA Leiden The Netherlands, ⁴. Department of Medicinal Chemistry, University Center of Pharmacy, University of Groningen, Antonius Deusinglaan 1 Groningen, NL-9713 AV Groningen, The Netherlands, ⁵. Division of Pharmacology, Leiden/Amsterdam Center for Drug Research, Einsteinweg 55, 2300 RA Leiden The Netherlands.

(submitted for publication, 2004)

ABSTRACT

The pharmacokinetics (PK) and the dopaminergic effect (PD) of 5-OH-DPAT *in vivo* were determined following transdermal iontophoretic delivery in rats on the basis of: 1) drug concentration in plasma (C_p), 2) drug concentration in striatum (C_s), and 3) the dopamine levels in striatum (C_{DA}). Data were analyzed on the basis of an integrated population PK-PD model. The PK analysis was based on the previously proposed compartmental model for transdermal iontophoresis whilst the PD analysis was based on the indirect response model with inhibition of response production, i.e. dopamine release (IDR type I). To determine the correlation of the *in vitro* transport with the *in vivo* PK-PD profiles during transdermal iontophoresis, the *in vitro* transport of 5-OH-DPAT was characterized in the transport in dermatomed rat skin (DRS) and in rat stratum corneum (RSC). A quantitative analysis of the transport was performed on the basis of an *in vitro* compartmental model for transdermal iontophoresis.

The integrated *in vivo* PK-PD model and the *in vitro* model allowed the estimation of PK, PD and *in vitro* iontophoretic transport parameters as well as successfully described the time course of C_p , C_s , C_{DA} , and the *in vitro* flux in DRS and in RSC. The population value of the steady state flux (J_{ss}) *in vivo* was comparable to J_{ss} *in vitro* in DRS ($31.8 \text{ nmol cm}^{-2} \text{ h}^{-1}$ versus $39.0 \text{ nmol cm}^{-2} \text{ h}^{-1}$, $p > 0.05$). On the other hand, the skin release rate constant (K_R) *in vivo* was

similar to the K_R in RSC (4.4 h^{-1} versus 2.58 h^{-1} , $p > 0.05$). The kinetic lag time (t_L) *in vivo* was negligible, which is close to the *in vitro* t_L in RSC (0.04 h , $p > 0.05$). Based on non-linear mixed effects modeling, the profiles of C_p , C_s , and C_{DA} were successfully predicted using *in vitro* values of J_{ss} in DRS with K_R and t_L in RSC. Transdermal delivery of 5-OH-DPAT by iontophoresis in rats yields a considerable dopaminergic effect. These findings strongly indicate that it is feasible to reach therapeutically effective concentrations of 5-OH-DPAT upon transdermal iontophoretic delivery.

A. INTRODUCTION

Recently, we have proposed a family of compartmental models for characterizing transdermal iontophoretic transport *in vitro* (1) and *in vivo* (2) in a strictly quantitative manner. These models constitute the theoretical basis for the evaluation of *in vitro-in vivo* correlations of transdermal iontophoretic transport.

5-OH-DPAT is one of the most potent dopamine agonists from 2-aminotetralin group (3). However, due to its low oral bioavailability (approximately 1% (4)), its utility in Parkinson's disease therapy is limited. Transdermal iontophoresis is an alternative route of delivery that can overcome these bioavailability issues. The method may offer the possibility to deliver a sufficient amount of the drug transdermally, as well as to optimize dose titration by controlling current intensity (5).

Previously, we reported the feasibility of transdermal iontophoretic delivery of 5-OH-DPAT *in vitro*, as determined by drug transport across human stratum corneum (HSC) and dermatomed human skin (DHS) (6). Assuming that a sufficient amount of 5-OH-DPAT can be delivered to the striatum, the activation of the dopaminergic receptors will result in a negative feedback on the dopamine production under the control of dopamine D_2 autoreceptors (7). In an animal model, such a feedback can be measured by the suppression of the dopamine release in the striatum using brain-microdialysis (4,8) The negative feedback mechanism can subsequently be characterized by an indirect response model with the inhibition of the input rate (IDR type I) (9,10)

In the present study we applied non-linear mixed effects modeling: 1) to characterize the transdermal iontophoretic transport of 5-OH-DPAT *in vivo* in rats; 2) to characterize the transdermal iontophoretic transport of 5-OH-DPAT *in vitro* in dermatomed rat skin (DRS) and in rat stratum corneum (RSC); 3) to explore the *in vitro-in vivo* correlation of transdermal iontophoretic transport as a predictor of the effect of 5-OH-DPAT on striatal dopamine release.

The use of non-linear mixed effects modeling enables assessment of population and individual pharmacokinetic-pharmacodynamic (PK-PD) parameter estimates using experimental data from separate experiments (11). This approach is therefore essential to optimally combine data from the various

in vitro and *in vivo* experiments required to establish an *in vivo-in vitro* correlation.

B. THEORY

The assessment of the correlation between *in vitro* drug transport and *in vivo* PK and PD profiles requires certain quantitative models. Previously, we have proposed the kinetic models based on compartmental mass transfer to describe the iontophoretic transport *in vitro* (1) and *in vivo* (2). In the present investigation these models are combined with the IDR type I into a comprehensive PK-PD model for the effect of 5-OH-DPAT delivered by transdermal iontophoresis on striatal dopamine release.

1. *In vitro* models

The schematic presentations of the *in vitro* models are depicted in Fig. 1A, in which panel I describes the transport in iontophoretic phase and panel II describes the transport in the post iontophoretic period. The equations derived for the *in vitro* flux from those models are presented in equation 1 and equation 2, respectively for the iontophoretic phase and for the post iontophoretic period.

$$J(t) = \frac{I_0}{S} (1 - e^{-K_R \cdot (t - t_L)}) \quad (1)$$

$$J(t) = \frac{P_{PI}}{S} (1 - e^{-K_R \cdot (t - T)}) + \frac{I_0}{S} (1 - e^{-K_R \cdot (T - t_L)}) \cdot e^{-K_R \cdot (t - T)} \quad (2)$$

In these equations $J(t)$ is the flux at time t , I_0 is the zero order drug input due to the iontophoretic driving force, P_{PI} is the zero order drug input due to the passive driving force in the post iontophoretic period, K_R is the skin release rate constant, S is the area of diffusion in DRS and in RSC, t_L is the kinetic lag time of the drug to enter skin compartment, and T is duration of current application.

2. *In vivo* PK model

The PK model used in this study differs slightly from the previously proposed *in vivo* models for transdermal iontophoresis (2). Modifications were introduced to account for the need to simultaneously fit 5-OH-DPAT concentrations in plasma (C_p), and in striatum (C_s), as well as dopamine concentration in striatum (C_{DA}) upon transdermal iontophoresis. Data from 5-OH-DPAT plasma concentrations following an intravenous infusion was also included in the analysis to improve estimation of relevant PK parameters, such as clearances and volumes of distribution. The schematic illustration of the model is presented in Fig. 1B.

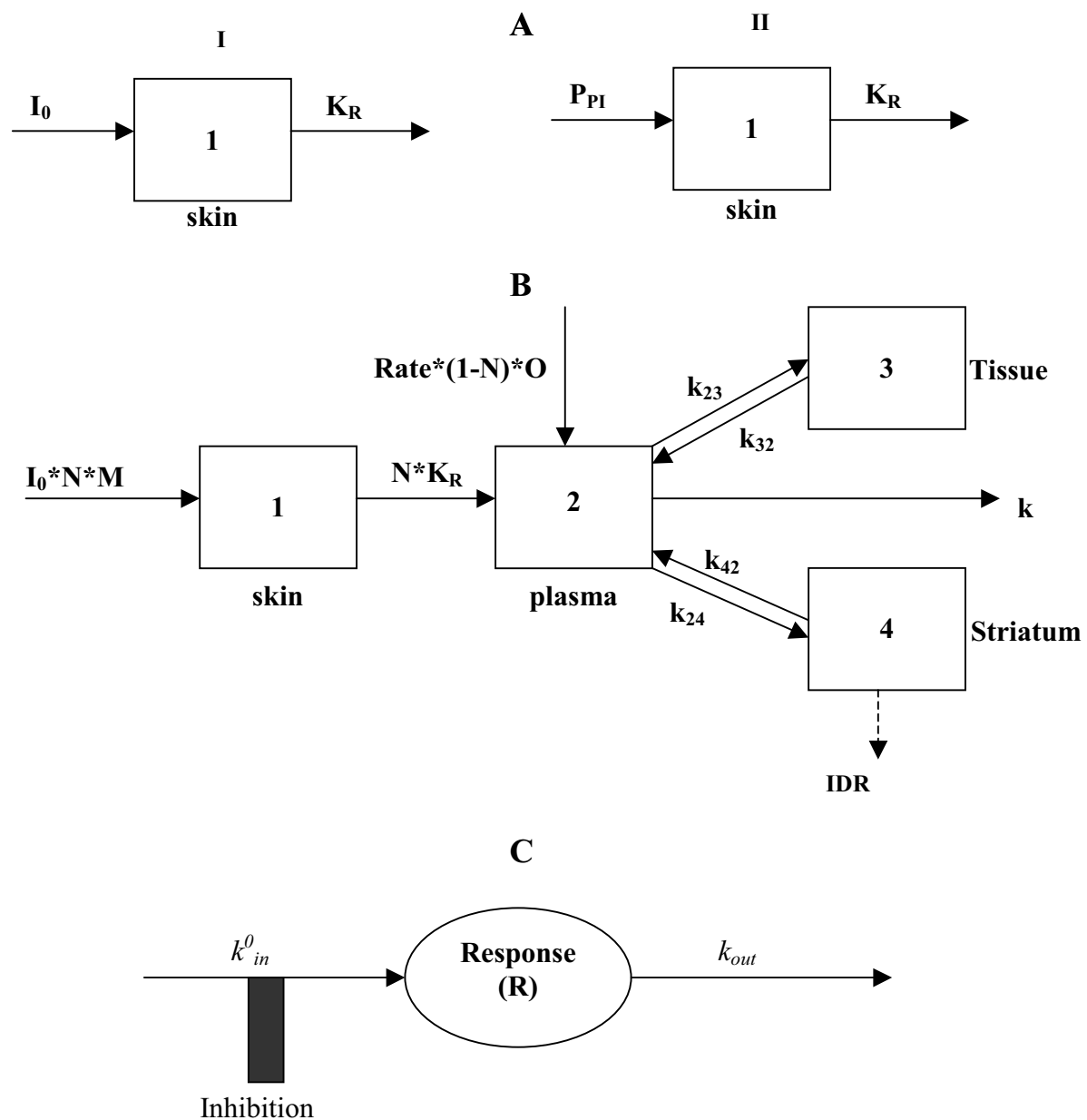


Fig. 1. The schematic representation of the compartment model of the iontophoretic transport *in vitro* (A), the pharmacokinetics models following intravenous infusion and transdermal iontophoresis (B) and the indirect response model (C).

Legends:

I_0 : the zero order input due to iontophoretic driving force, P_{PI} : the zero order input due to passive driving force post iontophoresis, K_R : Skin release rate constant, N : flag number, i.e. $N=0$ for intravenous infusion and $N=1$ for transdermal iontophoresis, M : flag number, i.e. $M=1$ for iontophoretic phase and $M=0$ at post iontophoretic period, O : flag number, i.e. $O=1$ during the duration of intravenous infusion and $O=0$ after termination of intravenous infusion, $Rate$: intravenous infusion rate, k : Elimination rate constant, k_{23} : plasma-tissue distribution rate constant, k_{32} : tissue-plasma distribution rate constant, k_{24} : plasma-striatum distribution rate constant, k_{42} : striatum-plasma distribution rate constant, IDR : Indirect response to inhibition of dopamine production

The *PREDPP* subroutine in NONMEM (12) was used to solve the ordinary differential equations (ODE). Data fitting was performed with the derived ODEs as presented in equation 3 to equation 6. In addition, preliminary analysis indicated that the kinetic lag time *in vivo* (t_L) was negligible. Therefore, the value of t_L was constrained to zero in all cases.

$$\frac{dX_1(t)}{dt} = N * (I_0 * M - K_R * X_1(t)) \quad (3)$$

$$\frac{dX_2(t)}{dt} = K_R * X_1(t) + Rate * (1 - N) * O - k * X_2(t) - k_{23} * X_2(t) - k_{24} * X_2(t) + k_{32} * X_3(t) + k_{42} * X_4(t) \quad (4)$$

$$\frac{dX_3(t)}{dt} = k_{23} * X_2(t) - k_{32} * X_3(t) \quad (5)$$

$$\frac{dX_4(t)}{dt} = k_{24} * X_2(t) - k_{42} * X_4(t) \quad (6)$$

In these equations, $\frac{dX_i(t)}{dt}$ is the rate of change in the amount of the drug in compartment i . $X(i)$ is the amount of the drug in compartment i , which refers to a skin compartment ($i=1$), plasma compartment ($i=2$), tissue compartment ($i=3$) and striatum compartment ($i=4$). The term *Rate* refers to the zero-order intravenous infusion rate, k is the first-order elimination rate constant, k_{23} , k_{32} , k_{24} and k_{42} are the first-order distribution rate constants, respectively from plasma to tissue, from tissue to plasma, from plasma to striatum, and from striatum to plasma. The terms N , M , and O are *flags* in the model and are defined as follows: $N=0$ for intravenous infusion and $N=1$ for transdermal iontophoresis; $M=1$ for the iontophoretic phase and $M=0$ in the post iontophoretic period; $O=1$ during the duration of intravenous infusion and $O=0$ after termination of intravenous infusion.

3. PD model

The IDR type I model was used to correlate the inhibition of dopamine release to 5-OH-DPAT striatum concentration (C_s). Since microdialysis enables the assessment of brain drug concentrations, the levels of 5-OH-DPAT in striatum could be linked directly to the dopaminergic effect instead of relying on 5-OH-DPAT concentrations in plasma (C_p). The IDR model is schematically presented in Fig. 1C. The equation and the ODE of the model is as follows:

$$\frac{dC_{DA}(t)}{dt} = k_{in}^0 * (1 - \frac{I_{max} * C_s(t)^H}{IC_{50}^H + C_s(t)^H}) - k_{out} * C_{DA}(t) \quad (7)$$

$$k_{in}^0 = k_{out} * C_{DA0} \quad (8)$$

In these equations $\frac{dC_{DA}(t)}{dt}$ is the rate of the change in the dopamine concen-

tration in striatum, I_{max} is the maximum inhibition of the dopamine production, $C_s(t)$ is the concentration of 5-OH-DPAT in striatum at time t , IC_{50} is the concentration of 5-OH-DPAT in striatum required to produce 50% of I_{max} , H is the Hill-slope coefficient (which is constrained to 1), k_{out} is the first-order rate constant for the loss of response, k_{in}^0 is the zero order rate constant for the production of response, C_{DA0} is the baseline values of dopamine (i.e., dopamine concentration *prior* to the inhibition effect of 5-OH-DPAT).

In this research two groups of animals were involved, i.e. group A (rats number 1-8, for PK and *in vitro* iontophoretic transport studies) and group B (rats number 9-12, for PD studies). To implement the PK-PD modeling, two important assumptions are used: 1) the pharmacokinetics of 5-OH-DPAT is identical in group A and group B, and 2) the recovery of the microdialysis probe is 100% for both 5-OH-DPAT and dopamine in all animals.

C. MATERIALS AND METHODS

1. Materials

5-OH-DPAT (HBr salt, purity >98%) was synthesized at the Department of Medicinal Chemistry of the University of Groningen, Groningen, The Netherlands. Silver and silver chloride (purity > 99.99%) were obtained from Aldrich (Borneum, Belgium). Ascorbic acid, trypsin (Type III, from a bovine pancreas) and trypsin inhibitor (Type II-S from soybean) were purchased from Sigma Chemicals (Zwijndrecht, The Netherlands). HPLC grade acetonitrile was obtained from Rathburn (Walkerburn, UK). All other chemicals and solvents were of analytical grade. All solutions were prepared in Millipore water with resistivity of more than 18M Ω .

2. Iontophoretic patches

The iontophoretic patches were prepared from elastic silicon materials (silicon: RTV-400T with the activator Beta19, GE Bayer Silicones, BV, Bergen op Zoom, The Netherlands). The active area of the patches was 2.5 cm² with a volume of 2.3 ml. The circle electrodes (diameter of the contact area was 0.5 cm) were placed in the upper side of the patch with a distance from the skin surface of approximately 1 cm. The anodes were made from the silver plate while the cathodes were made from silver plate after electrochemical coating with silver chloride at the side with contact to the PBS solution. The wound dressing film (Opsite* FLEXIGRID*, Smith&Nephew, Hoofddorp, The Netherlands) was used to attach the patch to the rat skin. The attachment of silicon patch to the back part of the Opsite* FLEXIGRID* was performed by using the medical silicon adhesive (MD7-4602, obtained as a gift from Dow Corning, Belgium). The design of the patch is depicted in Fig. 2.

3. Animals

The PK and the PD studies described below were approved by the ethical committee of Leiden University and by the ethical committee of the University of Groningen respectively.

The PK-PD studies were performed in male albino Wistar WU rats, weight 280-320g (Charless River, The Netherlands). The rats were housed in plexiglas cages, 6 animals in each cage with free access to water and standard laboratory chow. The cages were placed in a room with a controlled environmental conditions (temperature 21°C, humidity 60-65%, the duration of light on and off was 12 hours). The animals were housed at least one week *prior* to surgery. The surgery consisted of femoral artery and femoral vein cannulations (for PK studies) or implantation of the microdialysis probes in the striatum (PD studies). In the PK studies, rats were used for either intravenous infusion or transdermal iontophoresis studies immediately after the surgery. In PD studies, the rats were given a 10-24 hours recovery period *prior* to the microdialysis studies. After surgery, the animals were housed individually in cages and were supplied with a standard laboratory chow and a free access to water.

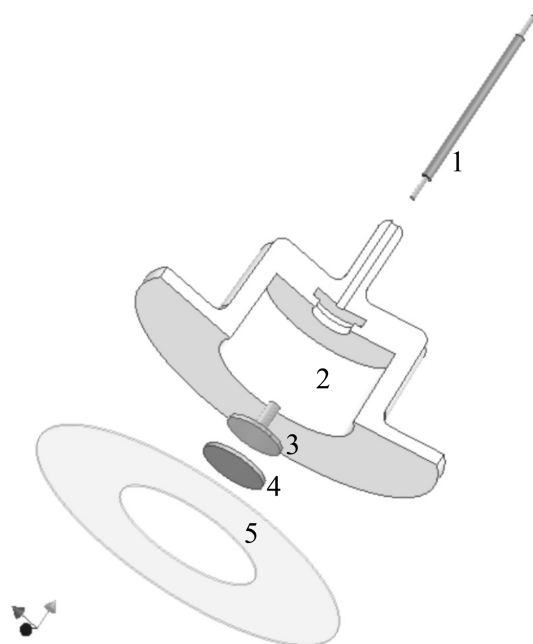


Fig. 2. The schematic design of the iontophoretic patch.

Legends:

1. Wire, 2. Active chamber from elastic silicon (volume 2.3 ml, active area 2.5 cm²), 3. Gold-plated connector, 4. Electrode, 5. Opsite* FLEXIGRID* adhesive layer.

4. PK studies following intravenous infusion and transdermal iontophoresis

The animals were anaesthetized with a combination of Dormicum[®] (midazolam 5 mg ml⁻¹, Roche Nederland, Mijdrecht, the Netherlands) and Hypnorm[®] (fentanyl citrate 0.315 mg ml⁻¹ + fluanizone 10 mg ml⁻¹, Janssen Pharmaceutica, Beerse, Belgium) at a dose of 0.5 mg kg⁻¹ rat weight. The permanent cannulation was performed using Polythene tubings (Rubler BV, Hilversum The Netherlands) with the diameter of 0.58 mm (ID) - 0.96mm (OD) and 0.28 mm (ID) - 0.61 mm (OD), respectively for femoral vein cannulation and femoral artery cannulation. The tubings were inserted approximately 3 cm inside the vessels. The cannulas were externalized through the skin at the back of the neck.

Directly after the surgery, the rats received 5-OH-DPAT solution either via an intravenous infusion or via transdermal iontophoretic delivery. During both studies, the rats were maintained in an anaesthetized condition with the same dose of Hypnorm and Dormicum.

The total intravenous dose administered to the rats was 75 µg over 15 minutes, with the exception of one animal, which received a total dose of 16.5 µg. The blood samples (0.2 ml) were transferred from the femoral vein cannula into the lithium-heparin containing tubes (microvette CB 200 LH-transparent, Sarstedt BV, Etten-Leur, The Netherlands) at 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, and 180 minutes. Subsequently, the plasma samples were separated from the blood cells by centrifugation at a speed of 1400 rpm for 10 minutes *prior* to storage at -20 °C until analysis.

Prior to transdermal iontophoretic studies, the hairs of the back of the rats were electrically clipped out. The skin surface was gently wiped with a tissue paper containing Millipore water to remove any contaminants from the skin surface. A pair of patches was then attached to the skin surface. At approximately 15 minutes after patch attachment, the solutions of 1.3 mg ml⁻¹ of 5-OH-DPAT and PBS pH 7.4 were filled, respectively into the anodal and cathodal patches using disposable syringes. After 15 minutes of passive diffusion, the current (0.25 mA cm⁻²) was switched on for 3 hours. During iontophoresis blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes. After current removal at 3 hours blood samples were collected at 195, 210, 240, 270, 300, and 360 minutes. These samples were treated identical to the protocol described above for the intravenous administration.

5. PD (On-line microdialysis) studies following transdermal iontophoresis

Prior to surgery, the rat was anaesthetized with Isofluran 2% in N₂O/O₂ (2/1). The rat was then mounted into a stereotaxic frame (Kopf). The incisor bar was placed in a position to allow the skull to be horizontally held. The skull was exposed and was anaesthetized with 10% lidocaine before the holes for probe insertion were drilled. An Y-shaped dialysis probe was used for the

experiments, with an exposed tip length of 3 mm. The dialysis tube (diameter: 0.22 mm (ID) - 0.31 mm (OD)) was prepared from polyacrylonitrile/sodium methallyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis membrane was implanted in the striatum. The dura was cut with a sharp needle. Two anchor screws were positioned in different bone plates nearby. The following coordinates were used according to the atlas of Paxinos and Watson (13): AP $\pm$ 0.5, LM $\pm$ 3.0 relative to bregma, and Vd - 6.0 below dura. Before insertion into the brain, the dialysis probe was perfused successively with Millipore water, methanol, Millipore water and Ringer solution (1.2 mM Ca²⁺). The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was cemented in this position with dental cement. After the surgery, the rats received fentanyl 1 mg kg⁻¹ subcutaneously as an analgesic agent. Thereafter the rats were housed individually.

The microdialysis experiments upon transdermal iontophoretic delivery of 5-OH-DPAT were performed in anaesthetized rats 17–48 h after implantation of the probes. The same protocol of transdermal iontophoresis for PK studies was applied. During the experiment, the animals were kept under anesthesia by a subcutaneous injection of 6% chloralhydrat. The striatum was perfused with Ringer solution (147 mmol l⁻¹ NaCl, 4 mmol l⁻¹ KCl, 1.2 mmol l⁻¹ CaCl₂, 1.1 mmol l⁻¹ MgCl₂) at a flow of 2 μ l minute⁻¹ (CMA/102 microdialysis pump, Sweden). The concentration of dopamine was quantified from the probe implanted at the left striatum by an on-line HPLC with electrochemical detection with the detection limit of 1 fMol/sample. *Prior* to application of the drug, four measurements of C_{DA} for the baseline values were performed. The HPLC pump (LC10-AD-Shimadzu) was used in conjunction with an electrochemical detector (Coulchem, ESA) working at oxidation + 250mV and reduction -275 mV. The analytical column was Supelco Supelcosil LC-18 (particle size: 3 μ m). The mobile phase consisted of a mixture of 4.1 g l⁻¹ sodium acetate (Merck), 85 mg l⁻¹ octane sulphonic acid (Sigma-Aldrich Chemie B.V, Zwijndrecht, The Netherlands), 50 mg l⁻¹ EDTA (Merck B.V, Amsterdam, The Netherlands), 8.5% methanol (Labscan, Hasselt, Belgium) and Millipore water (pH 4.1 with glacial acetic acid). In addition, the dialysate from the probe in the right striatum were collected hourly. The solution was then analyzed using the LC-MS-MS method for the concentration of 5-OH-DPAT (defragmentations are at m/z 248 followed by m/z 147).

6. Preparation of Dermatomed Rat Skin (DRS) and Rat Stratum Corneum (RSC)

At least 1.5 weeks after the last *in vivo* experiment, the rats were sacrificed by an intravenous injection of an overdose of pentobarbital. The hairs of the rats skin from the back of the rats (the place where the iontophoretic patches were situated during the *in vivo* studies) were removed by using the electric clipper. The skin was then collected using a surgical blade. After the

removal of the subcutaneous residual, the skin surface was carefully wiped with a tissue paper soaked in Millipore water. Dermatomed rat skin (DRS) was then obtained by dermatoming the skin to a thickness of approximately 300 μm using a Padgett Electro Dermatome Model B (Kansas City, USA). In order to obtain rat stratum corneum (RSC), DRS was treated with the same protocol to isolate the stratum corneum in human skin as described previously (6,14).

7. *In vitro* Iontophoretic Studies

The *in vitro* iontophoretic studies were performed according to the similar protocol for the studies in human stratum corneum (HSC) and dermatomed human skin (DHS) as described previously (6,14) with several exceptions as follows: 1) The donor solution was the solution of 1.3 mg ml^{-1} of 5-OH-DPAT with the composition identical to the solution used for *in vivo* studies (buffered at pH 5 with NaCl concentration of 0.07M); 2) The protocol of current application was identical to the *in vivo* studies; 3) The acceptor phase was maintained at 32 $^{\circ}\text{C}$

8. HPLC analysis

Rotigotine solution at a concentration of 500 ng ml^{-1} (volume of 10 μl), which was used as the internal standard, was vortex-mixed into 100 μl of plasma samples. Thereafter, 100 μl of 10% NaCO_3 was vortex-mixed to the plasma. After addition of 0.5 ml of Millipore water and 2 ml of dichloromethane/cyclohexane (45:55 v/v), the mixtures were shaken for 15 minutes at 1400 rpm (IKA-VIBRAX-VXR, Omnilabo International BV, Breda, The Netherlands). Subsequently, the mixtures were centrifuged at 3000 rpm for 5 minutes. The organic layer was separated from the water phase and was transferred into a clean glass-tube. To the water phase, the extraction using dichloromethane/cyclohexane (45:55) was repeated with the same protocol. The organic layer was then evaporated to dryness under a stream of nitrogen at 40 $^{\circ}\text{C}$. The residue was then reconstituted in 200 μl of 20% acetonitrile in Millipore water, and then analyzed using HPLC methods. The recoveries of 5-OH-DPAT and the internal standard in this protocol were more than 80%.

Collected samples from plasma extraction and from the *in vitro* transport studies were injected into the HPLC system. For separation, a Superspher® 60, RP-select B, 75 mm - 4 mm column (Merck KGaA, Darmstadt, Germany) was used and eluted with the mobile phase, consisting of acetonitrile/0.1M acetate buffer at pH 3.6 (40/60) v/v at a flow rate of 1.0 ml minute^{-1} or 0.7 ml minute^{-1} , respectively for plasma samples and samples of the *in vitro* transport studies. The detection was performed by a scanning fluorescence detector (Waters™ 474, Millipore Corporation, Milford, MA, USA) at excitation and emission wavelengths of 275 and 303 nm, respectively. In all cases the attenuation was set to 1, while the gain was set to 10 and 100, respectively for the *in vitro* transport studies analysis and the plasma samples analysis. The calibration

curves were linear ($r > 0.999$) in the concentration range of 0.01 to 10 $\mu\text{g ml}^{-1}$ (*in vitro* analysis) and 0.5 to 200 ng ml^{-1} (plasma samples analysis). The intra and inter-assay variation was less than 5% for all concentrations tested. The detection limit under these conditions was 3 and 0.5 ng ml^{-1} , respectively for the samples of the *in vitro* transport and plasma extraction analysis.

9. Data Analysis

The *in vivo* data following transdermal iontophoresis and intravenous infusion were combined for analysis. Fitting of data to the integrated PK/PD model according to equation 3-6) was performed using the subroutines *ADVANCE6 TRANS1 TOL=5* from *PREDPP* in NONMEM. The fixed effect parameters (θ) evaluated in this case were: I_0 , K_R , Clearance (CL), Inter compartmental clearance (Q), volume of central compartment (V_2), volume of peripheral compartment (V_3), k_{24} , k_{42} , k_{out} , I_{max} , and IC_{50} .

Inter-individual variability was modelled by an exponential error model as written in equation 9.

$$P_i = \theta \cdot \exp(\eta_i) \quad (9)$$

in which θ is the population value for the fixed effect parameter P , P_i is the individual Bayesian estimate value and η_i is the inter-individual variation, for which the values are assumed to be independently and normally distributed with mean zero and variance ω^2 . The interindividual variability was applied for I_0 , K_R , CL , V_2 , V_3 , k_{out} , I_{max} , and IC_{50} .

The residual error was characterised by the combination of the exponential and the additive error model as follows:

$$Fp_{ij} = Fo_{ij} \cdot \exp(\varepsilon_1) + \varepsilon_2 \quad (10)$$

where Fp_{ij} is the prediction of the j^{th} evaluated functions (C_p , C_s , or C_{DA}), Fo_{ij} is the measured value of the evaluated function (C_p , C_s , or C_{DA}), and ε represents the residual deviation of the predicted from the observed value and is assumed to be independently and normally distributed with mean zero and variance σ^2 . The analysis of the population parameters θ , ω^2 , and σ^2 was performed using the conventional first-order estimation method (FO).

The data of the *in vitro* iontophoretic transport of 5-OH-DPAT was also analyzed by non-linear effects modelling, using the proposed compartmental models. The parameters estimated in this analysis were I_0 , K_R , t_L , and J_{pas} . The inter-individual variability was introduced using the exponential equation model as proposed in equation 9. The residual error was determined using the combination of the exponential and the additive error model in equation 10. The estimation of the population parameters was also performed using the conventional first order (FO) method.

To compare the level of transport during transdermal iontophoresis *in vitro* and *in vivo*, the steady-state flux (J_{ss}) values were determined on the basis of I_0 values according to the equation below:

$$J_{ss} = \frac{I_0}{S} \quad (11)$$

The estimates of the *in vivo* fixed-effect of J_{ss} , K_R , and t_L were compared to the values obtained from the *in vitro* transport studies in DRS and in RSC. Due to the limited information, the statistical significance ($p < 0.05$) was estimated by a conservative method on the basis of the overlap of the 95% confidence interval of the fixed-effect parameter estimates (15).

The results of the population modeling were evaluated graphically using plots of correlation between population prediction (*PRED*) and the observed values of the dependent variable (*DV*) and the individual prediction (*IPRE*) versus *DV*. In addition, the bias and the precision of the models were also evaluated by estimating the “mean prediction error” (*mpe*) and “root mean squared prediction error” (*rmse*) (16) provided by Visual-NM software (17).

The ultimate objective of the population data analysis was to establish a link between *in vitro* and *in vivo* transport, which allow prediction of the time course of drug effect *in vivo* on the basis of the estimated transport parameters *in vitro*. To test this statement, C_p , C_s , and C_{DA} during iontophoresis of 5-OH-DPAT were simulated on the basis of the optimum *in vitro* transport parameters. Simulations were performed using the *\$SIMULATION* function provided in NM-TRANS. The Kolmogorov-Smirnov test was applied to evaluate differences in the time course of model predictions, as compared to the observed data. This test is based on the comparison between the cumulative density of the geometric means of the simulated and observed data ($p < 0.05$).

D. RESULTS

A. Profiles of C_p , C_s and C_{DA} following transdermal iontophoresis *in vivo*.

In most of the rats, the values of C_p during 15 minutes of passive diffusion *prior* to iontophoresis were negligible (data are not shown). At the start of iontophoresis, C_p steadily increased to reach a plateau in approximately 2 hours of iontophoresis (see Fig. 3). In principle, by current removal at 3 hours, the level of C_p should have dropped according to a first-order elimination process. Interestingly however, in most animals a short delay (approximately 15 minutes) was observed before concentrations started to decay.

As expected, during the passive diffusion, C_s was negligible (data are not shown). After the start of iontophoresis, there was a time delay before C_s steadily increased. The value of C_s directly decreased with the current removal at 3 hours (see Fig. 4).

The value of C_{DA} during passive diffusion was not different from base line values (data are not shown). Similarly to C_s , with the start of iontophoresis, except in one animal a delay for approximately 7.5 minutes was observed before C_{DA} began to decrease as depicted in Fig. 5. The reduction of the C_{DA} in the next 15 minutes was dramatic and reached a maximal inhibition after approximately

37 minutes of iontophoretic period. The maximum inhibition of dopamine release was approximately 80% of the average baseline values in each rat. Interestingly, although the current application has been removed at 3 hours, the level of dopamine did not return to the baseline even after 3 hours of post iontophoretic period. Only two animals showed a slight trend of recovery to the base line after 1 and 2 hours of current removal.

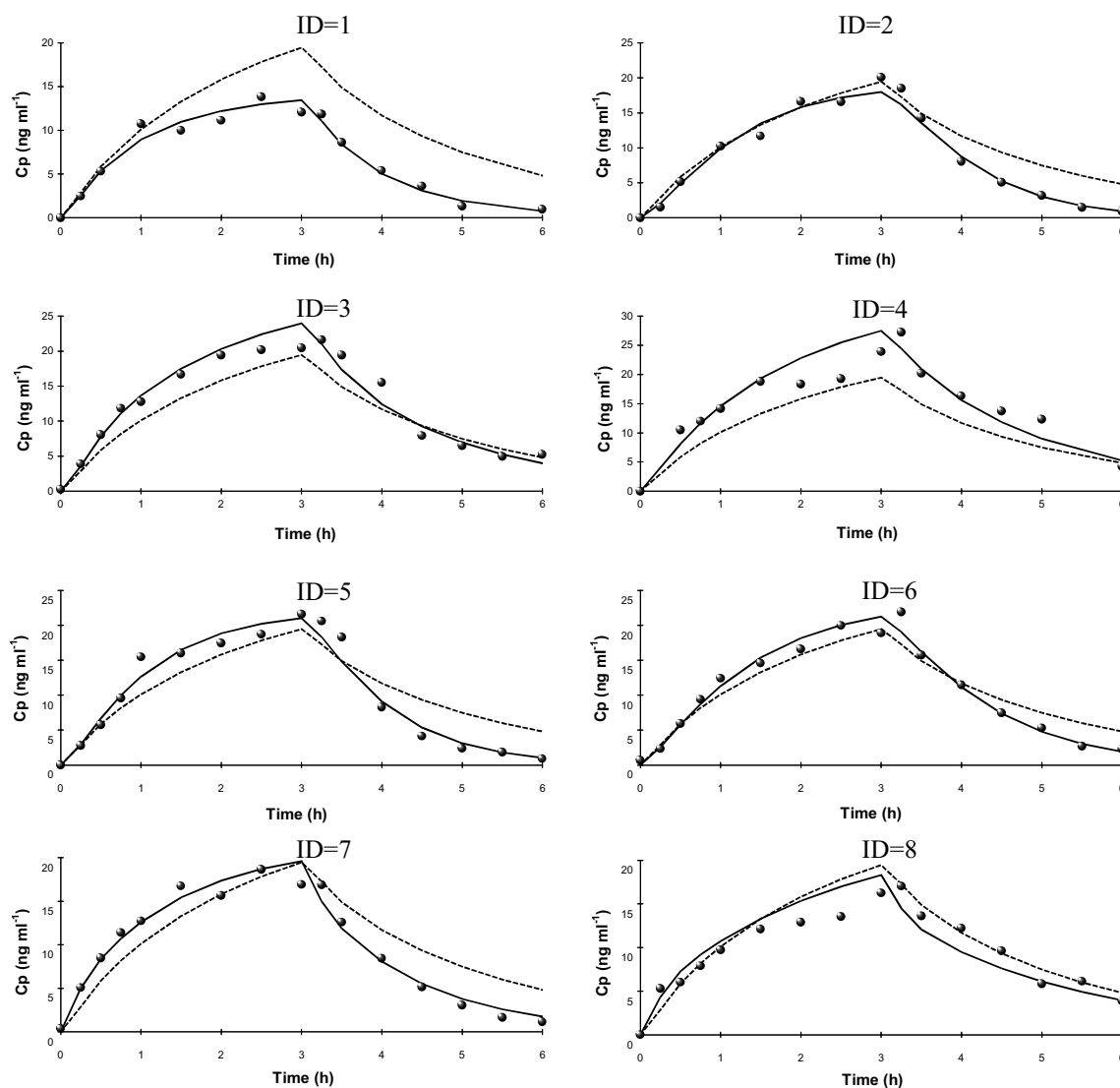


Fig. 3. The observed data of C_p (filled circles) following transdermal iontophoresis of 5-OH-DPAT presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

B. Simultaneous PK-PD analysis of C_p , C_s and C_{DA} following transdermal iontophoresis *in vivo*

The results of the population analysis of the profiles of C_p , C_s , and C_{DA} following iontophoresis of 5-OH-DPAT are presented in Fig. 3 (C_p profile), Fig. 4 (C_s profile) and Fig. 5 (C_{DA} profile). In these figures the observed data are presented together with the population prediction (dashed line) and the individual prediction (solid line). The fitted lines show that the simultaneous

modeling of the data adequately describe C_p , C_s , and C_{DA} . Fig. 6A (panel I and II) depicts two graphs showing the goodness of fit for the data (*PRED versus DV* and the *IPRE versus DV*). The quality of the fitting and model parameter estimation were also evaluated by bias and imprecision parameters as presented in Table I. Despite a slight bias in the population parameter values, this bias disappeared after interindividual variability was introduced in the post-hoc Bayesian estimates (*IPRE versus DV*, Fig 6A panel II). The estimated population parameters are presented in Table II. Except for the parameters k_{24} and k_{42} , estimates of all the fixed effect parameters were obtained with a reliable precision as demonstrated by the low values of %RSE. The inter-individual variability of J_{ss} , K_R , and CL was relatively high, as indicated by the values of their *etas*. The estimates for inter-individual variability of IC_{50} and k_{out} were found to be very high. This is likely to be caused by difficulties in parameter estimation, rather than true differences between individuals. As indicated by values of *sigma*, the residual error of this simultaneous fitting was relatively small.

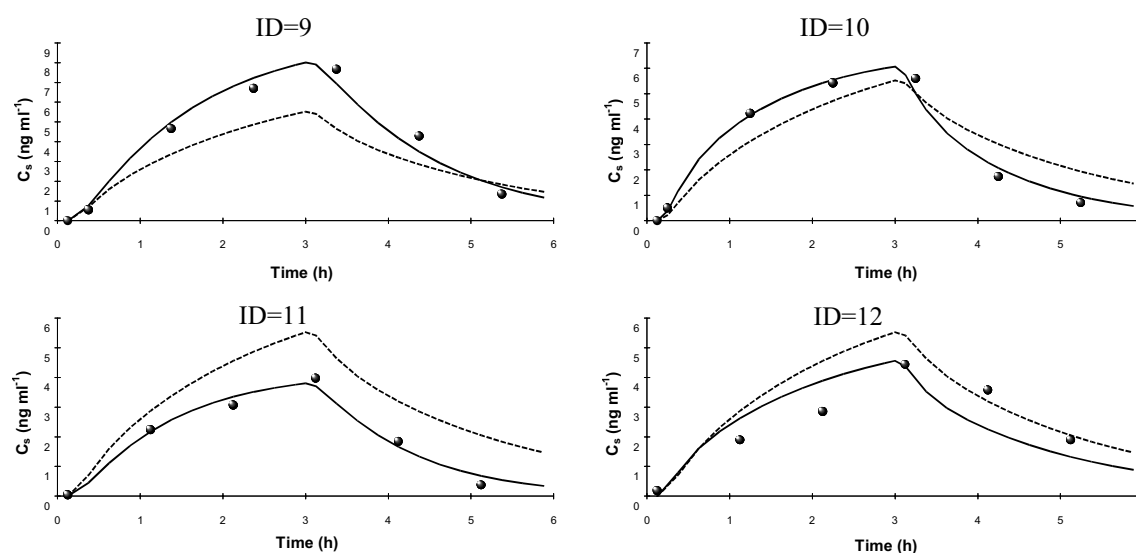


Fig. 4. The observed data of C_s (filled circles) following transdermal iontophoresis of 5-OH-DPAT presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

C. Transdermal iontophoretic transport *in vitro* of 5-OH-DPAT

To estimate the correlation of the *in vitro* transport of 5-OH-DPAT to its PK/PD profiles following transdermal iontophoresis, the *in vitro* iontophoretic transport of 5-OH-DPAT was studied across DRS obtained from rats number 1-8 (see Fig. 7). In this case, a delay to the start of the iontophoretic flux was observed after the current was switched on. The flux then gradually increased, but in most cases no steady state flux was reached during 3 hours of iontophoresis. Subsequently, the flux directly decreased when the current was switched off at 3 hours.

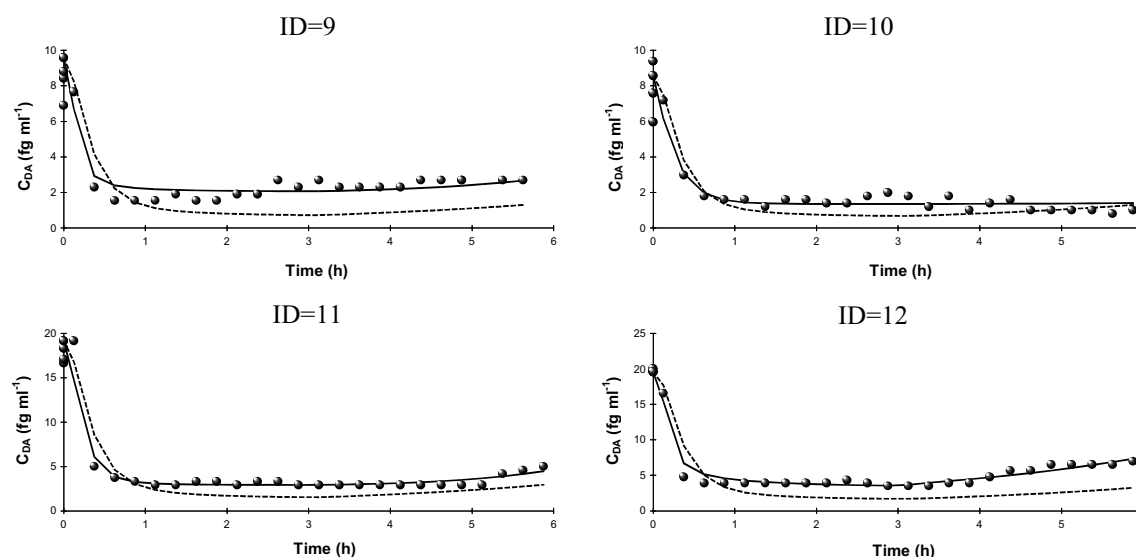


Fig. 5. The observed data of C_{DA} (filled circles) following transdermal iontophoresis of 5-OH-DPAT presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

Table I. Calculation of the absolute, % relative and 95% confidence interval of the bias and imprecision of the model *in vivo* and *in vitro*

model	Bias (<i>mpe</i>)			Imprecision (<i>rmse</i>)		
	absolute	%relative	95%CI	absolute	%relative	95%CI
<i>PRED-In vivo</i> -All	1.2*	12.0	0.8 – 1.7	4.8	46.5	-0.8 – 10.4
<i>IPRE-In vivo</i> -All	0.0	0.2	-0.2 – 0.2	2.2	21.3	0.7 – 3.7
<i>PRED_in vitro</i> -DRS	0.2	0.7	-1 – 1.4	8.6	29.3	-8.3 – 25.5
<i>IPRE-In vitro</i> -DRS	0.0	0.2	-0.2 – 0.3	1.5	5.0	0.5 – 2.4
<i>PRED_in vitro</i> -RSC	1.7	3.6	-0.8 – 4.2	11.1	23.2	-35.6 – 57.7
<i>IPRE-In vitro</i> -RSC	0.3	0.6	-0.4 – 1	3.1	6.6	-2.2 – 8.4
<i>PRED_in vitro</i> -DHS	0.3	0.9	-3.1 – 3.8	13.5	35.6	-49.7 – 76.8
<i>IPRE-In vitro</i> -DHS	0.2	0.5	-0.5 – 0.8	2.6	6.9	-0.8 – 6.0

Legends:

* indicates a significant bias ($p < 0.05$)

Moreover, for several subjects (rats number 1, 3, and 8), the *in vitro* iontophoretic transport of the dopamine agonist was also studied in RSC. As shown in Fig. 8, the increment of the flux was faster than in the transport across DRS and the steady-state flux was reached in all subjects. Similar to the transport in DRS the flux gradually decreased after switching off the current.

To quantitatively describe the *in vitro* iontophoretic transport of 5-OH-DPAT in DRS and RSC, the flux *versus* time profiles were analyzed on the basis of the *in vitro* compartmental model as described in the previous section. The results of the population prediction curves (dashed line) as well as the individual post-hoc Bayesian prediction curves (solid line) are presented

together with the observed data in Fig. 7 (*in vitro* transport in DRS) and in Fig. 8 (*in vitro* transport in RSC). The figures show that the compartmental model properly describes the flux *versus* time profiles *in vitro*. As clearly demonstrated in the *in vitro* transport in DRS, the model also describes the lag period where the flux was not yet observed (by parameter t_L).

Table II. The population estimates obtained from the simultaneous fitting of the *in vivo* data, the *in vitro* transport across DRS and the *in vitro* transport across RSC upon transdermal iontophoresis of 5-OH-DPAT.

Data	Parameter	Unit	Theta		Eta		Sigma	
			mean	%RSE	mean	%RSE	mean	%RSE
<i>In vivo</i>	J_{ss}	$\text{nmol cm}^{-2} \text{ h}^{-1}$	31.80	20	0.22	52	-	-
	K_R	h^{-1}	4.41	31	0.51	68	-	-
	CL	L h^{-1}	1.00	18	0.35	40	-	-
	Q	L h^{-1}	3.70	9	-	-	-	-
	V_2	L	0.46	15	0.12	51	-	-
	V_3	L	1.49	12	0.07	51	-	-
	K_{24}	h^{-1}	0.02	178	-	-	-	-
	K_{42}	h^{-1}	31.80	180	-	-	-	-
	K_{out}	h^{-1}	4.61	16	1.21	37	-	-
	IC_{50}	ng ml^{-1}	0.21	26	3.98	81	-	-
	I_{max}	-	0.95	3	0.03	30	-	-
	σ_1	-	-	-	-	-	0.02	19
	σ_2	-	-	-	-	-	0.06	35
<i>In vitro</i> across DRS	J_{ss}	$\text{nmol cm}^{-2} \text{ h}^{-1}$	39.00	5	0.02	31	-	-
	K_R	h^{-1}	1.45	6	0.19	32	-	-
	t_L	h	0.24	6	0.02	76	-	-
	J_{pas}	$\text{nmol cm}^{-2} \text{ h}^{-1}$	3.89	12	0.28	44	-	-
	σ_1	-	-	-	-	-	0.001	24
	σ_2	-	-	-	-	-	0.31	42
<i>In vitro</i> across RSC	J_{ss}	$\text{nmol cm}^{-2} \text{ h}^{-1}$	62.70	10	0.03	52	-	-
	K_R	h^{-1}	2.58	1	0.03	46	-	-
	t_L	h	0.04	66	0.51	187	-	-
	J_{pas}	$\text{nmol cm}^{-2} \text{ h}^{-1}$	2.49	5	0.01	34	-	-
	σ_1	-	-	-	-	-	0.004	20

Analogous to the *in vivo* data, goodness of fit for the *in vitro* iontophoretic transport was evaluated graphically (Figs. 6B and Fig 6C, respectively for the transport in DRS and in RSC) as well as statistically by calculating bias and precision parameters (Table I). No bias was observed for either population or individual parameter estimates ($p > 0.05$), confirming the accuracy of the model for iontophoretic transport in DRS and in RSC. A summary of the population parameters of the models of the *in vitro* iontophoretic transport across DRS and RSC is presented in Table II. In the

transport across DRS and RSC, all the fixed-effects parameters were estimated with a reliable precision. The residual errors of the *in vitro* transport across DRS and RSC were relatively small as indicated by the values of σ_1 in both models.

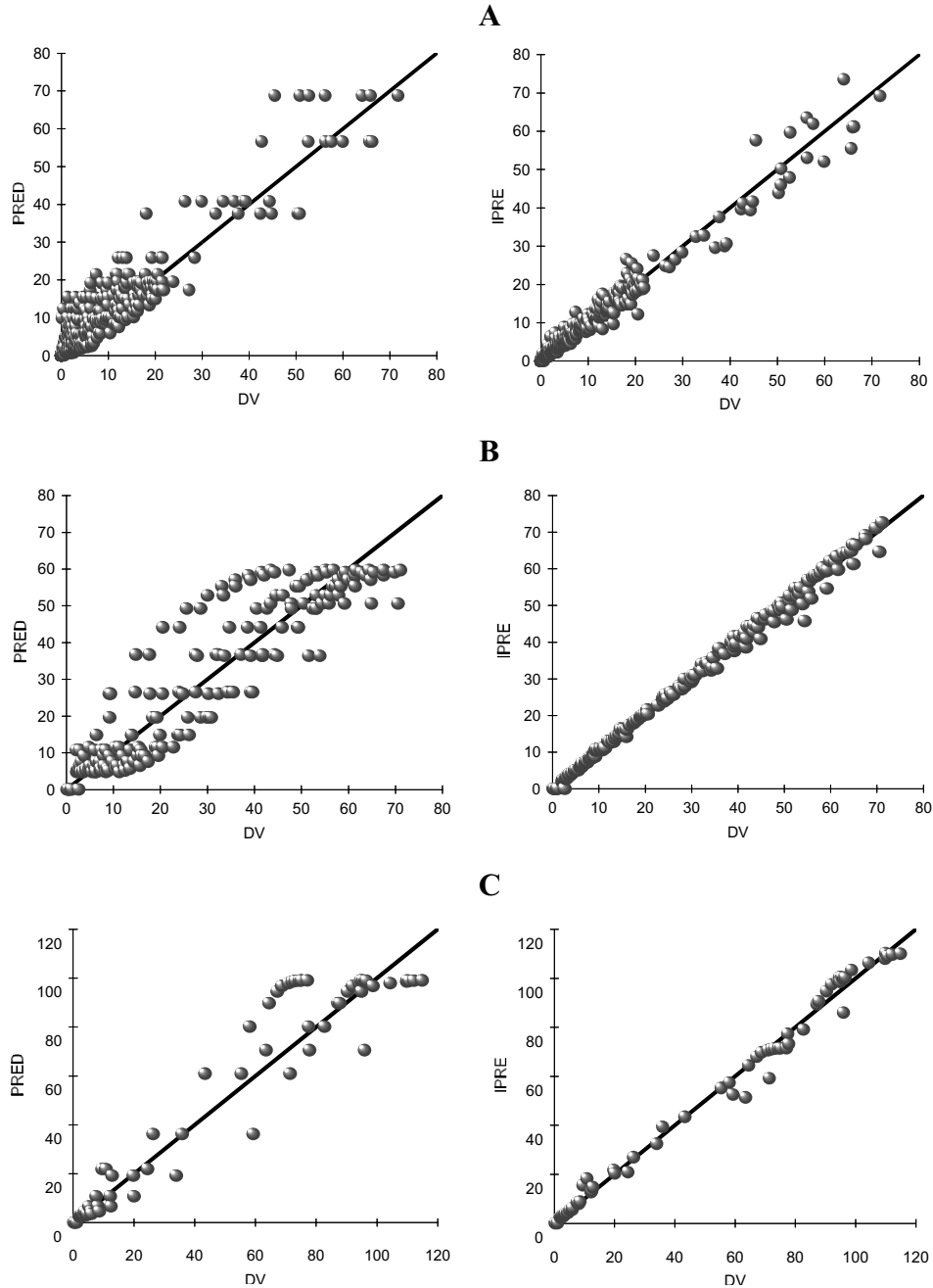


Fig. 6. Diagnostic of the simultaneous modeling of the C_p , C_s , and C_{DA} data following transdermal iontophoresis of 5-OH-DPAT in rats (Panel A), the *in vitro* transport in DRS (panel B), and the *in vitro* in RSC (panel C). Panels I represent the plot of the population prediction (PRED) versus the observed value (DV) and panels II represent the plot of the individual prediction (IPRE) versus DV.

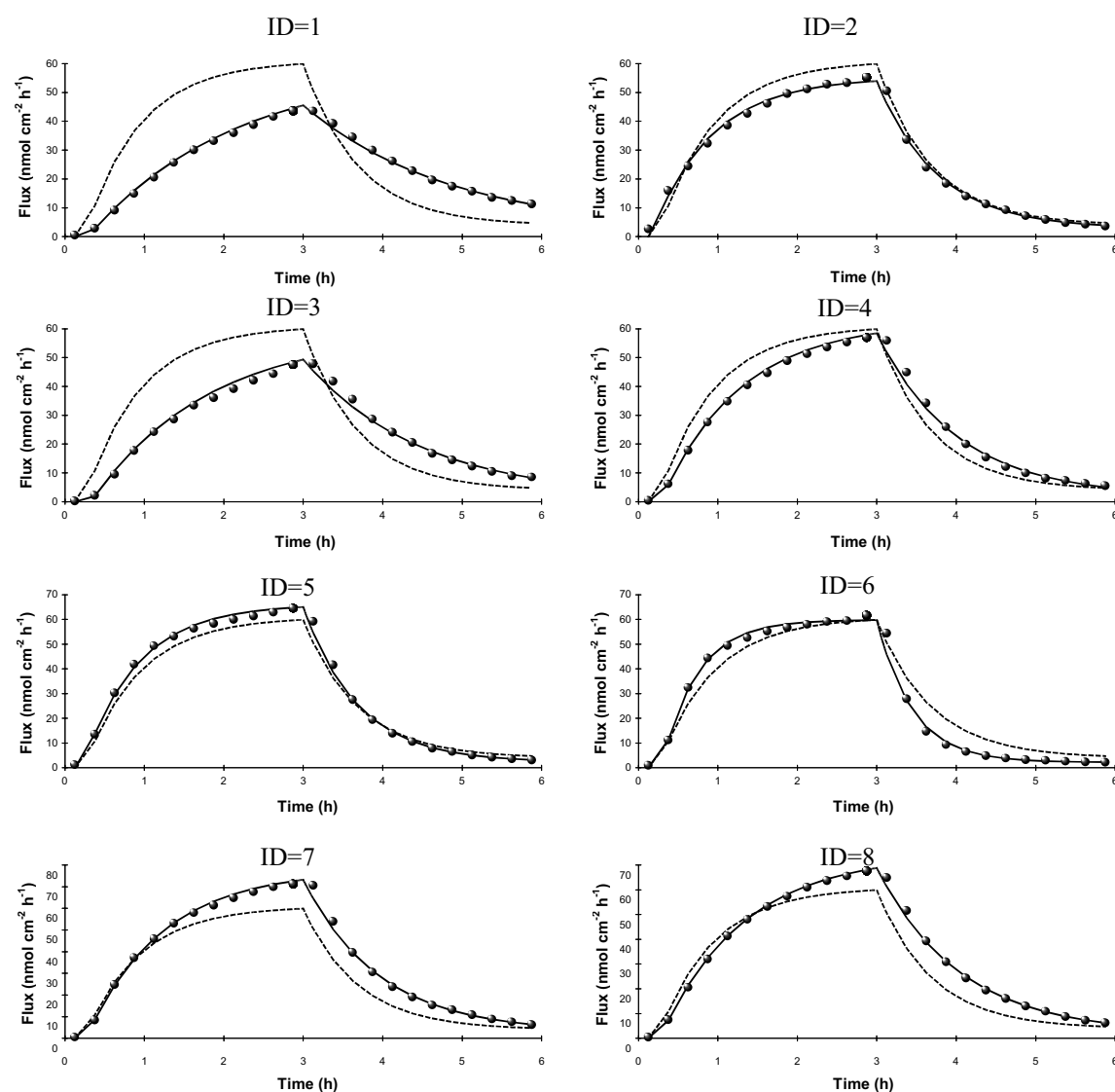


Fig. 7. The observed data of flux (filled circles) following transdermal iontophoresis *in vitro* of 5-OH-DPAT in DRS presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

D. Correlation of the *in vitro* transport to the PK-PD modeling of 5-OH-DPAT following iontophoresis

Overlap of the population estimates (geometric mean and 95% confidence intervals) showed that the values of three parameters characterizing the iontophoretic transport are similar in *in vivo* and *in vitro* skin preparations. The *in vivo* J_{ss} was not significantly different from J_{ss} of the *in vitro* transport across DRS. The *in vivo* K_R was not significantly different from the *in vitro* K_R in the transport across RSC. Despite the constrained t_L values *in vivo*, the estimated of t_L was close to the *in vitro* drug transport across RSC. These similarities suggest that prediction of *in vivo* PK-PD profiles may be derived from *in vitro* data.

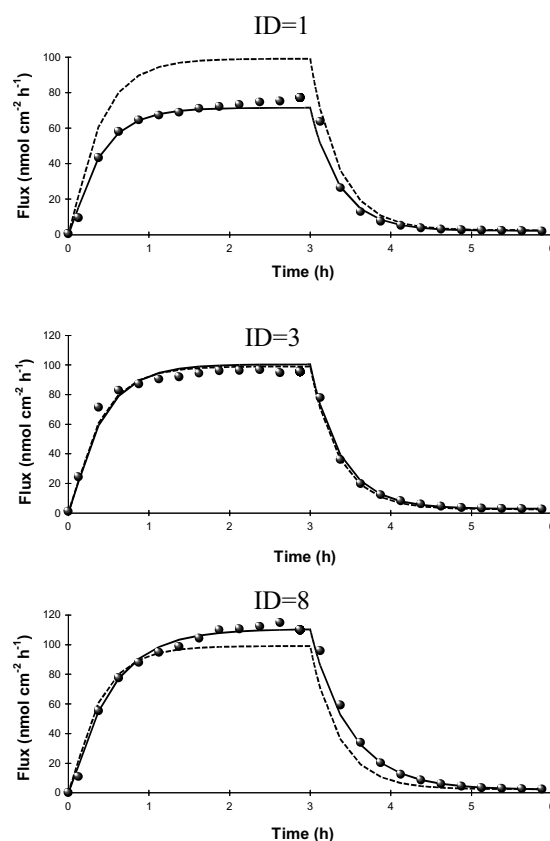


Fig. 8. The observed data of flux (filled circles) following transdermal iontophoresis *in vitro* of 5-OH-DPAT in RSC presented together with the population model prediction (dashed line) and the individual model prediction (solid line).

E. Prediction of the population PK-PD profiles on the basis of the optimum iontophoretic transport

To explore the predictive value of *in vitro* parameters, model-based simulations of the time course of C_p , C_s , and C_{DA} were performed following iontophoretic delivery of 5-OH-DPAT. The results of this approach are presented in Fig. 9 in panel A (simulation of C_p profiles), in panel B (simulation of C_s) and in panel C (simulation of C_{DA}). In these panels, the geometric mean of C_p , C_s , and C_{DA} are also depicted with 95% confidence intervals. As expected the observed data fall within the prediction intervals. In addition, this was formally compared using the Kolmogorov-Smirnov test. The profiles of the geometric means of the population data of C_p , C_s , and C_{DA} were not significantly different from the geometric mean of the simulated values ($p > 0.05$).

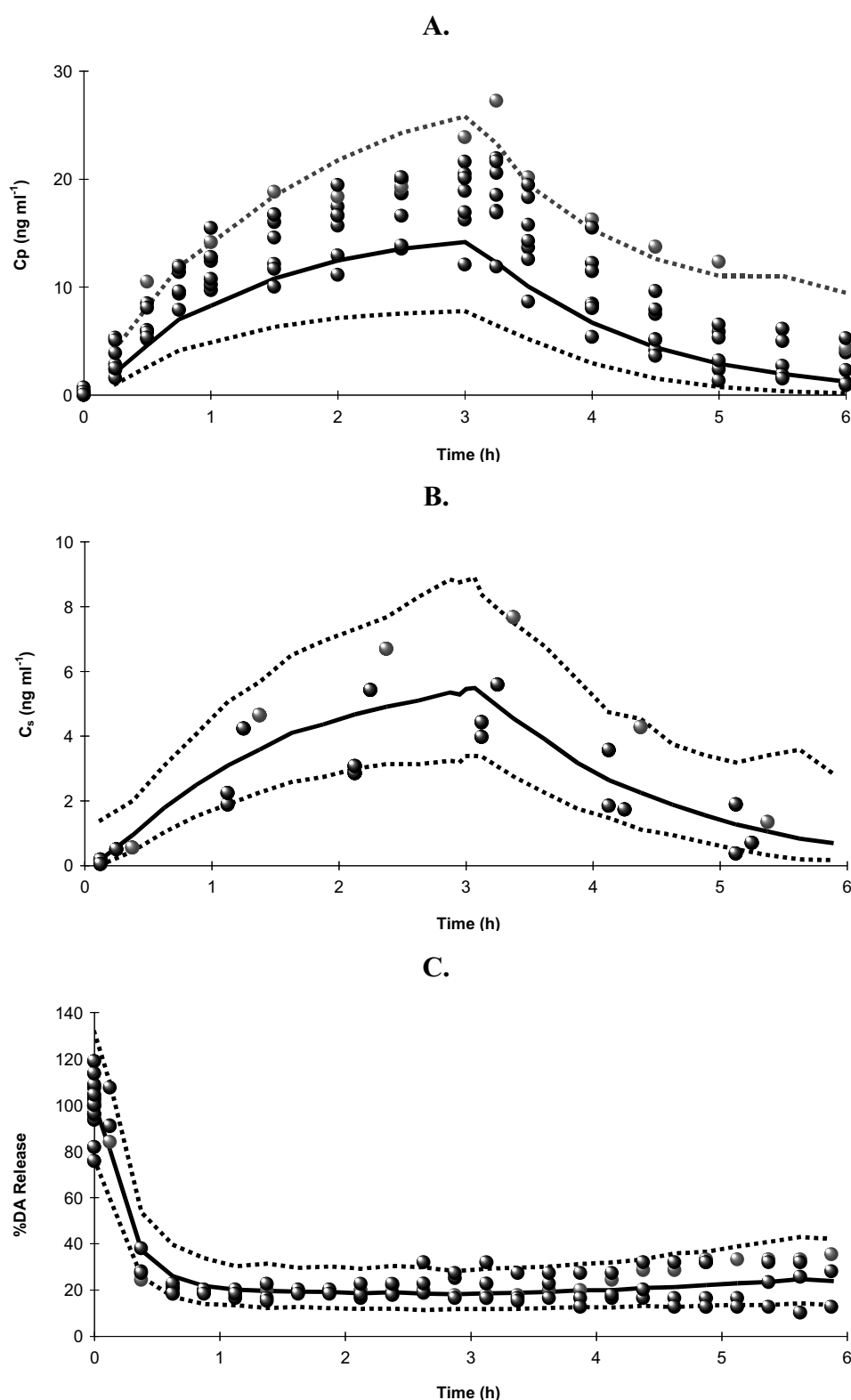


Fig. 9. The prediction of the C_p (panel A), C_s (panel B), and C_{DA} (panel C) following transdermal iontophoresis of 5-OH-DPAT on the basis of the optimum values of the *in vitro* transport parameters. The dashed lines are the range of 95% confidence interval of the population prediction of C_p , C_s , or C_{DA} . The solid line is the geometric mean of the population prediction of C_p , C_s , or C_{DA} . The filled circles are the observed data of C_p , C_s , and C_{DA} following transdermal iontophoretic delivery of 5-OH-DPAT.

E. DISCUSSION

In this study we showed the application of an integrated *in vitro-in vivo* approach to characterize the pharmacokinetics, pharmacodynamics and iontophoretic properties of 5-OH-DPAT in rats.

Despite the overall ability of the compartmental models in describing the time course of drug concentrations in plasma and in tissue compartments, we realize that the model may not be able to describe some features of drug transport, for example the delay in the C_p decay at 15 minutes after current termination as observed in most animals in the PK studies. This delay might be due to a depot effect, which may be present in the skin or in the subcutaneous tissue. This effect is consistent with the lipophilic nature of the drug ($\log P=2.19$ (6)). Further extension of the model as well as increased sampling scheme for C_p may be required to account for this phenomenon. Nevertheless, the profiles of C_s and C_{DA} are not affected by the presence of a possible depot effect, suggesting that the amount of drug deposited is minimal.

The recent approach has given us the possibility to correlate the *in vitro* to the *in vivo* transport (flux), and consequently to describe the time course of C_p , C_s , and C_{DA} . The characterization of the correlation of *in vitro* to *in vivo* transport offers the benefit of predicting the impact of *in vitro* drug properties in an *in vivo* experiment. Conversely, it is also possible to optimize the *in vitro* experimental conditions required to *mimic* relevant *in vivo* profiles.

Most importantly, this study demonstrated the fact that transdermal iontophoresis successfully delivers a therapeutic level of 5-OH-DPAT to achieve a very strong dopaminergic effect in rats. Approximately half of the maximum concentration of 5-OH-DPAT that can be prepared in the donor phase as well as half of the maximum current density that can be safely used in human (6), were used in this study. Although requires further study and analysis, by assuming similarity in the iontophoretic flux in human and in rat skin, (18,19) these findings might suggest the feasibility of transdermal iontophoretic delivery of 5-OH-DPAT in patients with Parkinson's disease.

The assessment of an *in vitro* - *in vivo* correlation and PK-PD relationship would not have been possible without nonlinear mixed effect modeling as integration of data from different experiments is not feasible or labour- and resource intensive using the standard data analysis methodology (11). This highlights the need for implementation of model libraries to support further research in transdermal iontophoretic delivery in future.

In summary the simultaneous fitting of the PK-PD data and the fitting of the *in vitro* iontophoretic transport of 5-OH-DPAT in DRS and RSC adequately characterize transdermal iontophoretic delivery of 5-OH-DPAT in rats. The time course of plasma and brain concentrations of 5-OH DPAT, as well as the inhibition of dopamine release upon transdermal iontophoresis can be predicted from *in vitro* parameter estimates of J_{ss} , K_R , and t_L . Transdermal iontophoresis

of 5-OH-DPAT successfully delivers sufficient amount of the drug into striatum to achieve a strong dopaminergic effect, which might indicate the promising future of transdermal iontophoresis of 5-OH-DPAT in patients with Parkinson's disease.

F. ABBREVIATIONS:

- DRS : Dermatomed rat skin
RSC : Rat stratum corneum
IDR type I: Indirect response model with the inhibition of production of response
 I_0 : The zero-order iontophoretic mass transfer from the donor phase/patch into the skin compartment
 $J(t)$: Flux at time t
 J_{ss} : Steady-state flux
 K_R : The first-order rate constant of drug release from the skin into acceptor compartment (*in vitro*) or to the systemic circulation (*in vivo*)
 P_{PI} : The zero order post-iontophoretic mass transfer due to PIDF
 S : Diffusion active area or patch area
 t_L : The kinetic lag time of the drug molecules to enter the skin compartment
 T : Duration of current application
 $X_n(t)$: Drug amount at time t in the compartment n , which refers to the skin ($n=1$), acceptor phase (*in vitro*) or plasma (*in vivo*) ($n=2$), tissue ($n=3$), and striatum ($n=4$) compartments.
 k : The elimination rate-constant
 k_{ab} : The distribution rate constant from compartment a to compartment b
 C_p : Concentration of 5-OH-DPAT in plasma
 C_s : Concentration of 5-OH-DPAT in striatum
 C_{DA} : Concentration of dopamine in striatum
 I_{max} : The maximum inhibition of the dopamine production
 IC_{50} : The concentration of 5-OH-DPAT in striatum required to produce 50% of I_{max}
 H : The Hill-slope coefficient (which is constrained to 1)
 k_{out} : The first-order rate constant for the loss of response
 k_{in}^0 : The zero order rate constant for the production of response
 C_{DA0} : The baseline values of C_{DA} prior to the inhibition effect of 5-OH-DPAT.

G. ACKNOWLEDGMENTS

This research was supported by QUE Project Batch III year of 2000 – 2004 Faculty of Pharmacy Gadjah Mada University Yogyakarta Indonesia.

H. REFERENCES

1. A. K. Nugroho, O. Della-Pasqua, M. Danhof, and J. A. Bouwstra. Compartmental Modeling of Transdermal Iontophoretic Transport: I. *In Vitro* Model Derivation and Application. *Pharm. Res.* **21**:1974-1984 (2004).
2. A. K. Nugroho, O. Della-Pasqua, M. Danhof, and J. A. Bouwstra. Compartmental modeling of transdermal iontophoretic transport: II. *In vivo* model derivation and application. *Pharm. Res.* **22**:335-346 (2005).
3. J. D. McDermed, G. M. McKenzie, and H. S. Freeman. Synthesis and Dopaminergic Activity of (±)-, (+)-, and (-)-2-Dipropylamino-5-hydroxy- 1,2,3,4-tetrahydronaphthalenel. *J. Med. Chem.* **19**:547-549 (1976).
4. N. Rodenhuis, W. Timmerman, H. V. Wikstrom, and D. Dijkstra. Thiophene analogs of naphthoxazines and 2-aminotetralins: bioisosteres with improved relative oral bioavailability, as compared to 5-OH-DPAT. *Eur. J. Pharmacol.* **394**:255-263 (2000).
5. B. H. Sage. Iontophoresis. In E. W. Smith, and H. I. Maibach (eds.). *Percutaneous penetration enhancers*, CRC Press, Boca Raton, 1995. pp. 351-368.
6. A. K. Nugroho, L. Li, D. Dijkstra, H. V. Wikstrom, M. Danhof, *et al.* Transdermal iontophoresis of dopamine agonist 5-OH-DPAT in human skin *in vitro*. *J. Control. Release* **103**:393-403 (2005).
7. B. H. Westerink, P. de Boer, W. Timmerman, and J. B. De Vries. *In vivo* evidence for the existence of autoreceptors on dopaminergic, serotonergic, and cholinergic neurons in the brain. *Ann. N. Y. Acad. Sci.* **604**:492-504 (1990).
8. N. Rodenhuis, D. Dijkstra, P. de Boer, E. S. Vermeulen, W. Timmerman, *et al.* Dopamine D(2) activity of R(-)-apomorphine and selected analogs: a microdialysis study. *Eur. J. Pharmacol.* **387**:39-45 (2000).
9. N. L. Dayneka, V. Garg, and W. J. Jusko. Comparison of four basic models of indirect pharmacodynamic responses. *J. Pharmacokinet. Biopharm.* **21**:457-478 (1993).
10. A. Sharma and W. J. Jusko. Characteristics of indirect pharmacodynamic models and applications to clinical drug responses. *Br. J. Clin. Pharmacol.* **45**:229-239 (1998).
11. P. M. C. Wright. Population based pharmacokinetic analysis: why do we need it; what is it; and what has it told us about anaesthetics? *Br. J. Anaesth.* **80**:488-501 (1998).
12. A. J. Boeckmann, L. Sheiner, and S. L. Beal. *NONMEM users guide - part V: Introductory guide*. NONMEM Project Group University of California at San Francisco, San Francisco, CA, USA, 1992.
13. G. Paxinos and C. Watson. *The rat brain in stereotaxic coordinates*. Academic Press, New York, 1982.
14. A. K. Nugroho, G. L. Li, A. Grossklaus, M. Danhof, and J. A. Bouwstra. Transdermal iontophoresis of rotigotine: influence of concentration, temperature and current density in human skin *in vitro*. *J. Control. Release* **96**:159-167 (2004).
15. N. Schenker and J. F. Gentleman. On judging the significance of differences by examining the overlap between confidence intervals. *Am Stat* **55**:182-186 (2001).
16. L. B. Sheiner and S. L. Beal. Some suggestions for measuring predictive performance. *J. Pharmacokinet. Biopharm.* **9**:503-512 (1981).
17. Anonymous, Visual-NM, RDPP, Montpellier, France, (1997).
18. 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).
19. J. B. Phipps, R. V. Padmanabhan, and G. A. Lattin. Iontophoretic delivery of model inorganic and drug ions. *J. Pharm. Sci.* **78**:365-369 (1989).

