Mechanism-Based Pharmacokinetic-Pharmacodynamic Modeling of 5-HT<sub>1A</sub> Receptor Agonists: Estimation of in Vivo Affinity and Intrinsic Efficacy on Body Temperature in Rats

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ABSTRACT

The pharmacokinetic-pharmacodynamic (PK-PD) correlations of seven prototypical 5-HT<sub>1A</sub> agonists were analyzed on the basis of a recently proposed semi-mechanistic PK-PD model for the effect on body temperature. The resulting concentration-effect relationships were subsequently analyzed on the basis of the operational model of agonism to estimate the operational affinity (pK<sub>i</sub>) and efficacy (log τ) at the 5-HT<sub>1A</sub> receptor in vivo. The values obtained in this manner were compared with estimates of the affinity (pK<sub>i</sub>) and intrinsic efficacy (log[agonist ratio]) in a receptor-binding assay. Between 5-HT<sub>1A</sub> agonists wide differences in in vivo affinity and efficacy were observed, with values of the pK<sub>i</sub> ranging from 5.67 for flesinoxan to 8.63 for WAY-100,635 [N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-2-pyridinyl-cyclohexanecarboxamide] and of the log τ ranging from −1.27 for WAY-101,135 [N-(1,1-dimethylethyl)-4-(2-methoxyphenyl)-α-phenyl-1-piperazine-propanamide] to 0.62 for R-(+)-8-hydroxy-2-[di-n-propylamino]tetralin. Poor correlations were observed between the in vivo receptor affinity (pK<sub>i</sub>) and the affinity estimates in the in vitro receptor binding assay (pK<sub>i</sub>; r<sup>2</sup> = 0.55, P < 0.05), which could in part be explained by differences in blood-brain distribution. In contrast, a highly significant correlation was observed between the efficacy parameters in vivo (log τ) and in vitro (log [agonist ratio]; r<sup>2</sup> = 0.76, P < 0.05). Thus by combining the previously proposed semi-mechanistic PK-PD model for the effect on body temperature with the operational model of agonism, a full mechanistic PK-PD model for 5-HT<sub>1A</sub> receptor agonists has been obtained, which is highly predictive of the in vivo intrinsic efficacy.

Recently a semi-mechanistic pharmacokinetic-pharmacodynamic model for the effects of 5-HT<sub>1A</sub> agonists on body temperature has been proposed, which is based on dynamical systems analysis. It has been demonstrated that this model can be used to estimate both the in vivo potency and the intrinsic activity of 5-HT<sub>1A</sub> agonists. In the mean time, this model has been successfully applied to characterize the in vivo concentration-effect relationships of several 5-HT<sub>1A</sub> agonists including R- and S-8-OH-DPAT, flesinoxan, and buspirone (Zuideveld et al., 2001, 2002a, 2002b). Furthermore, the in vivo affinity of the 5-HT<sub>1A</sub> receptor antagonist WAY-100,635 could be estimated on basis of the analysis of the competitive interaction with R-8-OH-DPAT (Zuideveld et al., 2002b).

The previously proposed model is semi-mechanistic in the sense that it uses a mechanistic model for the characterization of the transduction process. Specifically, 5-HT<sub>1A</sub> agonists cause lowering of body temperature by modulation of the set-point for maintenance of the body temperature in a direct concentration-dependent manner (Zuideveld et al., 2001). Therefore a mechanism-based set-point model, which is based on dynamical systems analysis, was proposed and successfully implemented in the integrated PK-PD model to describe the complex time profile of the effects of 5-HT<sub>1A</sub> agonists.
agonists on body temperature (Zuideveld et al., 2001, 2002b). However, the existing model still contains the Hill equation to characterize the interaction of the 5-HT1A receptor agonists at the receptor level. The Hill equation is an empirical equation describing concentration-effect relationships, since its parameters (E\text{max}, EC_{50}, and the Hill factor) are dependent on both drug-specific properties (receptor affinity and receptor intrinsic efficacy) and system-related properties (e.g., receptor density). This complicates the use of this equation for extrapolation and prediction (i.e., from in vitro test systems to the in vivo situation, for interspecies extrapolation and for the prediction of intra- and inter-individual variability in drug response) (Van der Graaf and Danhof, 1997a). Therefore, in previous investigations it has been proposed to incorporate principles from receptor theory for characterization of the drug concentration-effect relationship in mechanism-based PK-PD modeling (Van der Graaf and Danhof 1997a; Cox et al., 1998; Tuk et al., 1999, 2002; Garrido et al., 2000; Visser et al., 2002, 2003). In these investigations it has been shown that in particular the operational model of agonism (Black and Leff, 1983) is a useful expression for the characterization of the drug-receptor interaction, allowing a separation between drug-related and system-related parameters. It has been demonstrated that this model is very useful for the prediction of in vivo concentration-effect relationships on the basis of results from in vitro bioassays (Black and Leff, 1983; Van der Graaf and Danhof, 1997a), the prediction of tissue selectivity of drug effects (Van der Graaf et al., 1997b, 1999) and the understanding of inter-individual variability in drug effects (Van der Graaf and Danhof, 1997a).

The objective of the present investigation was to link the previously developed semi-mechanistic PK-PD model for 5-HT1A agonists with the operational model of agonism into a full mechanism-based PK-PD model. To this end the concentration-effect relationships of seven pro-typical 5-HT1A receptor agonists were simultaneously analyzed. This allowed estimation of the unique system related parameters such as the tissue maximum (E\text{max}) and slope factor of the transduction function (n). In addition for each compound the specific drug-related properties receptor affinity (K\text{A}) and intrinsic efficacy (\tau) were estimated. These drug-related properties were compared with estimates of the receptor affinity and intrinsic efficacy as determined in an in vitro binding assay.

Materials and Methods

In Vivo Pharmacological Experiments. The details of the pharmacokinetic-pharmacodynamic experiments have been described previously (Zuideveld et al., 2001, 2002a, 2002b, 2002c). Briefly, 8 days prior to the experiment, the rats were operated upon. Indwelling cannulae for drug administration and blood sampling were implanted into the right jugular vein and the left femoral artery, respectively. Furthermore, a telemetric transmitter (Physio-tek implant TA10TA-F40 system; Data Sciences International (DSI), St. Paul, MN) was implanted into the abdominal cavity for the measurement of core body temperature. In the PK-PD experiments, conscious freely moving rats received an i.v. infusion of vehicle (saline) or active drug. R-8-OH-DPAT was administered in a wide range of different doses: 1 mg/kg in 5 min (n = 6), 3 mg/kg in 5 min (n = 7), in 15 min (n = 5) and in 30 min (n = 6) and by computer-controlled infusions, with the concentration targeted at 160 ng/ml in blood for 2 h (n = 6). S-8-OH-DPAT was administered in a 5 mg/kg in 15 min (n = 6) and a 15 mg/kg in 15 min (n = 6) infusion. Flesinoxan was administered in 3 mg/kg in 5 min (n = 6), 10 mg/kg in 5 and 15 min (both n = 6) infusions. Buspirone was administered in a 5 mg/kg in 15 min (n = 6), WAY-100,135 (n = 7) infusion. 1-PP and WAY-100,135 were administered in a 10 mg/kg in 15 min infusion (both n = 6). WAY-100,635 (−5-HT1A antagonist) was administered in a 3 mg/kg in 15 min infusion and in computer-controlled infusions with the blood concentration targeted at 170, 85, and 20 ng/ml in blood, respectively (4 \times n = 6) during which a 1 mg/kg in 15 min infusion of R-8-OH-DPAT was administered. In each experiment each individual rat, approximately 15 to 18 serial blood samples of 50 µl were taken according to a fixed time schedule to determine the time course of the drug concentration. After the experiment samples were stored at −20°C pending HPLC analysis based on methods described previously (Zuideveld et al., 2000, 2002a, 2002b, 2002c) or by MS (e.g., WAY-100,135). Body temperature was measured continuously throughout the experiment using the telemetric system. Protein binding was determined ex vivo. Blood was collected and incubated with various compounds at 34° and 38°C. Concentrations of 50 and 1000 ng/ml for R-8-OH-DPAT and flesinoxan, 250 and 2500 ng/ml for buspirone and 1-PP, and 750 and 5000 ng/ml for S-8-OH-DPAT, buspirone, WAY-100,635, and WAY-100,135 were evaluated. Blood was centrifuged and from the remaining plasma, the free fraction was isolated using ultra filtration (Centrifree; Millipore Corporation, Bedford, MA).

Receptor Binding Assay. The interaction at the 5-HT1A receptor was determined in vitro in recombinant Ha 6 Ha5 5-HT1A cells in which the human receptor is expressed (Pauwels et al., 1993). It has been demonstrated that the rat 5-HT1A receptor is 89% similar to the human receptor (Albert et al., 1990). Recombinant Ha 5-HT1A cells were grown in adherent culture in roller bottle flasks in Dulbecco’s modified Eagle’s medium containing fetal bovine serum (50 ml/500 ml), 1-glutamine (5 ml/500 ml), antibiotic-antimycotic (5 ml/500 ml), and the antibiotic Geneticin (5 ml/500 ml; BioVectra, Prince Edward Island, Canada).

Receptor binding was determined on the basis of a scintillation proximity assay (SPA). Briefly this assay was conducted as follows. On reaching confluence, the cells were harvested, cell pellets were prepared by centrifugation and stored at −80°C until required. For the actual analysis, cell pellets were retrieved from storage, thawed on ice, and resuspended in membrane preparation buffer (50 mM Tris-HCl, pH 7.5 (4°C), 4 mM CaCl2, + 1 protease inhibitor tablet (per 50 ml; Roche Diagnostics, Indianapolis, IN) at approximately 10 µl of buffer per milliliter of pellet. The membrane were homogenized with a mechanical homogenizer using 15 full strokes in ice before centrifuging at 1000g for 20 min at 4°C. The supernatant was retained, and the pellet was homogenized and centrifuged at 1000g for 20 min at 4°C. The supernatants were combined, incubated at 37°C for 15 min and centrifuged at 48,000g for 20 min at 4°C. The pellets were resuspended in a small volume of membrane preparation buffer using the homogenizer as before. The volumes were adjusted to allow storage at −80°C at a protein concentration of around 0.5 mg/ml, as determined on the basis of a protein assay using the Microprotein kit (Sigma Chemical, Dorset, UK). Membrane homogenates were thawed on ice, diluted in incubation buffer if required, and homogenized at low speed using a Powergen 125 homogenizer (Fisher Scientific, Loughborough, UK). PII-Ya SPA beads ([H]8-OH-DPAT Assay) or WGA-Ya SPA beads ([H]WAY-100,635 assay), both from Amersham Biosciences UK Ltd (Little Chalfont, Buckinghamshire, UK) were resuspended at 50 mg/ml in incubation buffer. Beads were precoupled with membranes by incubating 3 µg of protein/mg of bead on a tilting tube roller for 2 h at 4°C. The coupled beads/membranes were then centrifuged at 120g in a Heraeus Multifuge 3 benchtop centrifuge (Kendro, Bishop’s Stortford, Hertfordshire, UK) for 2 min at 4°C. The supernatant was discarded, and the pellet was washed in incubation buffer and spun as before. The conjugated beads were then resuspended in incubation buffer at 15 mg of bead/ml. Radioligands [H]8-OH-DPAT and
[\({\text{[H]}}\text{WAY-100,635}\), 635] were incubated in dilution buffer (50 mM Tris-HCl, 4 mM CaCl\(_2\), 10 \(\mu\)M pargylene, 1 g/l ascorbic acid, 0.01% Tween 40, pH 7.5 at 25°C) to give a concentration of 3 nM and 1.5 nM, respectively (1 nM and 0.5 nM final assay concentration, respectively). For the determination of [\({\text{[H]}}\text{8-OH-DPAT}\), binding, non-specific binding (NSB) was defined using 10 \(\mu\)M WAY-100,635, while for [\({\text{[H]}}\text{WAY-100,635}\) binding NSB was defined using 10 \(\mu\)M R-8-OH-DPAT. Compounds were dissolved in 100% DMSO and diluted in assay buffer containing 3% DMSO using a Tecan Genesis liquid handling robot (Tecan, Maennedorf, Switzerland) to a top concentration of 30 \(\mu\)M in 3% DMSO (10 \(\mu\)M in 1% DMSO in well). Serial half-log dilutions of these stock solutions were made with assay buffer containing 3% DMSO. Samples (20 \(\mu\)l) were plated out in duplicate into 384 well optiplates to give 11-point concentration-effect curves. Twenty microliters of the total and NSB stocks were added to the plate, followed by the addition of 20 \(\mu\)l of the bead/membrane preparation to all wells using a mult滴. The bead/membrane preparation was kept in suspension using a stirring flasket. Finally, 20 \(\mu\)l of the [\({\text{[H]}}\text{8-OH-DPAT}\) or [\({\text{[H]}}\text{WAY-100,635}\) was added to each well of the Optiplate using the multidot. The plates were incubated on a plate shaker at room temperature for a total incubation time of 2 h for [\({\text{[H]}}\text{8-OH-DPAT}\) or 6 h for [\({\text{[H]}}\text{WAY-100,635}\), before being left to settle for 30 min before counting using the Topcount NXT for 45 s/well (PerkinElmer Life and Analytical Sciences, Boston, MA). All procedures were carried out at 4°C unless otherwise stated.

**Compounds.** R-8-OH-DPAT, S-8-OH-DPAT, and WAY-100,635 were purchased from Sigma/RBI (Natick, MA). Buspirone and 1-PP were generously donated by Bristol-Myers Squibb Co. (Princeton, NJ). Solvay Pharmaceuticals (Wexford, The Netherlands) generously donated forskolin. WAY-100,635 was synthesized by Pfizer (Sandwich, Kent, UK). Acetic acid, cell dissociation solution, CaCl\(_2\), Hepes, IBMX, KCl, L-glutamine, NaCl, pargylene, polyethylene-imine, and TRIS (hydroxymethyl) methylamine were purchased from Sigma Chemical. Glucose, KH\(_2\)PO\(_4\), and MgSO\(_4\) were purchased from VWR International Ltd. (Dorset, UK). [\({\text{[H]}}\text{8-OH-DPAT}\) and [\({\text{[H]}}\text{WAY-100,635}\) were obtained from Amersham Biosciences UK, Ltd.

**Data Analysis.** A population approach was used to quantify both the pharmacokinetics and pharmacodynamics of the 5-HT\(_{1A}\) receptor agonists. Modeling of the in vivo pharmacokinetic and pharmacodynamic data was performed using the nonlinear mixed effects modeling software NONMEM developed by Sheiner and Beal (version V 1.1, NONMEM Project Group, University of California, San Francisco, CA) (Boekeman et al., 1992). Individual predictions were obtained in a Bayesian post hoc step. The concentration-time profiles of the 5-HT\(_{1A}\) receptor ligand were described using 2- and 3-compartment pharmacokinetic models like those implemented in NONMEM’s ADVAN3 and ADVAN11, respectively (Zuideveld et al., 2001, 2002a, 2002b, 2002c). The pharmacokinetic parameter estimates were used to calculate individual agonist blood concentrations at the times of the temperature measurements.

The pharmacokinetic data were used to quantify the relationship between the time profile of the agonist blood concentration and the time course of the hypothermic effect. For this purpose, the data on the time course of the hypothermic effect for each individual rat were fitted to the semi-mechanistic PK-PD model, which we have recently proposed (Zuideveld et al., 2001, 2002a). In this model, the hypothermic effect by the 5-HT\(_{1A}\) receptor agonists is considered the result of the attenuation of a set-point control by the drug receptor interaction. The model further uses the concept of an indirect physiological response model (Daynek et al., 1993), and takes into account a 0th order rate constant associated with the warming of the body (\(k_{in}\)) and a first order rate constant associated with the cooling of the body (\(k_{out}\)). 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-HT\(_{1A}\) agonists elicit hypothermia by decreasing the set point value, whereby the extent of the decrease is a function of the drug concentration \(C\) (see below). This yields the following system of equations.

\[
\begin{align*}
\frac{dT}{dt} & = k_{in} - k_{out} \cdot T \cdot X^{-}, \\
\frac{dX}{dt} & = a(T_{0} \cdot [1-f(C)] - T),
\end{align*}
\]

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 \(a\). Where \(T_{SP} = T_{0} \cdot [1-f(C)]\). Hence, when the set-point value is lowered, the body temperature is perceived as too high and \(X\) is lowered. The thermostat signal affects the cooling of the body through an effector function \(X^{-}\), which multiplies the first order rate constant \(k_{in}\). Thus, a drop in set point temperature leads to an increase in thermostat signal and a lowering of the “effective rate constant,” which governs the cooling of the body. With four system parameters to be estimated, the degree of parameterization in eq. 1 is high, and this may lead to parameter identifiability problems. It can be shown that that one parameter can be eliminated in a procedure involving the introduction of dimensionless variables (Zuideveld et al., 2001). The procedure results in the establishment of the parameters \(A\) and \(B\) defined by,

\[
A = \frac{\alpha \cdot T_{0} \cdot X_{0}^{a}}{X_{0}} = \frac{h_{in}^{a} k_{in}^{1/a}}{k_{in}} \cdot T_{0}^{1/(1\alpha)} \quad \text{and} \quad B = \frac{h_{in}}{k_{in}},
\]

where \(T_{0}\) and \(X_{0}\) are the values for \(T\) and \(X\) when no drug is present. Thus, four physiological parameters are reduced to three, and the parameters become identifiable. Note that \(A\) and \(B\) represent the relative growth rate of, respectively, \(X\) and \(T\) when \(C = 0\) and \(T = T_{0}\). The maximal response, as defined by \(S_{max}\), equals 1 for a full agonist, such as R-8-OH-DPAT and 0 for an antagonist. As a result of the introduction of dimensionless quantities, the dependent variable \(T\) is rescaled with respect to \(T_{0}\), the average temperature during the hour prior to drug administration and the observed average minimal temperature of the individuals receiving the highest dose of R-8-OH-DPAT as described previously (Zuideveld et al., 2001).

In the original model, the effect of the 5-HT\(_{1A}\) receptor agonist at the receptor was described on the basis of the empirical Hill equation. Thereby the drug-receptor interaction is considered to generate a stimulus \(S\), which in turn lowers the body’s set point. The relationship between the concentration and the fractional lowering of the set point was formulated as a sigmoidal \(E_{max}\) model according to,

\[
S = f_{1}(C) = \frac{\alpha \cdot C_{vis}}{C_{IC} + C_{vis}},
\]

where \(S\) is the stimulus, \(\alpha\) is the maximum stimulus the drug can produce, \(C\) is the drug concentration or the potency, \(C_{IC}\) is the concentration required to produce 50% of the maximum stimulus, and \(n_{A}\) is the slope factor, which determines the steepness of the curve (also known as the Hill factor). To obtain a full mechanistic model the sigmoidal \(E_{max}\) model was replaced by the operational model of agonism (Black and Leff, 1983),

\[
f_{2}(C) = \frac{E_{max} \cdot \tau \cdot C_{vis}}{(K_{A} + C_{vis})^{n_{A}} + \tau \cdot C_{vis}},
\]

where \(E_{max}\) is the maximum effect achievable in the system, \(K_{A}\) is the agonist dissociation equilibrium constant, \(n\) is the slope index for the occupancy-effect relation, and \(\tau\) is the efficacy parameter, which is defined by the ratio of total receptor concentration and the concentration of agonist-receptor complex required to produce half-max-
imal effect. As \( f_1(C) \rightarrow \alpha \) and \( f_2(C) \rightarrow E_{\max} \cdot \frac{\alpha}{\theta^2 + 1} \) when \( C \rightarrow \infty \), the sigmoidal \( E_{\max} \) equation parameters can be expressed in terms of the operational model of agonism as follows (Black and Leff, 1983):
\[
\alpha = E_{\max} \cdot \frac{\theta}{\theta^2 + 1} \tag{5}
\]
and for \( f_2(C) = \nu \exp\): 
\[
SC_{50} = \frac{K_A}{(2 + \nu)^{1/n} - 1} \tag{6}
\]
Inspection of eq. 6 shows that \( SC_{50}/K_A \rightarrow 1/(2^{1/n} - 1) \) when \( \nu \rightarrow 0 \). Furthermore, with high-efficacy values, eq. 6 approximates to a simple linear relationship, \( SC_{50}/K_A = 1/\nu \) regardless of the value of \( n \) (Van der Graaf et al., 1999).

Leff et al. (1990) have shown that the operational model can be used to obtain estimates of affinity and efficacy of a partial agonist by comparison to full agonists. This comparative method (originally proposed by Barlow, 1967) is based on the idea that per definition, the intrinsic activity of a full agonist is identical to the maximum system response. Therefore \( E_{\max} \) is constrained to the estimate of the sigmoidal \( E_{\max} \) equation, \( \alpha \), for a full agonists, and \( K_A \) and \( \nu \) for partial agonists can be estimated by directly fitting the operational model of agonism to the concentration-effect data. Van der Graaf and Danhof (1997b) have shown that ignoring inter-individual variation in \( E_{\max} \) may result in erroneous estimates of affinity and efficacy. Therefore, the pharmacodynamic models were fitted to the data using nonlinear mixed effects modeling with the NONMEM software package (Boeckman et al., 1992). The model was implemented in NONMEM using ADVAN6. Inter-individual variability on the parameters was modeled to an exponential equation, 
\[
P_i = \theta \cdot \exp(\eta_i), \tag{7}
\]
where \( \theta \) is the population value for parameter \( P \), \( P_i \) is the individual value and \( \eta_i \) is the random deviation of \( P_i \) from \( P \). The values of \( \eta_i \) are assumed to be independently normally distributed with mean zero and variance \( \sigma^2 \). Interindividual variability of \( K_A \) was assumed to be insignificant because receptor affinity is generally considered to be constant across animals of the same strain. \( K_A \) and \( \nu \) were estimated as \( pK_A \) (−log \( K_A \)) and \( I_{50} \), respectively, because these parameters are assumed to be log normally distributed (Leff et al., 1990; Van der Graaf et al., 1997c). Residual error was characterized by a proportional error model 
\[
y_{mi} = y_{pi} \cdot (1 + e_i), \tag{8}
\]
where \( y_{mi} \) is the \( i \)th prediction for the \( i \)th individual predicted by the model, \( y_{pi} \) is the measurement, and \( e \) accounts for the residual deviance of the model predicted value from the observed value. The values for the population \( \theta \), \( \sigma^2 \), and \( \nu \sigma^2 \), were estimated using the centering first-order conditional estimation method with the first-order model in NONMEM. A conditional estimation method was used due to the high degree of nonlinearity 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 which 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 1/16th of the temperature data set was used for modeling, reducing the number of 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. This reduction did not void the integrity of the data profiles, as judged by cross validation of fitting the split data files.

Goodness-of-fit was analyzed using the objective function and various diagnostic methods. Model selection was based on the Akaike Information Criterion (Akaike, 1974) and assessment of parameter estimates and correlations. Goodness-of-fit between the different models (Hill equation versus the operational model of agonism) was not formally compared since they are structurally different.

Data from the in vitro binding assays were analyzed using the Cheng-Prusoff equation (Cheng and Prusoff, 1973) to obtain estimates of the \( pK_i \). Correlations between the apparent in vivo \( pK_A \) estimates with \( pK_i \) values from the in vitro 5-HT1A receptor binding assays (in the presence of either \(^{3}H\)WAY-100,635 or \(^{3}H\)8-OH-DPAT) and the in vitro parameter for efficacy in the receptor binding assays (log[agonist ratio]) to the in vivo measure for efficacy (log \( \tau \)) were calculated on the basis of mean values using an error-in-variables (e.i.v.) approach (Casella and Berger, 1990). The e.i.v. approach differs from a "regular" linear regression since it allows error in both the \( x \) and \( y \) direction to be taken into account. The e.i.v. model assumes that the expected \( y \) values (\( E(\eta_i) \)) depend linearly on the expected or "true" \( x \) values, i.e., \( E(y_i) = a + b \cdot E(x_i) \), which cannot be observed directly. The model used is the so-called functional relationship, where 
\[
y_i = a + b \cdot E(x_i) + e_i, \quad e_i \sim N(0, \sigma_i^2) \tag{9}
\]
where \( N(\mu, \sigma^2) \) denotes the normal distribution with expectation \( \mu \) and variance \( \sigma^2 \). For identifiability reasons, the usual assumption that \( \sigma_i^2 = \lambda \sigma^2 \), where \( \lambda \) is fixed and known, was made (Casella and Berger, 1990). A reasonable estimate for \( \lambda \) was obtained using:
\[
\lambda = \left( \frac{\bar{s}_2}{\bar{s}_1^2 + \bar{s}_2^2} \right), \tag{10}
\]
where \( \bar{s}_i \) is the mean standard error of all the \( x \) values and \( \bar{s}_i \) of all the \( y \) values. The Pearson correlation coefficient between the expected and the observed \( y \) values was used as a measure of explained variability. Confidence limits for the estimated slope \( b \) and intercept \( a \) were obtained by bootstrap methods (3000 replicates). The value 1 for the slope \( b \) and 0 for the intercept \( a \) not contained in the corresponding confidence interval means that the values are significantly different from 1 and 0, respectively (at the given level of the confidence interval). The e.i.v. analysis was written in S-PLUS (v.6.1; Insightful Corporation, Seattle, WA), and a copy of the script can be obtained from the authors.

**Results**

In Vivo Concentration-Effect Relationships. Figure 1, A and B, shows the average hypothermic effects versus time profiles of R- and S-8-OH-DPAT, WAY-100,635 (without and with R-8-OH-DPAT), WAY-100,135, buspirone, its metabolite 1-PP, flesinoxan, and vehicle treatment. The average baseline temperature (±S.D., \( n = 109 \)) was 37.95 ± 0.04°C. Upon drug administration, a significant decrease in the body temperature for all the 5-HT1A receptor ligands was observed except for WAY-100,635, which did not show a significant decrease from baseline. Administration of R-8-OH-DPAT, S-8-OH-DPAT, flesinoxan, buspirone, 1-PP, and WAY-100,135 resulted in a maximum decrease in temperature (mean ± S.D.) of 4.0 ± 1.0°C at 40 to 60 min, 3.2 ± 0.7°C at 40 to 60 min, 3.8 ± 0.8°C at 20 to 60 min, 2.8 ± 1.0°C at 40 to 50 min, 1.6 ± 0.5°C at 30 to 40 min, and 0.8 ± 0.8°C at 20 to 40 min, respectively. After reaching a maximal decrease a rapid recovery was observed, followed by a plateau phase, before the body temperature returned to baseline for all compounds except for the higher doses of R-8-OH-DPAT (3 mg/kg administrations), in which the plateau phase was not observed, and the body
temperature returned to baseline more gradually (Zuideveld et al., 2001, 2002a, 2002b, 2002c) (see Fig. 2 for examples of individual profiles).

The pharmacokinetic behavior of all the 5-HT₁A receptor agonists could be adequately described using regular 2- and 3-compartmental models. The population pharmacokinetic parameters with the inter-individual variability expressed as a coefficient of variation are depicted in Table 1. The estimates of clearance (CL) and volume of distribution at steady state (Vdss) are CL = 22.8, 7.86, 2.96, 17.6, 8.22, 150, and 28.4 ml/min, and Vdss = 2820, 10,900, 583, 635, 932, 8750, and 1560 ml, which resulted in a terminal half-life (t1/2) of 86, 900, 136, 25, 79, 20, and 33 min for R-8-OH-DPAT, S-8-OH-DPAT, flesinoxan, buspirone, 1-PP, WAY-100,135, and WAY-100,635, respectively (Zuideveld et al., 2001, 2002a, 2002b, 2002c).

The fraction unbound (mean ± S.D.) was 49 ± 4.2%, 52 ± 2.4%, 30 ± 4.0%, 34 ± 3.0%, 78 ± 2.6%, 20 ± 3.4%, and 19 ± 3.0% for R-8-OH-DPAT, S-8-OH-DPAT, flesinoxan, buspirone, 1-PP, WAY-100,135, and WAY-100,635, respectively. No differences were found between protein binding determined at 34°C or 38°C and across different concentrations (data not shown).

The pharmacokinetic parameter estimates were used to simultaneously fit the set point model to the individual (n =...
body temperature versus time profiles for each agonist (eq. 1) in combination with the sigmoidal $E_{\text{max}}$ model (eq. 3) to obtain estimates of the pharmacodynamic parameters ($A$, $k_{\text{on}}$, $\gamma$, $\alpha$, $\text{SC}_{50}$, and $n_{HT}$ based on whole blood concentrations). See Zuideveld et al. (2001, 2002a, 2002b, 2002c) for the values of the physiological parameters. The drug-related parameters, $\alpha$, $\text{SC}_{50}$, and $n_{HT}$ are represented in Table 2. In the subsequent analysis with the operational model of agonism, values for $A$, $k_{\text{on}}$, and $\gamma$ were constrained to the population estimates for each individual compound to avoid an increase in parameter precision due to over-parameterization of the model.

**Estimation of Apparent Affinity and Efficacy in Vivo.** Individual time-body temperature versus time profiles for all agonists were simultaneously analyzed on the basis of the full mechanistic model. Due to the relatively large between-experiment variability in the steepness of the concentration-effect curves, it was not possible to fit all data simultaneously with a single transducer slope parameter, $n$. Therefore the values of $n$ and the associated variance describing the inter-individual variability were fitted and allowed to vary between compounds. The model converged and estimates of in vivo affinity ($pK_A$) and efficacy ($\log \tau$) for each agonist were obtained (Table 3). Overall the population parameter estimates and the inter-individual variability were estimated with good precision. Figure 2 depicts representative fits for each of the compounds.

**Receptor Binding Assay.** The values of the binding affinity ($K_i$) of the various 5-HT$_{1A}$ agonists using the full agonist $[^3H]8$-OH-DPAT (racemic) and the silent agonist $[^3H]$WAY-100,635 as a radioligand as well as the ratio between the two (agonist ratio $= 10^{0.5 \cdot (pK_{iR-8-OH-DPAT} - pK_{iWAY-100.635})}$) are shown in Table 4. The values of the agonist ratio differed substantially with values ranging from 0.61 for WAY-100,635 to 10.53 for buspirone.

**In Vivo-in Vitro Correlations.** The in vivo-in vitro correlations for the different 5-HT$_{1A}$ receptor agonists were analyzed both with regard to potency and efficacy. Figure 3 shows the correlation between the apparent in vivo $pK_A$ estimates with $pK_i$ values found in vitro in the 5-HT$_{1A}$ receptor binding assay in the presence of either of $[^3H]$WAY-100,635 and $[^3H]8$-OH-DPAT, respectively. No statistically significant correlations were observed and the coefficients of determination were rather low ($r^2 = 0.55$,

---

**Table 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameters</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$CL_{2}$</td>
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<tr>
<td>R-8-OH-DPAT</td>
<td>22.8 (35)</td>
</tr>
<tr>
<td>S-8-OH-DPAT</td>
<td>7.86 (55)</td>
</tr>
<tr>
<td>Flesinoxan</td>
<td>2.96 (51)</td>
</tr>
<tr>
<td>Buspirone</td>
<td>17.6 (33)</td>
</tr>
<tr>
<td>1-PP</td>
<td>8.52 (31)</td>
</tr>
<tr>
<td>WAY-100,135</td>
<td>150 (22)</td>
</tr>
<tr>
<td>WAY-100,635</td>
<td>28.4 (39)</td>
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</table>
flesinoxan, and WAY-100,135, and [3H]WAY-100,635. The ratio between the two Ki values serves as a measure for the effects of R-8-OH-DPAT and WAY-100,635, 1-PP, buspirone, WAY-100,135, and WAY-100,635, respectively. The S.E.M. values for the pKi determined in the presence of [3H]WAY-100,635 values are too small to depict. The coefficient of variation for WAY-100,135 (C) are large and imprecise and have been left out.

**TABLE 2**

| Drug           | pK binding affinity | log τ | Parameter estimates [population mean ± coefficient of variation [%]] for the upper asymptote (a), potency (pSC50), and slope parameter (nH) were obtained by nonlinear mixed effects modeling. The coefficients of variation for WAY-100,135 are excessively large, and to the fact that out of six rats, there were two high, two intermediate, and two low responders. In the analysis of a low responder, the pSC50 may become very large, while α is essentially 0.
<table>
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<tbody>
<tr>
<td>R-8-OH-DPAT</td>
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<td>0.62 (53)</td>
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<tr>
<td>S-8-OH-DPAT</td>
<td>6.68 (5)</td>
<td>0.0223 (123)</td>
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</tr>
<tr>
<td>Flesinoxan</td>
<td>5.67 (15)</td>
<td>0.291 (70)</td>
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<tr>
<td>Buspirone</td>
<td>7.03 (32)</td>
<td>-0.0684 (32)</td>
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<td>1-PP</td>
<td>5.68 (32)</td>
<td>-0.291 (70)</td>
<td></td>
</tr>
<tr>
<td>WAY-100,135</td>
<td>7.74 (130)</td>
<td>-1.25 (88)</td>
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</tr>
<tr>
<td>WAY-100,635</td>
<td>8.63** (3)</td>
<td>NA</td>
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</tr>
</tbody>
</table>

N/A, not applicable.

**TABLE 3**

| Drug           | pK binding affinity | log τ | Parameter estimates [population mean ± coefficient of variation [%]] for the upper asymptote (a), potency (pSC50), and slope parameter (nH) were obtained by nonlinear mixed effects modeling. The coefficients of variation for WAY-100,135 are excessively large, and to the fact that out of six rats, there were two high, two intermediate, and two low responders. In the analysis of a low responder, the pSC50 may become very large, while α is essentially 0.
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N/A, not applicable.

**TABLE 4**

| Drug           | pK binding affinity | log τ | Parameter estimates [population mean ± coefficient of variation [%]] for the upper asymptote (a), potency (pSC50), and slope parameter (nH) were obtained by nonlinear mixed effects modeling. The coefficients of variation for WAY-100,135 are excessively large, and to the fact that out of six rats, there were two high, two intermediate, and two low responders. In the analysis of a low responder, the pSC50 may become very large, while α is essentially 0.
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N/A, not applicable.

**Discussion**

The recently developed semi-mechanistic PK-PD model for 5-HT1A agonists using the effect on body temperature as a pharmacodynamic endpoint utilizes the empirical Hill equation to characterize the actions of the 5-HT1A receptor agonists at the receptor in terms of potency (pSC50) and intrinsic activity (α) (Zuideveld et al., 2001, 2002a). Unfortunately the empirical Hill equation has only limited applicability as a model to predict the expression of agonism since both pSC50 and α contain mixed information on drug-specific properties and characteristics of the biological system. The prediction of the expression of agonism is of particular interest since it is believed that the pharmacological and therapeutical properties of 5-HT1A agonists are closely related to the degree of intrinsic activity they display at the 5-HT1A receptor (De Vry, 1995). It has been demonstrated that the operational model of agonism is a particularly useful tool to explain and predict differential expression of agonism in vivo (Black and Leff, 1983; Van der Graaf and Danhof, 1997a; Van der Graaf et al., 1997c, 1999; Cox et al., 1998; Garrido et al., 2000). The present study has therefore focused on the application of the
operational model of agonism in the analysis of the 5-HT$_{1A}$ receptor-mediated hypothermia. In this manner estimates of the in vivo affinity and efficacy of 5-HT$_{1A}$ receptor agonists could be obtained and compared with the values found in the receptor binding assay and the cAMP assay.

Estimates of in vivo and in vitro affinity (pK$_A$ and pK$_B$, respectively) are represented in Tables 3 and 4. The pK$_B$ was estimated on the basis of displacement of both labeled antagonist and agonist. Since the antagonist binds to all the receptors in the inactive state, this pK$_B$ is believed to be the most representative measure for affinity (Assie et al., 1999). The correlation found between the pK$_A$ and pK$_B$ based on $[^3]$HWAY-100,635 was rather poor compared with similar in vivo-in vitro correlations observed for adenosine A$_1$ agonists, synthetic opiates, and GABA$_A$ receptor agonists. In fact, the correlation was not statistically significant ($P > 0.05$), which was also the case when using the values obtained with $[^3]$HS-8-OH-DPAT ($P > 0.1$) as the radioligand. Interestingly, no specific pattern or frame shift could be observed for the correlation. This indicates that the poor correlation cannot be explained by a systematic difference. Also, no improvement in the correlation was found after correction for the degree of protein binding in vivo ($[^3]$HWAY-100,635: $r^2 = 0.51$; $P > 0.1$ and $[^3]$HS-8-OH-DPAT: $r^2 = 0.24$; $P > 0.2$). However, close inspection of Fig. 3A shows that it is flesinoxan, which particularly deviates from the line of identity. In fact, the correlation between the pK$_A$ and pK$_B$ became statistically significant when flesinoxan was excluded from the analysis ($[^3]$HWAY-100,635: $r^2 = 0.84$; $P < 0.05$ and $[^3]$HS-8-OH-DPAT: $r^2 = 0.66$; $P < 0.05$). Recently Van der Sandt et al. (2001) have shown that active transport mechanisms (i.e., P-glyco-protein) at the blood-brain barrier are an important determinant of the brain distribution for flesinoxan. It appears therefore that the in vivo pK$_A$ which has been determined on the basis of blood concentrations, is not representative for the flesinoxan concentrations at the site of the 5-HT$_{1A}$ receptor in the brain.

The estimates of in vivo and in vitro efficacy (log $\tau$ and agonist ratio) are shown in Tables 3 and 4. G protein-coupled receptors can exist in two states, with different affinities for agonists and inverse agonists but similar ones for neutral antagonists. The difference in affinity for a compound for the different states is believed to provide a measure of their intrinsic efficacy (Birdsall and Lazareno, 1997). It has been shown by Assie et al. (1999) and Watson et al. (2000) that this ratio is indeed representative for intrinsic activity displayed by agonists at the 5-HT$_{1A}$ receptor. Between the in vivo and in vitro efficacy (log $\tau$ and log[agonist ratio]) a significant correlation was found ($P < 0.05$, $r^2 = 0.76$, Fig. 4A). The correlation between log $\tau$ and log[agonist ratio] shows further that the in vivo “test assay” is more sensitive in detecting 5-HT$_{1A}$ activity than the agonist ratio. For example, the significant in vivo agonist activity demonstrated for WAY-100,135 was not detected in vitro. Interestingly, despite the strong correlation, the log[agonist ratio] for buspirone does not appear to be a good predictor of its log $\tau$. The log[agonist ratio] of buspirone is higher than that of the full agonist R-8-OH-DPAT despite the fact that in vivo the intrinsic efficacy of buspirone is similar to that of the partial agonist S-8-OH-DPAT. This discrepancy might be caused by buspirone’s activity at other receptors present in the in vivo test assay (New, 1990).

Figure 4B shows the relationship between the intrinsic activity in vivo for the effect on body temperature in rats found for the 5-HT$_{1A}$ receptor and in vitro measure for efficacy log[agonist ratio]. The solid line shows the predicted relationship that was derived from the operational model of agonism fitting results. The symbols correspond to the agonists R-8-OH-DPAT (■), S-8-OH-DPAT (▲), flesinoxan (▲), buspirone (●), 1-PP (▲), WAY-100,135 (○) and WAY-100,635 (▲). Expressed are population and mean values with error bars that represent a derived standard deviation for log $\tau$ (and $\alpha$) and a 95% confidence interval for the log[agonist ratio].
Acknowledgments

The generous donation of buspirone and 1-PP by Bristol-Myers Squibb and flesinoxan by Solvay Pharmaceuticals is highly appreciated.

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Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K i) and the concentration of inhibitor which causes 50 per cent inhibition (I 50) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108.


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