A Competitive Interaction Model Predicts the Effect of WAY-100,635 on the Time Course of \(R^+\)-8-Hydroxy-2-(di-\(n\)-propylamino)tetralin-Induced Hypothermia

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ABSTRACT

The objective of this investigation was to characterize quantitatively the pharmacodynamic interaction between \(N\)-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-\(N\)-2-pyridinyl-cyclohexanecarboxamide (WAY-100,635) and \(R^+\)-8-hydroxy-2-(di-\(n\)-propylamino)tetralin (8-OH-DPAT) in vivo. The 8-OH-DPAT-induced change in body temperature was used as a pharmacodynamic endpoint. Four groups of rats each received 1 mg/kg 8-OH-DPAT in 5 min during computer-controlled infusions of physiological saline or WAY-100,635, targeted at steady-state concentrations of 20, 85, and 170 ng/ml. Body temperature was monitored continuously with a telemetric system, and frequent blood samples were obtained to determine the pharmacokinetics of both drugs. Large differences in pharmacokinetics were observed between WAY-100,635 and 8-OH-DPAT, reflected in values of the terminal elimination half-life of 33 and 143 min, respectively. Infusion of WAY-100,635 had no influence on the pharmacokinetics of 8-OH-DPAT. With regard to the pharmacodynamics, clear antagonism of the 8-OH-DPAT-induced hypothermia was observed. The complex pharmacological effect versus time profiles of 8-OH-DPAT were analyzed on the basis of an indirect physiological response model with set point control coupled to a competitive interaction model for an agonist and antagonist acting at a common receptor. This model converged, yielding precise estimates of the pharmacodynamic parameters of both WAY-100,635 and 8-OH-DPAT, which were independent of the infusion rate of WAY-100,635. The estimated in vivo binding constant of WAY-100,635 was 0.98 ng/ml (2.3 nM), which is very similar to the reported value from in vitro receptor binding assays. The findings of this investigation show that, in contrast to earlier reports in the literature, WAY 100,635 behaves as a pure competitive antagonist at the 5-hydroxytryptamine\(_{1A}\) receptor in vivo.

Over the years, several, more or less, stable agonists at the 5-HT\(_{1A}\) receptor have been developed that differ in both affinity and intrinsic efficacy. Of these 5-HT\(_{1A}\) ligands, \(N\)-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-\(N\)-2-pyridinyl-cyclohexanecarboxamide (WAY-100,635) was found to act as a highly selective "silent" antagonist with high affinity for the 5-HT\(_{1A}\) receptor (Forster et al., 1995). Several studies have shown that this compound may act as an antagonist of the pharmacological actions of 5-HT\(_{1A}\) receptor agonists (Forster et al., 1995; Khawaja et al., 1997; Newman Tancredi et al., 1996, 1997). In contrast in vivo the mechanism of the interaction might be more complex. It has for example been reported that in vivo WAY-100,635 behaves as an insurmountable antagonist and that different doses are needed to antagonize the effects of different 5-HT\(_{1A}\) agonists (Cryan et al., 1999).

Typically, in vivo drug-drug interaction studies are based on an analysis of the change in the dose-response relationship. A complicating factor in such studies is that pharmacokinetic factors may make the interpretation of the result difficult. Previous investigations with benzodiazepines and adenosine A\(_1\) agonists have shown that when the differences in pharmacokinetics between the compounds are taken into account...
PK-PD Modeling of WAY-100,635 on 5-HT1A-Mediated Hypothermia

consideration, the interactions with antagonists can indeed be reliably described on the basis of a competitive interaction model (Mandema et al., 1992; Appel et al., 1995). In addition, particularly with 5-HT1A agonists, it seems important that time dependencies in pharmacodynamics are taken into consideration (Zuijdeveld et al., 2001).

It is well established that agonists for the 5-HT1A receptor induce a hypothermic response in rats (Hjorth, 1985; Goodwin et al., 1987; Hadrava et al., 1996), which is mediated via post-synaptic hypothalamic 5-HT1A receptors (Radja et al., 1992; Salmi and Ahlenius, 1998). Overall the change in body temperature is considered a robust biomarker of 5-HT1A receptor-mediated responses (Millan et al., 1993; Cryan et al., 1999). Recently we have developed an integrated pharmacokinetic-pharmacodynamic (PK-PD) model for the effects of 5-HT1A agonists on body temperature in rats. (Zuijdeveld et al., 2001). This model is based on dynamical systems analysis and utilizes the principle of set point control in combination with an indirect physiological response model. In this model the 5-HT1A Receptor agonist acts directly on the set point for temperature via a sigmoidal transducer function to explain the hypothermic response observed in rats. This model has been applied successfully to predict the complex effect versus time profiles following administration of the reference agonists R-8-OH-DPAT (R-(+)-8-hydroxy-2-(di-n-propylamino)tetrinal) and S-8-OH-DPAT in rats (Zuijdeveld et al., 2001). An integrated PK-PD approach further takes into account pharmacokinetic factors, such as differences in elimination half-life.

In the present study we show that administration of different infusions of WAY-100,635 in combination with the prototypical 5-HT1A receptor agonist R-8-OH-DPAT reduces the hypothermic effect by producing a rightward shift in the concentration effect curve (Fig. 5; actual results). It is further shown that the rightward shift allows for the determination of precise estimates of the pharmacodynamic parameters of both WAY-100,635 and R-8-OH-DPAT, independent of the infusion rate of WAY-100,635. The findings of this investigation show that WAY-100,635 behaves as a pure competitive antagonist at the 5-HT1A receptor in vivo.

Materials and Methods

Experiments were performed on male Wistar rats (Broekman BV, Someren, The Netherlands) weighing 316 ± 5 g (mean ± S.E.M., n = 35) and were approved by the Leiden University Ethics Committee. The animals were housed in standard plastic cages (six per cage before surgery and individually after surgery). They were kept in a room with a normal 12-h light/dark cycle (lights on at 7:00 AM and lights off at 7:00 PM) and a temperature of 21°C. During the light period, a radio was on for background noise. Acidified water and food (laboratory chow; Hope Farms, Woerden, The Netherlands) was provided ad libitum before the experiment.

Surgical Procedure

Eight days before the experiment, the rats were operated upon. The animals were anesthetized with an intramuscular injection of 0.1 ml/kg Domitor (1 mg/ml medetomidine hydrochloride; Pfizer, Capelle a/d IJssel, The Netherlands) and 1 ml/kg Ketalar (50 mg/ml Ketamine base; Pfizer, Hoofddorp, The Netherlands). Indwelling pyrogen-free cannulae (Polythene, 14 cm, 0.52-mm i.d., 0.96-mm o.d.) were implanted into the right jugular vein, for infusions of R-8-OH-DPAT and WAY-100,635, and in the left femoral vein (Polythene, 20 cm, 0.52-mm i.d., 0.96-mm o.d.) for computer-controlled infusions of WAY-100,635. For blood sampling, the left femoral artery was cannulated (Polythene, 4 cm, 0.28-mm i.d., 0.61-mm o.d. + 20 cm, 0.58-mm i.d., 0.96-mm o.d.). Cannulae were tunneled subcutaneously to the back of the neck and exteriorized. To prevent coagulation of blood, the cannulae were filled with a 25% (w/v) solution of polyvinylpyrrolidone (PVP) (Brockacef, Maarsen, The Netherlands) in a 0.9% (w/v) pyrogen-free sodium chloride solution (NPBI, Emmers Compascuum, The Netherlands) that contained 50 IU/ml heparin (Leiden University Medical Center, Leiden, The Netherlands). Prior to the experiment, the PVP solution was removed and the cannulae were flushed with saline containing 20 IU/ml heparin. The skin in the neck was stitched with normal sutures, and the skin in the groin was closed with wound clips. Furthermore, a telemetric transmitter (Physiotel implant TA10TA-F40 system; Data Sciences International, St. Paul, MN) (weighting ~ 7 g), which had been made pyrogen-free with CIDEK (22 g/l glutaraldehyde; Johnson and Johnson Medical Ltd., Gargrave, Skipton, UK) for at least 2 h, was implanted into the abdominal cavity for the measurement of core body temperature. After surgery, an injection of the antibiotic ampicillin (0.6 ml/kg of a 200 mg/ml; A.U.V., Cuijk, The Netherlands) was administered to aid recovery.

Experimental Protocol

Dosage Regimen. The experiments were performed 8 days after surgery. Rats received infusions of R-8-OH-DPAT and WAY-100,635.

One group of rats received a bolus infusion of 3 mg/kg WAY-100,635 in 15 min (n = 5); another group received a R-8-OH-DPAT bolus infusion of 1 mg/kg in 5 min (n = 6) during a computer-controlled infusion of physiological saline. The other groups received 1 mg/kg R-8-OH-DPAT in 15 min in combination with a computer-controlled infusion of WAY-100,635, which was targeted at 20 (n = 6), 85 (n = 6), and 170 ng/ml (n = 5) of WAY-100,635. This computer-controlled infusion started 15 min before the administration of R-8-OH-DPAT and lasted 2.5 h. Finally, six rats received a vehicle treatment that consisted of an equivalent amount of saline as in the computer-controlled infusion regimen of WAY-100,635. For the computer-controlled infusions, the STANPUMP software (Shafer and Gregg, 1992) was utilized. The STANPUMP program was running on an IBM compatible computer (486 processor) and connected to a Harvard 22-syringe pump (Harvard Apparatus Inc., South Natick, MA) through a RS232 interface. The concentration was targeted using population pharmacokinetic parameters obtained with the 3 mg/kg WAY-100,635 infusion. All the experiments started between 9:00 and 9:30 AM.

Blood Sampling. In the groups that received just an infusion of either WAY-100,635 or R-8-OH-DPAT, approximately 15 to 18 serial blood samples of 50 μl were taken according to a fixed time schedule to determine their pharmacokinetics. In the interaction experiments, both the pharmacokinetics of R-8-OH-DPAT and WAY-100,635 were determined by taking two 50-μl samples at each sampling time. When WAY-100,635 concentrations were targeted to 20 ng/ml, 200-μl samples were taken. To reduce the total amount of blood drawn, a sparse sampling protocol was utilized, in which approximately seven samples were taken at randomly distributed time points. For each sample, the exact amount of blood was measured with a calibrated capillary (Servoprax, Wesel, Germany) and transferred into a glass centrifuge tube containing 400 μl of Millipore water for hemolysis. During the experiment, the samples were kept on ice. After the experiment, samples were stored at −20°C pending analysis.

Data Acquisition

Temperature Measurements. To measure the body temperature of the rat, a telemetric system (Physiotel telemetry system; DSI) was used. A telemetric transmitter (Physiotel implant TA10TA-F40; DSI) was implanted in the abdominal cavity of the rat. The transmitter measured the body temperature every 30 s for a 2-s period and

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signaled it to a receiver (Physiotel receiver, model RPC-1; DSI). The receiver was connected to the computer through a BCM 100 consolidation matrix (DSI). The computer processed the data and visualized the temperature profiles using LabProTM software (DSI) running under OS/2 Warp (IBM) as it did for room temperature (C10T temperature adapter; DSI).

**HPLC Analysis of R-8-OH-DPAT.** The blood concentrations of R-8-OH-DPAT were assayed by an enantioselective HPLC method as described previously (Zuideveld et al., 2000). Briefly, detection with the HPLC system was with an electrochemical detector (DECADE, Antec Leyden, Zoeterwoude, The Netherlands) operating in DC mode at 0.63 V, at a temperature of 30°C. Chromatography was performed on a Chiralcel OD-R column (Daicel Chemical Industries, Tokyo, Japan). The mobile phase was a mixture of 50 mM phosphate buffer (pH 5.5)/acetonitrile (80:20, v/v) and contained a total concentration of 5 mM KCl and 20 mg/l of EDTA. The analytes were extracted from blood using a liquid-liquid clean-up step and filtration on Bakerbond solid phase extraction NARC-2 columns (J.T. Baker, Phillipsburg, NJ). Calibration curves in the concentration range of 0.1 to 5000 ng/ml were analyzed with each run and peak-area ratios of analyte over internal standard were calculated. Calibration curves were constructed by weighted linear regression (weight factor = 1/(peak-area ratio))². Quality control samples of fixed concentration were analyzed to determine intra- and intervariability. Intr variability was less than 12%, and intervariability was less than 11% over a range of 50 to 1000 ng/ml. Extraction yield as determined by the internal-external standard method was 52% over a range of 50 to 1000 ng/ml. Using 50 µl of blood, the limit of detection for R-8-OH-DPAT was 0.5 ng/ml (signal-to-noise ratio = 3).

**HPLC Analysis of WAY-100,635.** The blood concentrations of WAY-100,635 were determined by a HPLC using fluorescent detection. The HPLC system consisted of a Shimadzu LC-10 AD pump (Waters, Milford, MA), an Alltima C18 5-µm column (4.6 mm × 150 mm) (Alltech, Breda, The Netherlands), a FP-1520 fluorescence detector (Jasco Corporation, Tokyo, Japan) set at a detection wavelength of 250 nm and an emission wavelength of 350 nm, and a C-R3A Chromatopac integrator (Shimadzu Corporation). The mobile phase was comprised of a mixture of a citric acid buffer (25 mM, pH 2.5) and acetonitrile (50:50, v/v) at a flow rate of 1 ml/min. The analytes were extracted from blood using a liquid-liquid extraction. To 50 µl of blood hemolyzed in 400 µl of water, 20 µl of the internal standard solution (250 ng/ml BMY-7787) was added. After mixing, 0.5 ml of borate buffer (0.2 M, pH 11.5) and 5 ml of a petroleum ether/dichloromethane mixture (55:45, v/v) was added and vortexed for 2 min. The mixture was centrifuged for 8 min at 4000 rpm, and the water phase was disposed by means of suction. The remaining water was removed by freezing and transferring the organic phase to a clean tube. The organic phase was evaporated to dryness under vacuum at 40°C. The residue was redissolved in 100 µl of mobile phase of which 50 µl was injected into the HPLC system. On the day of analysis, a 9-point calibration curve was prepared by spiking 50 µl of blood hemolyzed in 400 µl of water with 50 µl of a WAY-100,635 solution. This resulted in a blood concentration range of 25 to 5000 ng/ml. Samples were prepared as described and peak-area ratios of WAY-100,635/BMY-7787 (8-2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl) was 50 (signal-to-noise ratio = 3). Quality control samples of fixed concentration were prepared to determine intra- and intervariability. Intra variability was less than 10% and intervariability was less than 8% over a range of 60 to 1000 ng/ml. Extraction yield as determined by the internal-external standard method was 98% over a range of 100 to 1000 ng/ml. Using 50 µl of blood, the limit of detection for WAY-100,635 was 50 ng/ml (signal-to-noise ratio = 3).

**Chemicals.** R-8-OH-DPAT and WAY-100,635 were purchased from Research Biochemicals International (Natick, MA) and BMY-7787 from Tocris Cookson (Bristol, UK). All other chemicals used were of analytical grade (Baker, Deventer, The Netherlands).

**Data Analysis.** A population approach was utilized to quantify both the pharmacokinetics and pharmacodynamics of R-8-OH-DPAT and WAY-100,635. Using this approach, the population is taken as the unit of analysis while taking into account both intraindividual variability in the model parameters as well as interindividual residual error. Modeling was performed using the nonlinear mixed effects modeling software NONMEM (version V 1.1, NONMEM Project Group, University of California, San Francisco, CA) developed by Sheiner and Beal (Boeckman et al., 1992). Individual predictions were obtained in a Bayesian post hoc step.

**Pharmacokinetic Analysis.** The concentration-time profiles of R-8-OH-DPAT were best described using a standard 3-compartment pharmacokinetic model. The 3-compartment model as implemented in NONMEM’s ADVAN11, TRANS4 was used. In this model, the pharmacokinetics are described in terms of the compartments’ volumes of distribution (V₁, V₂, and V₃), clearance (CL), and the intercompartamental clearances (CL₂₃ and CL₃₄). The concentration-time profiles of WAY-100,635 were best described using a standard 2-compartment pharmacokinetic model as implemented in NONMEM’s ADVAN3 TRANS4 routine. Again the pharmacokinetics were described in terms of the compartments’ volumes of distribution, clearance, and intercompartmental clearance. Interindividual variability on the parameters was modeled by an exponential equation

\[ P_i = \theta \cdot \exp(n_i) \]  

where \( \theta \) is the population value for parameter \( P \), \( P_i \) is the individual value and \( n_i \) is the random deviation of \( P_i \) from \( P \). The values of \( n_i \) are assumed to be independently normally distributed with mean zero and variance \( \sigma^2 \). Residual error was characterized by a proportional error model

\[ C_{M,i} = C_{P,i} \cdot (1 + \varepsilon_i) \]  

where \( C_{P,i} \) is the ith plasma concentration for the ith individual predicted by the model, \( C_{M,i} \) is the measured concentration, and \( \varepsilon \) accounts for the residual deviance of the model predicted value from the observed concentration. Different \( \varepsilon \) values were allowed to be estimated for different dosing groups of WAY-100,635. The values for \( \varepsilon \) were assumed to be independently normally distributed with mean zero and variance \( \sigma^2 \). The values for the population \( \theta \), \( \sigma^2 \), and \( \sigma^2 \) were estimated using the first-order method in NONMEM.

**Pharmacodynamics Analysis.** Recently we have developed a physiological model that describes the hypothetic effect mediated by 5-HT₁₄ receptor agonists (Zuideveld et al., 2001). The model incorporates a set-point control that can be attenuated by the drug receptor interaction. The model utilizes the concepts of an indirect physiological response model (Dayneka et al., 1993), and takes into account a 0th-order rate constant associated with the warming of the body (\( k_w \)) and a first-order rate constant associated with the cooling of the body (\( k_c \)). The thermostat-like regulation is implemented as a continuous process in which body temperature (\( T \)) is compared with a fixed reference or set-point temperature (\( T_{SP} \)). 5-HT₁₄ agonists elicit hypothermia by decreasing the set-point value, of which the magnitude is a direct function of drug concentration \( f(C) \). This system is characterized by the following equations:

\[ \begin{align*} 
\frac{dT}{dt} &= k_m - k_{out} \cdot T \cdot X^{-\gamma}, \\
\frac{dX}{dt} &= a(T_{SP} - [1 - f(C)] - T), 
\end{align*} \]  

in which \( X \) denotes the thermostat signal, which is driven by the difference between the body temperature \( T \) and the set-point temperature \( T_{SP} \) on a time scale governed by \( a \). Hence when the set-point
value is lowered, the body temperature is perceived as too high, and X is lowered. The decreasing signal relates to the drop in body temperature via an effector function $X^{-}$, in which $\gamma$ determines the amplification. Raising this function to the loss term $k_{\text{out}} \cdot T$ therefore facilitates the loss of heat. With four system parameters ($k_{\text{in}}$, $k_{\text{out}}$, $\gamma$, and $a$) to be estimated, the degree of parameterization in eq. 3 is high and may lead to parameter identifiability problems. It can be shown that one parameter can be eliminated in a procedure involving redefinition of variables (Zuideveld et al., 2001). The procedure results in the definition of the parameters $A$ and $B$ where

$$A = \frac{a \cdot T_0}{X_0} = a \left( \frac{k_{\text{in}}}{k_{\text{out}}} \right)^{\frac{1}{2}} T_0^{-\frac{1}{2}}, \quad B = \frac{k_{\text{in}}}{T_0},$$

(4)

where $T_0$ and $X_0$ are the values for $T$ and $X$ when no drug is present. Hence, four physiological parameters are reduced to three, and parameter unidentifiability is abolished.

The combined effects of $R\text{-8-OH-DPAT}$ and WAY-100,635 at the $5\text{-HT}_{1A}$ receptor were characterized by the following equation for an agonist and an antagonist acting at a common receptor (Waud, 1975):

$$f(C) = \frac{S_{\text{max}} \cdot C_{\text{R}}}{SC_{50} \cdot (1 + C_{\text{W}}/K_{\text{B}}) + C_{\text{R}}}.$$  

(5)

where $S$ is the stimulus to the system, $S_{\text{max}}$ is the maximum stimulus the agonist can produce, $C_{\text{R}}$ and $C_{\text{W}}$ are the concentrations of the agonist, $R\text{-8-OH-DPAT}$, and the antagonist, WAY-100,635, respectively, $K_{\text{B}}$ denotes the affinity of the antagonist, WAY-100,635, for the $5\text{-HT}_{1A}$ receptor, $SC_{50}$ a measure of potency for $R\text{-8-OH-DPAT}$ and $\eta$ the slope coefficient of its concentration-response curve (see Leff and Dougall, 1993). Equation 5 also contains the exponent $b$, equivalent to the Schild regression slope, to test for the competitive nature of the antagonism, such as described in the Arunlaksana-Schild equation (Arunlaksana and Schild, 1959)

$$1 + \frac{C_{\text{W}}^b}{K_{\text{B}}} = \left( \frac{SC_{50}^\eta}{SC_{50}} \right)^n.$$  

(6)

In the Arunlaksana-Schild equation, $SC_{50}^\eta$ represents the apparent potency, and its ratio is often called the drug ratio ($DR^\eta$). When the regression-slope factor $b$ is not significantly different from 1, the $K_{\text{B}}$ represents the affinity of a competitive antagonist for the receptor (see Kenakin, 1993).

The maximal response as defined by the $S_{\text{max}}$ equals 1 for a full agonist, such as $R\text{-8-OH-DPAT}$ and 0 for an antagonist, consequently $S_{\text{max}}$ was fixed to 1 (Zuideveld et al., 2001). As a result of the introduction of dimensionless quantities, the dependent variable $T$ is rescaled depending on $T_{\text{sys}}$, the average temperature from the hour before drug administration and $T_{\text{max}}$ the average minimal temperature of the individual rats receiving a high dose of the $R\text{-8-OH-DPAT}$ as described previously (Zuideveld et al., 2001).

The model was implemented in NONMEM using ADVAN6. Parameterization is different from eq. 4, where $B$ is purely phenomenological. Since the individual values for $T_{\text{sys}}$ are known, the parameter $B$ can be calculated from $k_{\text{in}}$ (eq. 4). Therefore, the estimated physiological parameters were $k_{\text{in}}, a, \gamma$. Interindividual variability on the parameters was modeled to an exponential equation, such as described in eq. 1. Residual error was characterized by a proportional error model

$$y_{m_{ij}} = y_{p_{ij}} \cdot (1 + \epsilon_{ij}),$$  

(7)

where $y_{p_{ij}}$ is the $i$th prediction for the $i$th individual predicted by the model, $y_{m_{ij}}$ is the measurement, and $\epsilon$ accounts for the residual deviance of the model predicted value from the observed value. The values for the population $\theta$, $\omega^2$, and $\alpha^2$ are estimated using the centering first-order conditional estimation method with the first-order model in NONMEM. A conditional estimation method was used because of the high degree of non-linearity of the model and the high density of the data. The centering option gives the average estimate of each element of $\eta$ together with a $P$ value that can be used to assess whether this value is sufficiently close to zero. The occurrence of an average $\eta$ that is significantly different from zero indicates an uncentered or a biased fit. This method was not chosen because the average estimates of each element of $\eta$ were expected to be different from zero, but rather to greatly decrease computing time as required with just the conditional estimation method (Lindstrom and Bates, 1990; Boeckman et al., 1992). To further decrease computing time, only one-sixteenth of the temperature data set was used for modeling, reducing the temperature measurements from over 900 measurements per individual to approximately 60. The implication of this reduction is that there is a data point every 8 min, as opposed to every 0.5 min; however, this reduction did not void the integrity of the data profiles.

**Statistical Analysis.** Goodness-of-fit was analyzed using the objective function and various diagnostic methods as present in Xpose version 3.04 [S-plus-based model building aid (Jonsson and Karlsson, 1997)]. Model selection was based on the Akaike Information Criterion (Akaike, 1974) and assessment of parameter estimates and correlations.

**Results**

The average time-effect profiles for the effect on body temperature following administration of vehicle, WAY-100,635, $R\text{-8-OH-DPAT}$, and combinations of WAY-100,635 with $R\text{-8-OH-DPAT}$ are depicted in Fig. 1. The average baseline temperature ($\pm$S.E.M., $n = 35$) was $37.9 \pm 0.02^\circ$C. Following administration of 3 mg/kg WAY-100,635 in a 15-min infusion, a maximal increase of $0.7 \pm 0.13^\circ$C was observed after $77 \pm 9$ min. Although this rise was significantly different from the baseline temperature prior to the start of the infusion, it was similar to the control group, which had received an equivalent amount of vehicle. Administration of 1 mg/kg $R\text{-8-OH-DPAT}$ resulted in a maximal decrease in body temperature of $3.9 \pm 0.35^\circ$C after $42 \pm 3$ min. After reaching its

![Fig. 1. Average temperature-time profiles ($\pm$ S.E.M.) for a control experiment consisting of a regular infusion plus a computer-controlled infusion containing an equivalent amount of saline ($n = 6$, $\bullet$), 3 mg/kg WAY-100,635 in a 15-min infusion ($n = 6$, $\Delta$), a 1 mg/kg $R\text{-8-OH-DPAT}$ in a 15-min infusion during a computer-controlled infusion of WAY-100,635 targeted at 170 ($\bullet$), 85 ($\Delta$), and 20 ($\bullet$) ng/ml in blood, respectively (3 times $n = 6$), and 1 mg/kg $R\text{-8-OH-DPAT}$ in a 5-min infusion ($n = 6$, $\bullet$). The computer-controlled infusions started at $t = -15$ while all regular infusions started at $t = 0$.](image-url)
profiles for both WAY-100,635 and ples were taken to construct individual concentration-time infusions of WAY-100,635 were targeted at concentrations 20, 85, and 170 ng/ml in blood, respectively.

**Pharmacokinetics.** During the experiments, blood samples were taken to construct individual concentration-time profiles for both WAY-100,635 and R-8-OH-DPAT. Individual concentration profiles were predicted on the basis of fitting the data to a population pharmacokinetic model. On the basis of time-concentration curves, goodness-of-fit, and the minimum value of the objective function, a two-compartment model was selected for WAY-100,635, and a three-compartment model for R-8-OH-DPAT. Both the individually measured concentrations and the individually predicted profiles following the regular and computer-controlled infusions of WAY-100,635 are depicted in Fig. 2. The average steady-state concentrations of WAY-100,635 in blood were 22 ± 2.2, 44 ± 2.5, and 220 ± 39 ng/ml for the computer-controlled infusions targeted at 20, 85, and 170 ng/ml, respectively, for 2.5 h. In Fig. 3, the individually measured concentrations and the population-predicted curve for R-8-OH-DPAT are represented. All pharmacokinetic parameters were estimated in their mixed effect form, with the random effects incorporated in exponential error models. The values of the pharmacokinetic parameter estimates are represented in Table 1. No statistically significant correlation between the parameter estimates and between the parameters in different dosing groups were detected. Table 1 further displays the interindividual variation, and the intraindividual variation in the parameter estimates for both WAY-100,635 and R-8-OH-DPAT. For the different infusions of WAY-100,635 different intraindividual variations were predicted, which significantly improved the fit. The precision of the parameter estimates is represented by their 95% confidence intervals.

**Pharmacodynamics.** The effect-time profiles for R-8-OH-DPAT and combinations of R-8-OH-DPAT with WAY-100,635 were analyzed by fitting the data to the set-point model. Parameters were estimated using the centering first-order conditional estimation method with the first-order model. The average values for all η values were not significantly different from zero. Figure 4 shows representative fits of the set-point model to the time course of the effects of R-8-OH-DPAT on body temperature in the presence of 0, 20, 85, and 170 ng/ml of WAY-100,635. Population pharmacodynamic parameter estimates are shown in Table 2. Initially, the Schild regression parameter b was allowed to vary but fixed to unity in subsequent fits because this significantly improved the goodness of fit. Individual post hoc predictions of the parameters were not biased in the different treatment groups. The intraindividual variation on SC50 of R-8-OH-DPAT is slightly larger than reported previously (Zuideveld et al., 2001). On the basis of the results in Table 2, the concentration-effect relationships for the direct effect of R-8-OH-DPAT at the 5-HT1A receptor can be derived. The population mean concentration-effect relationships are shown in Fig. 5. The estimated population mean concentration-effect relationships, shown in Fig. 1, demonstrate the concentration-dependent, parallel shift of the R-8-OH-DPAT concentration-effect curve by WAY-100,635. The linear Schild regression plot, based on the individual predictions of the SC50 with a unity slope shown in Fig. 6 further underscores the competitive nature of the WAY-100,635-induced antagonism.

**Discussion**

The present study provides novel information on both the pharmacokinetics of the 5-HT1A receptor antagonist WAY-100,635 and the pharmacological behavior of this compound in vivo. Numerous authors have shown in in vitro investigations that WAY-100,635 is a highly selective, competitive antagonist at the 5-HT1A receptor (Forster et al., 1995; New-
PK-PD Modeling of WAY-100,635 on 5-HT1A-Mediated Hypothermia

**TABLE 1**

Population pharmacokinetic parameters and inter- and intraindividual variabilities of WAY-100,635 and R-8-OH-DPAT

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameter</th>
<th>Value</th>
<th>CV inter-indiv.</th>
<th>95% CI</th>
<th>Unit</th>
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<tbody>
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<td>WAY-100,635</td>
<td>CL1</td>
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<td>22.0–34.7</td>
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<tr>
<td></td>
<td>CV intra-indiv: 44% (3 mg/kg), 39% (170 ng/ml), 16% (85 ng/ml), 105% (20 ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| R-8-OH-DPAT   | CL         | 18.7  | 30              | 14.4–23.0 | ml/min     |
|               | CL2        | 25.5  | 50              | 19.0–31.0 | ml/min     |
|               | CL3        | 114   | 51              | 86.2–141  | ml/min     |
|               | V1         | 32.3  | >1              | 4.7–59.9  | ml         |
|               | V2         | 3110  | 38              | 1960–4260 | ml         |
|               | V3         | 711   | 38              | 530–892   | ml         |
|               | CV intra-indiv: 19.8% |

CV inter-indiv, interindividual coefficient of variation; CV intra-indiv, intraindividual coefficient of variation, where the dose is represented in brackets; 95% CI, 95% confidence interval over the precision of the estimated parameter.

Fig. 4. Selected representative fits of the body temperature versus time profile for different treatments of WAY-100,635 with R-8-OH-DPAT. Open circles represent measured body temperature, solid lines represent individual prediction, and the dashed line represents the population prediction. Note that the second decrease in temperature in panel C is caused by the fact that the concentration R-8-OH-DPAT is still at a level at which it can induce a hypothermic response; interestingly the model takes this into account very well. Infusions all started at t = 60.

In vivo investigations of the pharmacokinetics of WAY-100,635 have not been taken into consideration, the nature of the in vivo interaction is poorly understood. This has led to conflicting reports in literature. It has for example been suggested that in vivo WAY-100,635 behaves as an insurmountable antagonist and that different doses of WAY-100,635 are needed to antagonize different 5-HT1A agonists (Cryan et al., 1999).

In the present study we present a mechanism-based PK-PD analysis of the interaction between WAY-100,635 and R-8-OH-DPAT. This analysis shows that both compounds interact in a competitive manner at the 5-HT1A receptor. The hypothermic response was used as a pharmacodynamic endpoint and the results were analyzed on the basis of a recently developed physiological response model with set-point control (Zuideveld et al., 2001) to account for time dependencies in the effect of R-8-OH-DPAT on body temperature. Furthermore, to overcome complexities due to differences in pharmacokinetics between the compounds, WAY-100,635 was administered by computer-controlled infusion, resulting in stable steady-state concentrations of WAY-100,635 for at least 2.5 h. This facilitates the estimation of the apparent affinity of WAY-100,635 to the 5-HT1A receptor in vivo on the basis of concentrations. A similar approach has been successfully applied before to other receptor systems (Mandema et al., 1992; Appel et al., 1995). In these studies, the direct effect of the electroencephalogram and heart rate were used as pharmacodynamic end points to estimate the affinity of the antagonists flumazenil and 8-cyclopentyltheophylline to the γ-aminobutyric acid A and adenosine A1 receptor, respectively. The present study is to our knowledge the first in which a competitive drug interaction is characterized in conjunction with an indirect physiological response model.

Large differences in pharmacokinetics of R-8-OH-DPAT versus WAY-100,635 were observed. The clearance of WAY-100,635 is fast, resulting in a terminal half-life of 33 min versus 143 min for R-8-OH-DPAT. This has important implications for the design of drug interaction studies. This marked difference in terminal half-life underscores the need for computer-controlled infusion techniques in drug-interaction studies in vivo. When administered as bolus infusions, R-8-OH-DPAT will be present in the body at effective concentrations for a much longer time than the antagonist WAY-100,635. With the aid of a computer-controlled infusions, it is possible to maintain stable concentrations of WAY-100,635 for a prolonged period of time.

Pharmacokinetic analysis has further shown that co-administration of WAY-100,635 did not influence the pharmacokinetics of R-8-OH-DPAT. Likewise there were no indications that R-8-OH-DPAT influenced the pharmacokinetics of WAY-100,635 to a significant extent. The small deviations of the measured concentrations versus the targeted concentrations of the computer-controlled infusions are most likely due to individualization of the infusion protocols, i.e., the programming per rat, and underscores the need to measure the...
blood concentrations during the experiment. The observed intraindividual variation in the computer-controlled infusion that was targeted at 20 ng/ml is rather high. This can most likely be explained by the sparse-sampling protocol, where a limited number of 200-μl samples were taken to overcome the detection limit of 50 ng/ml for 50-μl samples. For the other infusion rates, more frequent blood samples were collected. Furthermore, as the computer-controlled infusions were programmed with preliminary pharmacokinetic parameters obtained from the 3 mg/kg dosing group, some variation in dosing exists, which is most evident in the 170 ng/ml target group (Fig. 2). The pharmacokinetics of R-8-OH-DPAT did not differ significantly with values reported previously (Zuideveld et al., 2000, 2001).

To characterize antagonism of WAY-100,635, the R-8-OH-DPAT-induced hypothermic response was chosen as a pharmacodynamic end point, as it is considered to be a robust marker for 5-HT1A activity (Millan et al., 1993; Cryan et al., 1999). Furthermore, the hypothermic response behaves as an ideal biomarker, as it is continuous, reproducible, sensitive enough to discriminate between a full and a partial agonist (Hadrava et al., 1996, Zuideveld et al., 2001), and selective (Millan et al., 1993; Cryan et al., 1999). We have recently developed a mechanism-based pharmacokinetic-pharmacodynamic model that characterizes the time course of 5-HT1A receptor-mediated hypothermia (Zuideveld et al., 2001). The model is an indirect physiological response model (Daynaka et al., 1993) with a set-point control, in which 5-HT1A agonists act by lowering the set-point in a direct concentration-dependent manner. This model is considered physiological and accurately reflects the mechanism of R-8-OH-DPAT-induced hypothermia. It is well established that the 5-HT1A receptors play a role in maintaining the body's set-point temperature (Bligh, 1979; Lin et al., 1983; Zeisberger, 1990; 1998; Jessen, 1996; Schwartz et al., 1998). Numerous reports suggest this set point is regulated through an interplay between the 5-HT1A (hypothermia) and 5-HT2A/C (hyperthermia) receptor system (Gudelsky et al., 1986; Abdel-fattah et al., 1995; Schwartz et al., 1995; 1998; Salmi and Ahlenius, 1998). The set point model allows 5-HT1A receptor agonist to attenuate the set point via a sigmoidal transducer function. It has been shown that the effect of 5-HT1A agonists is determined by the administered dose and the affinity and intrinsic efficacy at the 5-HT1A receptor (Zuideveld et al., 2001). In the present study, it is shown that administration of increasing levels of the antagonist in computer-controlled infusions diminishes the hypothermic effect elicited by R-8-OH-DPAT, whereas WAY-100,635 itself has no significant effect on body temperature. It has been suggested that R-8-

### Table 2

Population pharmacodynamic parameters and inter- and intraindividual variabilities of the interaction experiments between R-8-OH-DPAT and WAY-100,635

<table>
<thead>
<tr>
<th>Type/Drug</th>
<th>Parameter</th>
<th>Value</th>
<th>CV inter-indiv</th>
<th>95% CI</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological</td>
<td>k_in</td>
<td>1.35</td>
<td>48%</td>
<td>0.9–1.8</td>
<td>°C/min</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.0435</td>
<td>&gt;1</td>
<td>0.0337–0.0533</td>
<td>min⁻¹</td>
</tr>
<tr>
<td></td>
<td>γ</td>
<td>1.00</td>
<td>12%</td>
<td>0.486–1.51</td>
<td>N.A.</td>
</tr>
<tr>
<td>R-8-OH-DPAT</td>
<td>SC₅₀</td>
<td>40.3</td>
<td>83%</td>
<td>24.8–55.8</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>1.38</td>
<td>35%</td>
<td>1.08–1.67</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>B_max</td>
<td>1 (fixed)</td>
<td>23%</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>WAY-100,635</td>
<td>Kₐ</td>
<td>0.98</td>
<td>2.6</td>
<td>0.162–1.79</td>
<td>ng/ml</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>1 (fixed)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

CV-inter, coefficient of variation; CV-intra, coefficient of the random error; N.A., not applicable.

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**Fig. 5.** Simulated concentration-effect curves for R-8-OH-DPAT during the different WAY-100,635 treatments based on the population averages. The different types of lines denote the different treatments, hence each rightward shift indicates a increase in WAY-100,635 steady-state level (0, 20, 85, and 170 ng/ml).

**Fig. 6.** A Schild regression analysis, the drug-ratio (log(DR-1)) as determined by the ratio of EC₅₀ apparent over the EC₅₀ is plotted against the average blood concentration of WAY-100,635 during the computer-controlled infusion. The solid line represents the fit where b is fixed to 1, together with the estimated parameters. The markers represent the individual predictions.
OH-DPAT exhibits affinity for the 5-HT$_{1A}$ receptor (Hoyer et al., 1994). However, this study contributes to the body of evidence that the hypothecic effect is solely caused by the 5-HT$_{1A}$ receptor since WAY-100,635 has little affinity for the 5-HT$_{3}$ receptor [over 74-fold selectivity over 5-HT$_{1A}$ (Forster et al., 1995)].

This experimental setup has facilitated the determination of the competitive nature of WAY-100,635, using an interaction model in the set point model. The fit with the Schid regression parameter ($b_2$) fixed to 1, greatly improved the objective function. The $K_B$ obtained, 0.98 ng/ml ($= 2.3$ nM, $K_B = 8.6$), can therefore be regarded as the equilibrium constant for a competitive antagonist. Another important observation in accordance with competitive behavior is the fact that the slope factor characterizing the concentration-effect curve of R-8-OH-DPAT did not differ between treatment groups. Interestingly, the $K_B$ obtained is practically identical to values found in vivo ($K_B = 9.0$ in rat hypothalamic (Kawajaha et al., 1997) and 9.0 in whole brain (Assie and Koek, 1996)) and falls within residual error (Table 2). Furthermore, despite the diminishing effect of the hypothepic response caused by R-8-OH-DPAT with increasing concentrations of WAY-100,635, the effect was consistent with the effect being surmountable, contrary to previous observations (Forster et al., 1995). The physiological and drug parameters estimated for R-8-OH-DPAT are similar to values found previously. The interindividual variation on some of the parameters is slightly larger than reported previously (Zuideveld et al., 2001), mainly caused by the fact that the small hypothertic effect of R-8-OH-DPAT in the 170 ng/ml WAY-100,635 infusion group is hard to characterize. Furthermore, in previous studies, multiple dose ranges were investigated, which has greatly improved precision (Zuideveld et al., 2001).

In summary, the present study, an estimate of the in vivo binding affinity of the selective 5-HT$_{1A}$ receptor antagonist WAY-100,635 was obtained by quantification of the competitive interaction with the protypical agonist R-8-OH-DPAT on the hypothertic response in rats. The recently developed physiological set point model for the quantification of 5-HT$_{1A}$ receptor agonists, combined with a model describing agonist-antagonist interaction acting on a common receptor, described the inhibition of agonist response in the absence and presence of antagonist well. The current study has further demonstrated the necessity of a thorough pharmacokinetic analysis, enabling interaction studies in which the concentration are controlled. Controlling the concentration profiles proved essential in demonstrating that the observed effect and the parameters obtained are consistent with the effect showing surmountable antagonism in vivo. The integrated pharmacokinetic-pharmacodynamic model offers a novel approach for the characterization and quantification of 5-HT$_{1A}$ receptor antagonists in vivo.

Acknowledgements

We thank Erica Tukker for technical assistance in the animal experiments.

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Hjorth S (1985) Hypothermia in the rat induced by the potent serotoninergic agent 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT. Science 228:131–133.


Lindstrom MJ and Bates DM (1990) Nonlinear mixed effects models for repeated observations in accordance with competitive behavior is the fact that the slope factor characterizing the concentration-effect curve of R-8-OH-DPAT did not differ between treatment groups. Interestingly, the $K_B$ obtained is practically identical to values found in vivo ($K_B = 9.0$ in rat hypothalamic (Kawajaha et al., 1997) and 9.0 in whole brain (Assie and Koek, 1996)) and falls within residual error (Table 2). Furthermore, despite the diminishing effect of the hypothertic response caused by R-8-OH-DPAT with increasing concentrations of WAY-100,635, the effect was consistent with the effect being surmountable, contrary to previous observations (Forster et al., 1995). The physiological and drug parameters estimated for R-8-OH-DPAT are similar to values found previously. The interindividual variation on some of the parameters is slightly larger than reported previously (Zuideveld et al., 2001), mainly caused by the fact that the small hypothertic effect of R-8-OH-DPAT in the 170 ng/ml WAY-100,635 infusion group is hard to characterize. Furthermore, in previous studies, multiple dose ranges were investigated, which has greatly improved precision (Zuideveld et al., 2001).

In summary, the present study, an estimate of the in vivo binding affinity of the selective 5-HT$_{1A}$ receptor antagonist WAY-100,635 was obtained by quantification of the competitive interaction with the prototypical agonist R-8-OH-DPAT on the hypothertic response in rats. The recently developed physiological set point model for the quantification of 5-HT$_{1A}$ receptor agonists, combined with a model describing agonist-antagonist interaction acting on a common receptor, described the inhibition of agonist response in the absence and presence of antagonist well. The current study has further demonstrated the necessity of a thorough pharmacokinetic analysis, enabling interaction studies in which the concentrations are controlled. Controlling the concentration profiles proved essential in demonstrating that the observed effect and the parameters obtained are consistent with the effect showing surmountable antagonism in vivo. The integrated pharmacokinetic-pharmacodynamic model offers a novel approach for the characterization and quantification of 5-HT$_{1A}$ receptor antagonists in vivo.

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References

Zuideveld et al.


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