A set-point model with oscillatory behavior predicts the time course of 8-OH-DPAT-induced hypothermia

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Zuideveld, Klaas P., Hugo J. Maas, Nicoline Treijtel, Joost Hulshof, Piet H. van der Graaf, Lambertus A. Peletier, and Meindert Danhof. A set-point model with oscillatory behavior predicts the time course of 8-OH-DPAT-induced hypothermia. Am J Physiol Regulatory Integrative Comp Physiol 281: R2059–R2071, 2001.—Agonists for the 5-hydroxytryptamine (HT)1A receptor induce a hypothermic response that is believed to occur by lowering of the body’s set-point temperature. We have developed a physiological model that can be used to predict the complex time course of the hypothermic response after administration of 5-HT1A agonists to rats. In the model, 5-HT1A agonists exert their effect by changing heat loss through a control mechanism with a thermostat signal that is proportional to the difference between measured and set-point temperature. Agonists exert their effect in a direct concentration-dependent manner, with saturation occurring at higher concentrations. On the basis of simulations, it is shown that, depending on the concentration and the intrinsic efficacy of a 5-HT1A agonist, the model shows oscillatory behavior. The model was successfully applied to characterize the complex hypothermic response profiles after administration of the reference 5-HT1A agonists R-8-hydroxy-2-(di-n-propylamino)tetralin (R-8-OH-DPAT) and S-8-OH-DPAT. This analysis revealed that the observed difference in effect vs. time profile for these two reference agonists could be explained by a difference in vivo intrinsic efficacy.

pharmacokinetic-pharmacodynamic modeling; 5-hydroxytryptamine1A receptor; R-8-hydroxy-2-(di-n-propylamino)tetralin; S-8-hydroxy-2-(di-n-propylamino)tetralin

It is well established that the 5-hydroxytryptamine (HT)1A receptor plays a role in the physiological regulation of body temperature (40, 54). Consequently, the 5-HT1A agonists, which are used therapeutically as antidepressants and antianxiety drugs (16), cause hypothermia (4, 20, 27, 50). For the prototype 5-HT1A agonists it has been demonstrated that this hypothermic response is indeed mediated specifically through the 5-HT1A receptor because it can be blocked in a dose-dependent manner by the selective, competitive 5-HT1A receptor antagonist WAY-100,635 (14, 17) and not by antagonists for other G protein-coupled receptors (34).

In a number of investigations, the time course of the hypothermic response after administration of 5-HT1A receptor agonists has been studied in detail. In these studies, complex effect vs. time patterns have been observed, suggesting the involvement of homeostatic control mechanisms (51, 54). So far, however, no mathematical models have been developed to characterize these complex time profiles of the hypothermic response in a strict quantitative manner. Specifically, there have been no attempts to link existing temperature regulation models (25, 49) to pharmacokinetic models describing the time course of the drug concentration in the body.

In recent years important progress has been made in the area of integrated pharmacokinetic-pharmacodynamic (PK-PD) modeling (11). PK-PD modeling has even allowed estimation of the in vivo affinity and intrinsic efficacy of drugs (43–46). Specifically, models have been proposed that allow for a delay between drug concentration and the pharmacological response (15, 19, 30). So far, however, very few of these models incorporate complex regulatory behavior (7, 18, 48). In this report we propose a new physiological PK-PD model, in which 5-HT1A receptor agonists exert their effect on body temperature by lowering of the set-point temperature. The model was applied to characterize hypothermic response vs. time profiles after administration of different doses of the reference 5-HT1A receptor agonists R- and S-8-OH-DPAT. It is shown that differences in the observed hypothermic response profiles can be explained by differences in intrinsic efficacy between the two compounds.

Glossary

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Rate of change in the set-point signal (min−1·°C−1)</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike Information Criterion</td>
</tr>
<tr>
<td>C</td>
<td>Drug concentration in the central compartment (ng/ml)</td>
</tr>
</tbody>
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SET-POINT MODEL FOR 8-OH-DPAT-INDUCED HYPOTHERMIA

The proposed set-point model for the effect of 5-HT1A agonists on body temperature is shown in Fig. 1. As the agonist binds to its receptor, a stimulus is generated. This stimulus in turn drives physiological processes that lower the temperature. This stimulus, which is determined by the drug-receptor interaction and hence the drug’s affinity and efficacy, can be described by a sigmoidal function $f(C)$

\[
  f(C) = \frac{S_{\text{max}} \cdot C^n}{SC_{50}^{n} + C^n}
\]

where $S_{\text{max}}$ represents the maximum stimulus the drug can produce, $C$ is the drug concentration, $SC_{50}$ is the concentration required to produce 50% of the maximum stimulus, and $n$ is a slope factor, which determines the steepness of the curve. As the drug concentration changes with time, the stimulus changes as well. As the stimulus $S$ is assumed to be inhibitory, it is defined as $S = 1 - f(C)$. The behavior of the concentration $C$ and the corresponding stimulus $S$ is shown in Fig. 2, A and B. This stimulus induces the hypothermic response. The changing drug concentrations therefore govern the first time scale of the model.

Here $k_{\text{in}}$ represents the zeroth-order rate constant associated with the warming of the body and $k_{\text{out}}$ represents a first-order rate constant associated with the cooling of the body.

The indirect physiological response model is combined with the thermostat-like regulation of body temperature. This regulation is implemented as a continuous process in which the body temperature is compared with a reference or set-point temperature ($T_{\text{SP}}$) (Fig. 1). It is accepted that 5-HT1A agonists elicit hypothermia by decreasing the value of $T_{\text{SP}}$, and hence $T_{\text{SP}}$ depends on the drug concentration $C$: $T_{\text{SP}} = T_{\text{SP}}(C)$. It is assumed that $T_{\text{SP}}$ is controlled by the drug concentration $C$ through $Eq. 3$

\[
  T_{\text{SP}} = T_0[1 - f(C)]
\]

where $T_0$ is the set-point value in the absence of any drug: $T_0 = T_{\text{SP}}(0)$. Combining the indirect physiological

---

**Fig. 1.** Proposed full model for describing 5-HT1A-receptor mediated hypothermia. The model is based on the concepts of the indirect physiological response model (15) and takes into account rate constants associated with the warming of the body ($k_{\text{in}}$) and cooling of the body ($k_{\text{out}}$). The indirect physiological response model is combined with the thermostat-like regulation of body temperature, in which body temperature ($T$) is compared with a fixed reference or set-point temperature ($T_{\text{SP}}$) at rate $a$, generating a set-point signal $X$. The extent to which the set-point value decreases is a function of drug concentration $f(C)$, which decreases $X$ by the amplification factor $\gamma$. The changing drug concentrations therefore govern the first time scale of the model. The second time scale on which the model operates is governed by physiological principles. The model that describes the hypothermic response utilizes the concepts of the indirect physiological response model as proposed by Dayneka et al. (15) and Gabrielson et al. (19). In this model the change in temperature ($T$) is described as an indirect response to either the inhibition of the production of body heat or the stimulation of its loss ($Eq. 2$)

\[
  \frac{dT}{dt} = k_{\text{in}} - k_{\text{out}} \cdot T
\]
response model with the thermostat-like regulation therefore yields

\[
\begin{align*}
\frac{dT}{dt} &= k_{in} - k_{out} \cdot T \cdot X^{-\gamma} \\
\frac{dX}{dt} &= a(T_0 \cdot [1 - f(C)] - T)
\end{align*}
\] (4)

in which \(X\) denotes the thermostat signal. In the model as described in Eq. 4, the change in \(X\) is driven by the difference between the body temperature \(T\) and \(T_{SP}\) on a time scale that is governed by \(a\). Hence, when the set-point value is lowered, the body temperature is perceived as too high and \(X\) is lowered. To relate this decreasing signal to the drop in body temperature, an effector function \(X^{-\gamma}\) was designed, in which \(\gamma\) determines the amplification. Raising this function to the loss term \(k_{out} \cdot T\) therefore facilitates the loss of heat. In Eq. 4, body temperature and set-point temperature are interdependent, and a feedback loop is created that can give rise to oscillatory behavior, as will be shown later. When the body temperature is at its initial set point, the no-drug situation, the equilibrium set-point signal \(X_0\) can be defined in terms of \(k_{in}\), \(k_{out}\), \(\gamma\), and \(T_0\) (see APPENDIX).

With four system parameters to be estimated, the degree of parameterization in Eq. 4 is high and thus may lead to parameter unidentifiability. It can be shown that one parameter can be eliminated by combining parameters into dimensionless quantities. Thus, we set

\[
x = \frac{X}{X_0} \quad \text{and} \quad y = \frac{T}{T_0}
\] (5)

where \(X_0\) is the reference signal of \(X\) when no drug is present and \(T_0\) has been defined in Eq. 3. For these dimensionless variables, we obtain the system

\[
\begin{align*}
\frac{dx}{dt} &= A\left([1 - f(C)] - y\right) \\
\frac{dy}{dt} &= B\left(1 - \frac{y}{x}\right)
\end{align*}
\] (6)

where

\[
A = \frac{a \cdot T_0}{X_0} = a\left(\frac{k_{in}}{k_{out}}\right)^{\gamma/2} T_0^{1 - (\gamma/2)} \quad \text{and} \quad B = \frac{k_{in}}{T_0} \quad (7)
\]

are the new dimensionless parameters. For the derivation of the new system, refer to APPENDIX. After reparameterization, the number of physiological parameters has been reduced from four \((a, k_{in, o}, k_{out, o}, \text{and} \gamma)\) in Eq. 4 to three \((A, B, \text{and} \gamma)\) in Eq. 6, and as a result, parameter unidentifiability has been abolished.

Under certain conditions, Eqs. 4 and 6 produce damped oscillations, around the equilibrium point \((\bar{x}, \bar{y})\), defined by

\[
\bar{x} = (1 - f(C))^{1/\gamma} \quad \text{and} \quad \bar{y} = 1 - f(C) \quad (8)
\]

The local behavior near this point can be characterized by the eigenvalues of the Jacobian matrix \(\mathbf{M}\), from which the discriminant \(D\) can be determined (see APPENDIX). For Eq. 6 the discriminant becomes

\[
D = \frac{B^2}{\bar{x}^{2\gamma}} - 4\frac{\gamma AB}{\bar{x}} \quad (9)
\]

When \(D < 0\), the eigenvalues are complex, and the system of differential equations will exhibit oscillatory behavior. When \(D \geq 0\), which is the case for a
relatively large stimulus, the eigenvalues are real and the system of differential equations will be “overdamped.” In both cases the eigenvalues have a negative real part, so that \((\dot{x}, \dot{y})\) is asymptotically stable. The behavior of the model is depicted in a phase plot (Fig. 2C) for low and high doses. In this picture, as the temperature starts to drop, the curves are transversed from the point \((x, y) = (1, 1)\) in a clockwise fashion to the slowly moving equilibrium points \((\ddot{x}, \ddot{y})\). This behavior results in temperature-time profiles as depicted in Fig. 2D. The complex behavior of the model is further depicted in a three-dimensional graph in Fig. 3.

As the drug concentrations will be known as time progresses, substituting the expressions of Eq. 8 into Eq. 6 gives a relationship that predicts the qualitative behavior of the model at different parameter combinations. Initial estimates of the model were further obtained with the use of simulations using different parameter values, an overview of which can be found in Fig. 4. It turns out that the model predicts the behavior as described above when \(S_{\text{max}}\) approaches or equals 1, whereas it predicts an oscillation at all doses for \(S_{\text{max}}\) between 0 and 1, i.e., partial agonist. This concept was implemented in Eq. 6 by defining the ranges of \(S_{\text{max}}\) and the dependent variable \(y\). In the model the \(S_{\text{max}}\) value of a full agonist equals 1 and that of an antagonist equals 0.

The procedure for calculating the redefined \(y\) values on the basis of the observed temperatures is represented in Eq. 10

\[
y = \frac{T - T_{\min}}{T_{\text{SP}} - T_{\min}}
\]

In Eq. 10, \(T\) is the temperature at time \(t\), \(T_{\text{SP}}\) is the average temperature from the hour before drug administration, and \(T_{\min}\) is the average minimal temperature of the individuals receiving a high dose of the full agonists.

![Fig. 3. Three-dimensional representation of the behavior of the models applied for a low dose (solid line) and a high dose (dashed line) in the temperature-set point-time scene. Inputs used were the same as for Fig. 2. The drop lines or curtain is added for clarity. The model predicts oscillatory behavior particularly for the low-dose administrations.](http://ajpregu.physiology.org/)

**EXPERIMENTAL METHODS**

Experiments were performed on male Wistar rats (Broekman BV, Someren, The Netherlands) weighing 297 ± 3.4 g (mean ± SE, \(n = 68\)) and were approved by the Leiden University Ethics Committee. The animals were housed in standard plastic cages (6 per cage before surgery and individually after surgery). They were kept in a room with a normal 12:12-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 on. The animals were anesthetized with an intramuscular injection of 0.1 ml/kg Domitor (1 mg/ml medetomidine hydrochloride, Pfizer, Capelle a/d IJs, The Netherlands) and 1 ml/kg Ketalar (50 mg/ml ketamine base, Parke-Davis, Hoofddorp, The Netherlands). Indwelling pyrogen-free cannulas were implanted into the right jugular vein (Polythene, 14 cm, 0.52-mm ID, 0.96-mm OD) for drug administration and into the left femoral artery (Polythene, 4 cm of 0.28-mm OD, 0.61-mm ID) plus 20 cm of 0.58-mm ID, 0.96-mm OD) for blood sampling. Cannulas were tunneled subcutaneously to the back of the neck and exteriorized. To prevent coagulation of blood, the cannulas were filled with a 25% (wt/vol) solution of polyvinylpyrrolidone (PVP) (Brockaef, Maarssen, The Netherlands) in a 0.9% (wt/vol) pyrogen-free sodium chloride solution (NPBI, Emmer-Compascuum, The Netherlands) that contained 50 IU/ml of heparin (Leiden University Medical Center, Leiden, The Netherlands). Just before the experiment, the PVP solution was removed, and the cannulas were flushed with saline containing 20 IU/ml of heparin. The skin in the neck was stitched with normal sutures, and the skin in the groin was closed with wound clips. Furthermore, a telemetry transmitter [Physiotel implant TA107TA-F40 system, Data Sciences Internationals (DSI), St. Paul, MN], with a weight of ~7 g, which had been made pyrogen free with CIDEX (22 g/l glutaraldehyde, Johnson and Johnson Medical, Gargrave, Skipton, United Kingdom) 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 solution, AUV, Cuijk, The Netherlands) was administered to aid recovery.

**Experimental Protocol**

**Dosage regimen.** Eight days after surgery, the experiments were performed. Rats received different doses in different infusion rates of R-8-OH-DPAT or S-8-OH-DPAT. For R-8-OH-DPAT, bolus infusions of 1 mg/kg in 15 min \(n = 7\), 3 mg/kg in 5 min \(n = 7\), 3 mg/kg in 15 min \(n = 5\), and 3 mg/kg in 30 min \(n = 6\) were given. In addition, a computer-controlled infusion was administered over 6 h, by which a stable concentration of 160 ng/ml was maintained in blood \(n = 6\). For S-8-OH-DPAT, infusions of 5 mg/kg \(n = 6\) and 15 mg/kg \(n = 6\) in 15 min were administered. Twenty-four rats received vehicle treatments, in which an equivalent amount of saline was infused.

For the bolus infusions, an external cannula was filled with a solution of the drug in an amount of saline calculated according to the weight of the rat, and the cannula was connected to the infusion pump (BAS beehive, Bioanalytical Systems). For the computer-controlled infusions, STAN-PUMP software (42) was used running on an IBM-compatible

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computer (486 processor) and connected to a Harvard 22-
syringe pump (Harvard Apparatus, South Natick, MA)
through an RS232 interface. The concentration was clamped
using population pharmacokinetic parameters obtained in
the bolus infusion experiments. All experiments started be-
tween 9:00 AM and 9:30 AM.

Blood sampling. Approximately 15–18 serial blood sam-
ples of 50 ml were taken according to a fixed time schedule to
determine the concentration vs. time profile of the drug. The
exact amount was measured with a capillary (Servoprax,
Wesel, Germany) and transferred into a glass centrifuge tube
containing 400 ml of purified water for hemolysis. During the
experiment the samples were kept on ice. After the experi-
ment, samples were stored at \(-20^\circ C\) pending analysis.

Data Acquisition

Temperature measurements. To measure the body temper-
ature of the rat, a telemetric system (Physiotel Telemetry
System, DSI) was used. The transmitter measured the body
temperature every 30 s for a 2-s period and signaled it to a
receiver (Physiotel Receiver, model RPC-1, DSI). The re-
ceiver was connected to the computer through a BCM 100
consolidation matrix (DSI). The computer processed the data
and visualized the temperature profiles [Dataquest LabPro
software (DSI) running under OS/2 Warp, IBM] as it did for
room temperature (C10T temperature adapter, DSI).

HPLC analysis of R- and S-8-OH-DPAT. The blood con-
centrations of R- and S-8-OH-DPAT were assayed by an
enantioselective HPLC method as described previously (55).
Briefly, detection with the HPLC system was obtained using
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 (Daicel Chemical Industries,
Tokyo, Japan). The mobile phase was a mixture of 50 mM
phosphate buffer (pH 5.5)-acetonitrile (80/20, vol/vol) and
contained a total concentration of 5 mM KCl and 20 mg/l of
EDTA. The analytes were extracted from blood using a liq-
uid-liquid cleanup step and isolated on Bakerbond solid
phase extraction NARC-2 columns (Baker, Phillipsburg, NJ).
Calibration curves in the concentration range of 0.1–5000
ng/ml were analyzed with each run, and peak area ratios of
analyte over internal standard [R-(-)-7-hydroxy-2-(di-n-
propylamino)tetralin (R-7-OH-DPAT)] were calculated. Using
50 ml of blood, the limit of detection was 0.5 ng/ml.

Chemicals. R-8-OH-DPAT, S-8-OH-DPAT, and R-7-OH-
DPAT were purchased from Research Biochemicals Interna-
tional. All other chemicals used were of analytical grade
(Baker, Deventer, The Netherlands).

Data Analysis

A nonlinear mixed-effects modeling approach was used to
quantify both the pharmacokinetics and pharmacodynamics
of R- and S-8-OH-DPAT sequentially. With 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 (39).
Modeling was performed using the nonlinear mixed-effects
modeling software NONMEM developed by Sheiner and col-
leagues (10) (version V1.1, NONMEM Project Group, Univer-
sity of California, San Francisco, CA). Individual predictions
were obtained in a Bayesian post hoc step. The concentra-
tion-time profiles of R-8-OH-DPAT as well as S-8-OH-DPAT
were best described using a standard three-compartment
pharmacokinetic model, such as implemented in NONMEM
ADVAN11, TRANS4. With this routine, the pharmacokinet-
ics were described in terms of the compartment volumes of
distribution (V1, V2, and V3), clearance (CL), and the inter-
compartmental clearances (CL2 and CL3). The set-point
model (Eq. 6) was implemented in NONMEM using AD-
VAN6. Parameterization was different from Eq. 6, where B is
purely phenomenological. However, as the individual TSP
values were known, the parameter $B$ could be calculated from $k_m$ (see APPENDIX, Eq. A9). Therefore, the estimated physiological parameters were $k_m$, $A$, and $\gamma$. The $S_{\max}$ was fixed to 1 for R-8-OH-DPAT. The observed dependent variable temperature measurements were redefined as described in Eq. 10. Interindividual variability on the parameters was modeled by an exponential equation

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

where $\theta$ is the population value for parameter $P$, $\eta_i$ 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 $\omega^2$. The covariance structure of the variability parameters was assumed to be diagonal. For the pharmacokinetics, residual error was characterized by an exponential error model

$$C_{mij} = C_{p(i)} \cdot \exp(\epsilon_{ij})$$

where $C_{p(i)}$ is the $j$th plasma concentration for the $i$th individual predicted by the model, $C_{mij}$ is the measured concentration, and $\epsilon$ accounts for the residual deviance of the model-predicted value from the observed concentration. For the pharmacodynamics, residual error was characterized by a proportional error model

$$y_{mij} = y_{p(i)} \cdot (1 + \epsilon_{ij})$$

where $y_{p(i)}$ is the $j$th prediction for the $i$th individual predicted by the model, $y_{mij}$ is the measurement, and $\epsilon$ accounts for the residual deviance of the model-predicted value from the observed value. The values for $\epsilon$ are assumed to be independently normally distributed with mean zero and variance $\omega^2$. Population pharmacokinetic values of $\theta$, $\omega^2$, and $\sigma^2$ are estimated using the first-order method in NONMEM. The values for the population pharmacodynamic $\theta$, $\omega^2$, and $\sigma^2$ are estimated using the centering first-order conditional estimation method with the first-order model in NONMEM. A conditional estimation method is used because of 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 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 chosen to greatly decrease computing time as required with just the conditional estimation method (10, 33). To further decrease computing time, only 1/16th of the temperature data set was used for modeling, reducing the temperature measurements from over 900 measurements per individual to ~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. Model selection was based on the Akaike Information Criterion (AIC; Ref. 2) and assessment of parameter estimates and correlations. 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; Ref. 29).

RESULTS

The average effect-time profiles for the hypothermic response after administration of vehicle, R-8-OH-DPAT, and S-8-OH-DPAT are represented in Fig. 5. In the control group (Fig. 5A), rats received the amount of saline equivalent to the drug infusions used and were subjected to blood sampling. They showed no hypothermic response. The administration of R-8-OH-DPAT resulted in a maximum decrease in temperature of $4 \pm 0.3^\circ$C at 40 to 60 min (Fig. 5, B and D). After an infusion of R-8-OH-DPAT of 1 mg/kg in 5 min, a complex effect vs. time profile was observed. The body temperature quickly rose after reaching its minimum; subsequently, a plateau phase was observed, after which temperature returned to baseline. For the

Fig. 5. Average temperature-time profiles (±SE) for vehicle treatments (A; $n = 24$); for R-8-OH-DPAT regular infusions (B: infusion 1, 1 mg/kg in 5 min ($n = 7$); infusion 2, 3 mg/kg in 5 min ($n = 7$); infusion 3, 3 mg/kg in 15 min ($n = 5$); and infusion 4, 3 mg/kg in 30 min ($n = 6$)): for S-8-OH-DPAT infusions (C: infusion 1, 5 mg/kg in 15 min ($n = 6$) and infusion 2, 15 mg/kg in 15 min ($n = 6$)); and for computer-controlled infusions of R-8-OH-DPAT (D), where the concentration was clamped at 160 ng/ml in blood ($n = 6$). All infusions started at $t = 0$; horizontal bars represent duration of the infusion.
higher doses (3 mg/kg in 5, 15, and 30 min) the plateau phase was not observed, and the body temperature returned to baseline more gradually. In the experiments in which the R-8-OH-DPAT concentration was maintained at a concentration of 160 ng/ml for 6 h, a similar plateau phase was observed, and as the infusion was turned off, the body temperature returned to baseline in a similar fashion.

On administration of S-8-OH-DPAT, a maximum decrease in body temperature of 3.2 ± 0.2°C was observed within 40–60 min (Fig. 5C). For S-8-OH-DPAT a plateau phase was observed, such as with the low dose of R-8-OH-DPAT, before returning to baseline for both infusions (5 and 15 mg/kg in 15 min).

**Pharmacokinetics**

During the experiments, blood samples were taken to construct individual concentration-time profiles. After the regular infusions of both R- and S-8-OH-DPAT, a distribution phase and an elimination phase were observed (Fig. 6). On the basis of predicted concentration curves, goodness-of-fit plots, and the AIC, the three-compartment model was selected over a two-compartment model in the pharmacokinetic analysis. The individual concentration-time profiles for R-8-OH-DPAT for both the regular infusions and the computer-controlled infusion are depicted in Fig. 6. The population prediction for the regular infusions and for the computer-controlled infusion as well as the individually predicted curves is represented. The individual concentration-time profiles for S-8-OH-DPAT are depicted in Fig. 7. The values of the pharmacokinetic parameter estimates are represented in Table 1. No statistically significant correlation between the parameters was detected. Table 1 further displays the interindividual variation and the intraindividual variation for both R- and S-8-OH-DPAT.

**Fitting the Set-Point Model**

The effect-time profiles for R- and S-8-OH-DPAT were described by fitting the set-point model to the data. Parameters were estimated using the centering first-order conditional estimation method with the first-order model. The average values for all ηs were not significantly different from 0. The population parameter estimates are shown in Table 2. Individual post hoc predictions of the parameters were found to be unbiased with respect to the different doses and infusions administered. Figure 8 depicts some typical individual and population predictions for different doses and infusions. Table 2 displays the interindividual variation and the intraindividual variation, which were both found to be reasonable for both R- and S-8-OH-DPAT. The precision with which the parameters were predicted was reasonable for both R- and S-8-OH-DPAT.

**DISCUSSION**

**Development of the Set-Point Model**

It is well established that temperature regulation in the rat takes place in the anterior hypothalamus (8, 25, 26, 47). Furthermore, it has been recognized that serotonin plays an active role in temperature regulation and in particular in the maintenance of the body’s set point (9, 28, 32, 40, 53, 54). More recently, numerous...
pharmacological studies have suggested that homeostasis is achieved through an interplay between the 5-HT1A and 5-HT2A/C receptor systems (1, 23, 38, 40, 41). This is further supported by the fact that both receptors are located in the hypothalamus (3, 37) amongst other regions in the brain. Administration of a 5-HT1A-receptor agonist induces a hypothermic response both in humans and rats. This is considered to be mediated by postsynaptic 5-HT1A receptor sites in this region (6, 20, 27, 34, 35).

Table 1. Population pharmacokinetic parameters and inter- and intraindividual variabilities of R- and S-8-OH-DPAT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Interindividual CV, %</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>CL3, ml/min</td>
<td>76.7</td>
<td>32</td>
<td>65.8–87.6</td>
</tr>
<tr>
<td>V1, ml</td>
<td>21.8</td>
<td>34</td>
<td>18.9–27.6</td>
</tr>
<tr>
<td>V2, ml</td>
<td>126</td>
<td>35</td>
<td>90.2–162</td>
</tr>
<tr>
<td>V3, ml</td>
<td>2,090</td>
<td>116</td>
<td>478–2,700</td>
</tr>
<tr>
<td>CL2, ml/min</td>
<td>19.2</td>
<td>35</td>
<td>12.7–25.5</td>
</tr>
<tr>
<td>CL1, ml/min</td>
<td>69.2</td>
<td>35</td>
<td>44.0–94.4</td>
</tr>
<tr>
<td>V3, ml</td>
<td>603</td>
<td>35</td>
<td>440–765</td>
</tr>
<tr>
<td>k\text{in}, °C/min</td>
<td>0.952</td>
<td>36</td>
<td>0.851–1.05</td>
</tr>
<tr>
<td>A1, min⁻¹</td>
<td>0.0239</td>
<td>20</td>
<td>0.0228–0.0251</td>
</tr>
<tr>
<td>γ</td>
<td>2.00</td>
<td>32</td>
<td>1.83–2.17</td>
</tr>
<tr>
<td>SC50, ng/ml</td>
<td>43.2</td>
<td>30</td>
<td>40.7–45.7</td>
</tr>
<tr>
<td>n</td>
<td>1.31</td>
<td>84</td>
<td>1.25–1.37</td>
</tr>
</tbody>
</table>

Intraindividual CV was 17% for R-8-OH-DPAT parameter values and 11% for S-8-OH-DPAT parameter values. \(k\text{in}, \) zeroth-order rate constant associated with production of body heat; \(\gamma, \) amplification of set-point signal; \(SC50, \) concentration at 50% of maximum stimulation; \(S_{\text{max}}, \) maximum stimulation the drug can produce; \(n, \) slope factor.

To characterize 5-HT1A-agonist-induced hypothermia, we have developed a mathematical model that describes the hypothermic effect on the basis of the concept of a set point (8, 12, 54) and a general physiological response model (15, 19, 30). The model developed is able to reproduce the observed complex effect vs. time profile with regard to both the delay relative to the maximal drug concentration and the plateau phase. It appears that the plateau phase originates from damped oscillations that occur around the equilibrium point on returning to baseline, when the model is not fully “pushed” into the maximal effect. When the model is fully pushed into its maximal effect, such as is the case for a relatively high dose of a full agonist, the system becomes overdamped, thereby losing its oscillatory behavior. Hence, the observed plateau phase is an intrinsic part of the regulatory mechanism related to the oscillatory behavior found in many regulatory systems (22, 31).

Because the model as represented by Eq. 4 is overparameterized, the model was simplified by introducing dimensionless quantities. Such operation does not change the behavior of the model but merely reduces the parameters that are correlated. One major drawback of this approach is, however, that the parameters become difficult to interpret. On the other hand, a major advantage is that it becomes possible to estimate the parameters with a higher degree of certainty. To fully utilize the properties of the model, the temperature data were rescaled as well. This was done under the assumption that R-8-OH-DPAT is the fullest agonist and that the maximal temperature decrease observed was indeed the maximum. Despite the fact that it is well known that temperature is regulated within a narrow range, it
has been shown that when the body temperature does drop below approximately 4–4.5°C below its homeostatic temperature, either very long recovery times are observed (>10 h) or the animals die. Examples of such experiments include administration of the adenosine agonist 5′-(N-ethylcarboxamido)adenosine and high but nonlethal doses of pentobarbital or clozapine (unpublished results). It is therefore reasonable to assume that the absolute drop as observed with R-8-OH-DPAT is the absolute maximum for the range in which the set-point system operates.

Because it is known from both in vitro and in vivo data that R- and S-8-OH-DPAT are, respectively, full and partial agonists for the hypothalamic 5-HT{sub 1A} receptor (14) and behave as such for the hypothermic response (24), we were interested in estimating the intrinsic activity and potency of these compounds on the basis of our model. Despite the fact that it has been suggested that the hypothermic effect of S-8-OH-DPAT is mediated via the dopamine D2 receptor (52), we have not been able to reproduce this result and were able to block the S-8-OH-DPAT-mediated hypothermic effect using the selective 5-HT{sub 1A} receptor antagonist WAY-100,635 (results not shown). Furthermore, we could not block the S-8-OH-DPAT-mediated response using the α-selective dopamine receptor antagonist haloperidol (results not shown). Finally, we were unable to block an effective dose of the selective dopamine D3 receptor agonist R-7-OH-DPAT, which does induce a hypothermic response in our studies (results not shown). These findings show that the observed hypothermic response after S-8-OH-DPAT is mediated exclusively through the 5-HT{sub 1A} receptor.

Fitting the Set-Point Model

To construct a concentration-effect relationship for R- and S-8-OH-DPAT, it was necessary to study the pharmacokinetics of both compounds. Both concentration-time profiles were estimated using a population-based three-compartment pharmacokinetic model.
model. The pharmacokinetics of R-8-OH-DPAT could be estimated with a high degree of precision, whereas the precision of the estimation of S-8-OH-DPAT pharmacokinetics was somewhat poorer. The reason for this may be the large half-life of S-8-OH-DPAT (15 h) relative to the duration of the study. In this respect, it is interesting to note that the difference in pharmacokinetics between R-8-OH-DPAT (half-life of 86 min) and S-8-OH-DPAT indicate a highly stereoselective metabolism. In the analysis of the effect on body temperature, the individual post hoc pharmacokinetic parameters were used for interpolation to estimate the drug concentration at every measurement of body temperature. The proposed physiological PK-PD model was able to describe the plateau phase observed for the low dose of R-8-OH-DPAT and not for the high dose in a single analysis. Additionally, the computer-controlled infusions in which an R-8-OH-DPAT concentration of 160 ng/ml is maintained in blood for 6 h follow a behavior that is also fitted by the model. Furthermore, it predicts a plateau phase for both doses of the partial agonist S-8-OH-DPAT. Overall, the individual predictions describe the shape of the observed responses well, and the parameters obtained are independent of dose or infusion. The parameters obtained show reasonable interindividual variances and intraindividual variance. Despite the fact that a number of parameters associated with the physiological part of the model are different between R- and S-8-OH-DPAT, they are within a physiological range of each other, whereas the \( S_{\text{max}} \) and \( S_{\text{50}} \) are markedly different.

**General Conclusions**

The parameters obtained from describing both the pharmacokinetics and the pharmacodynamics of both R- and S-8-OH-DPAT clearly indicate a stereoselective metabolism, a difference in potency, and a marked difference in intrinsic activity (Fig. 9) for the hypothermic effect. These findings comply with in vitro data, where affinity for both compounds is very similar (5, 36) and an efficacy of \( \sim 50\% \) is found for S-8-OH-DPAT in functional assays (13). The model that was developed incorporates the set-point hypothesis in temperature regulation to explain the hypothermic response to the 5-HT\(_{1A}\) receptor in terms of its oscillatory behavior.

**Perspectives**

This study shows that it is possible to predict the time course of drug effects in vivo in situations where complex homeostatic control mechanisms are operative. As such, it forms the basis for the development of an entirely new class of PK-PD models. These models are important for the development of new drugs and the application of such drugs in clinical practice. For example, on the basis of this kind of model, it becomes possible to predict whether withdrawal symptoms will occur on cessation of (chronic) drug treatment. Hence, these models may provide a scientific basis either for the selection of alternate drug candidates or the design of dosing regimens that show less pronounced withdrawal phenomena. It is further anticipated that such models will provide a basis for PK-PD modeling with disease progression.
APPENDIX

Derivation of the Set-Point Model

The model utilizes the concepts of the indirect physiological response model (15)
\[
\frac{dT}{dt} = k_{in} - k_{out} \cdot T \quad (A1)
\]
This model is combined with the thermostat-like regulation of body temperature. The thermostat principle is explained in Eq. A2
\[
\frac{dX}{dt} = a(T_{SP} - T) \quad (A2)
\]
Equation A2 describes the continuous process in which body temperature is compared with a reference or set-point temperature (\(T_{SP}\)). Differences in these temperatures generate a signal \(X\), which in turn has an effect on body temperature.

Through the action of the drug on the receptor, the set-point temperature is determined by the concentration \(C\) of the drug
\[
T_{SP}(C) = T_{d}[1 - f(C)] \quad (A3)
\]
Equations A2 and A3 together yield
\[
\frac{dX}{dt} = a[T_{d} \cdot [1 - f(C)] - T] \quad (A4)
\]
As a result of lowering the set-point temperature, the thermostat signal \(X\) decreases. To relate this decreasing signal to the drop of temperature observed on 5-HT\(_{1A}\) agonist administration, an effector function \(g(X)\) was introduced in the term modeling the heat loss of the body
\[
\frac{dT}{dt} = k_{in} - k_{out} \cdot T \cdot g(X) \quad (A5)
\]
For the function \(g(X)\), we chose
\[
g(X) = X^{-\gamma} \quad \text{where} \quad \gamma > \frac{1}{2} \quad (A6)
\]
The power \(\gamma\) in the function \(g(X)\) can be viewed as an amplification factor of the thermostat signal, which must be greater than \(\frac{1}{2}\), as will be explained later. Equations A4 and A6 together make up the basic system of equations
\[
\begin{align*}
\frac{dT}{dt} &= k_{in} - k_{out} \cdot T \cdot X^{-\gamma} \\
\frac{dX}{dt} &= a[T_{d} \cdot [1 - f(C)] - T]
\end{align*}
\quad (A7)
\]
When the body temperature is at its initial set point, the equilibrium set-point signal \(X_0\) can be defined in terms of \(k_{in}\), \(k_{out}\), \(\gamma\), and \(T_{SP}\), as given in Eq. A8
\[
T_0 = T_{SP} \quad X_0 = \left(\frac{k_{out} \cdot T_{SP}}{k_{in}}\right)^{1/\gamma} \quad (A8)
\]
Making the Model Dimensionless

The following shows that one parameter can be eliminated from Eq. A7 in a procedure involving redefinition of variables and subsequent substitution of \(X_0\). The variables \(X\) and \(T\) are converted into dimensionless quantities \(x\) and \(y\)
\[
X = X_0 \cdot x \quad \text{and} \quad T = T_0 \cdot y
\]
where \(X_0\) is chosen as in Eq. A8. The system of differential equations (Eq. A7) then becomes
\[
\begin{align*}
\frac{dx}{dt} &= \frac{a \cdot T_0}{X_0} \cdot [1 - f(C)] - y \\
\frac{dy}{dt} &= \frac{k_{in} - k_{out} \cdot y}{T_0} \cdot X_0 \cdot x
\end{align*}
\quad (A9)
\]
These yield the system
\[
\begin{align*}
\frac{dx}{dt} &= A([1 - f(C)] - y) \\
\frac{dy}{dt} &= B \left(1 - \frac{y}{x^\gamma}\right)
\end{align*}
\quad (A10)
\]
where
\[
A = \frac{a \cdot T_0}{X_0} = a \left(\frac{k_{in}}{k_{out}}\right)^{1/\gamma} \quad \text{and} \quad B = \frac{k_{in}}{T_0} \quad (A11)
\]
Discriminant Analysis

Under certain conditions, Eqs. A7 and A10 produce damped oscillations, converging to the equilibrium point \((\bar{x}, \bar{y})\), defined as
\[
\bar{x} = [1 - f(C)]^{1/\gamma} \quad \bar{y} = 1 - f(C) \quad (A12)
\]
This is demonstrated for Eq. A10. The local behavior near the equilibrium point can be characterized by the eigenvalues of the Jacobian matrix \(M\)
\[
M = \begin{pmatrix}
\gamma B \bar{x} & -A \\
\gamma B & -B \bar{x}^\gamma
\end{pmatrix}
\]
where we have used the fact that \(\bar{y} = \bar{x}^\gamma\) at the equilibrium point. The eigenvalues \(\lambda\) are then computed from
\[
|M - \lambda I| = \begin{vmatrix}
-\gamma & -A \\
\gamma B & -B \bar{x}^\gamma - \lambda
\end{vmatrix} = \lambda^2 + B \bar{x}^\gamma + \frac{\gamma AB}{\bar{x}}
\]
where \(I\) is the identity matrix. Thus the characteristic equation is
\[
\lambda^2 + B \bar{x}^\gamma + \frac{\gamma AB}{\bar{x}} = 0 \quad (A13)
\]
from which the eigenvalues can be calculated by taking its roots
\[
\lambda_{1,2} = \frac{1}{2} \left(-B \bar{x}^\gamma \pm \sqrt{B^2 \bar{x}^{2\gamma} - 4 \frac{\gamma AB}{\bar{x}}}\right) \quad (A14)
\]
These roots contain important information about the behavior of the system. The discriminant of Eq. A14 is given by Eq. A15
\[
D = \frac{B^2 \bar{x}^{2\gamma} - 4 \frac{\gamma AB}{\bar{x}}} \quad (A15)
\]
When \(D < 0\), the eigenvalues \(\lambda_1\) and \(\lambda_2\) are complex. In this case the solution of the system of differential equations exhibits oscillatory behavior. If \(\gamma \neq \frac{1}{2}\), the sign of \(D\) depends on the value of \(\bar{x}\). In particular, if \(\gamma > \frac{1}{2}\), there exists a critical signal value \(\bar{x}^*\), such that if \(\bar{x} < \bar{x}^*\), the eigenvalues are real valued and there are no oscillations; and if \(\bar{x} > \bar{x}^*\), the eigenvalues are complex and oscillations occur. In both cases the real part of the eigenvalues \(\lambda_1\) and \(\lambda_2\) is negative, so that solutions converge to \((\bar{x}, \bar{y})\) and oscillations die out.
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