Pharmacokinetic-Pharmacodynamic Modeling of Buspirone and Its Metabolite 1-(2-Pyrimidinyl)-piperazine in Rats

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ABSTRACT

The objective of this investigation was to compare the in vivo potency and intrinsic activity of buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine (1-PP) in rats by pharmacokinetic-pharmacodynamic modeling. Following intravenous administration of buspirone (5 or 15 mg/kg in 15 min) or 1-PP (10 mg/kg in 15 min), the time course of the concentrations in blood were determined in conjunction with the effect on body temperature. The pharmacokinetics of buspirone and 1-PP were analyzed based on a two-compartment model with metabolite formation. Differences in the pharmacokinetics of buspirone and 1-PP were observed with values for clearance of 13.1 and 8.2 ml/min and for terminal elimination half-life of 25 and 79 min, respectively. At least 26% of the administered dose of buspirone was converted into 1-PP. Complex hypothermic effects versus time profiles were observed, which were successfully analyzed on the basis of a physiological indirect response model with set-point control. Both buspirone and 1-PP behaved as partial agonists relative to R-(+)-8-hydroxy-2-(di-n-propylamino)tetralin (R-8-OH-DPAT) with values of the intrinsic activity of 0.465 and 0.312, respectively. Differences in the potency were observed with values of 17.6 and 304 ng/ml for buspirone and 1-PP, respectively. The results of this analysis show that buspirone and 1-PP behave as partial 5-hydroxytryptamine1A agonists in vivo and that following intravenous administration the amount of 1-PP formed is too small to contribute to the hypothermic effect.

Buspirone is a selective 5-HT1A agonist that is used clinically as an anxiolytic drug (for reviews on its pharmacological properties, see New, 1990 and Fulton and Brogdon, 1997). In vivo buspirone is metabolized into 1-(2-pyrimidinyl)-piperazine (1-PP), which also possesses affinity to the 5-HT1A receptor. This metabolite could therefore contribute to the effect of buspirone (Bianchi et al., 1988; Manahan-Vaughan et al., 1995; Cao and Rodgers, 1997). At present there is limited quantitative information on the magnitude of this effect. In theory the contribution of the metabolite to the effect is determined by the relative concentrations of buspirone and 1-PP, their relative in vivo potency and intrinsic activity, and the nature of the pharmacodynamic interaction between the two.

Recently we have developed a physiological pharmacokinetic-pharmacodynamic (PK-PD) model to quantitatively characterize the pharmacodynamics of 5-HT1A receptor agonists in vivo using the hypothermic effect as a pharmacodynamic endpoint (Zuideveld et al., 2001, 2002a). The hypothermic response is considered a robust marker of 5-HT1A activity (Millan et al., 1993; Cryan et al., 1999). It has been shown that the PK-PD model allows for the estimation of the relative in vivo potency and intrinsic activity of a wide array of different 5-HT1A agonists such as R-8-OH-DPAT, S-(−)-8-hydroxy-2-(di-n-propylamino)tetralin (S-8-OH-DPAT), N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-n-2-pyrindinyl-cyclohexanecarboxamide (WAY-100,635), and Flesinoxan (Zuideveld et al., 2001, 2002a, 2002b). In the present investigation we apply this model to determine the relative in vivo potency and intrinsic activity of buspirone and its metabolite 1-PP, taking into consideration the potential pharmacodynamic interaction between the two. In this respect it is important to consider the mechanism of the hypothermic response of buspirone and 1-PP. The hypothermic effect of buspirone has been widely studied (Higgins et al., 1988;
Koenig et al., 1988; Young et al., 1993) and is generally considered a specific 5-HT₁₄ receptor-mediated response (Koenig et al., 1988; Kommisarev et al., 1990; Cryan et al., 1999). 1-PP however has affinity for both the 5-HT₁₄ receptor (Kᵦ = 1035 nM = 94 ng/ml⁻¹) and the α₁-adrrenergic receptor (α₁-AR) (Kᵦ = 40 nM = 3.6 ng/ml⁻¹) (Gobert et al., 1995) and it is therefore possible that this effect is not mediated by the 5-HT₁₄ receptor (Kommisarev et al., 1990).

Materials and Methods

Experiments that were approved by the Ethics Committee of the University of Leiden were performed on male Wistar rats (Broekman BV, Someren, The Netherlands) weighing 315 ± 3 g (mean ± S.E.M., n = 27). They were housed in standard plastic cages (six per cage before surgery and individually after surgery). They were housed under normal 12-h light/dark cycle (lights on at 7 AM and lights off at 7 PM) and a temperature of 21°C. During the light period, a radio was turned on for background noise. Acidified water and food (laboratory chow; Hope Farms, Woerden, The Netherlands) were 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 for drug administration were implanted into the right jugular vein (Polythene, 14 cm, 0.52-mm i.d., 0.96-mm o.d.) for infusions of buspirone and 1-PP. For blood sampling, the left femoral artery (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.) was cannulated. 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) (Brocacef, Maarssen, The Netherlands) in a 0.9% (w/v) sodium chloride solution of polyvinylpyrrolidone (PVP) (Brocacef, Maarssen, The Netherlands) in a 0.9% (w/v) pyrogen-free sodium chloride solution (NPB, Emmer-Compascuum, The Netherlands) that contained 50 IU/ml heparin (Leiden University Medical Center, Leiden, The Netherlands). Just before 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. A telemetry transmitter (Physiotel implant TA10TA-F40; Data Sciences International) was implanted in the abdominal cavity of the rat. The transmitter measured the body temperature every 30 s for a 2-s period and signaled the data to a receiver (Physiotel receiver, model RPC-1; Data Sciences International). The receiver was connected to the computer through a BCM 100 consolidation matrix (Data Sciences International). The temperature profiles and the room temperature data (C10T temperature adapter; Data Sciences International) were processed and visualized using the Dataquest LabPro software (Data Sciences International, running under OS/2 Warp, IBM).

HPLC Analysis of Buspirone and 1-PP. The blood concentrations of buspirone and 1-PP were determined simultaneously by HPLC using UV detection. The HPLC system consisted of a Shimadzu LC-10 AD pump (flow rate: 1 ml/min; Shimadzu Corporation, Kyoto, Japan), a Waters 712WISP autosampler (Waters, Milford, MA), a Waters Sperisorb S5 OD/CD column (mixed phase 4.6 mm × 250 mm) together with a Sperisorb SSCN guard column (all Waters), a Spectroflow 757 UV absorbance detector set at 240 nm (Kratos Analytical, Ramsey, NJ), and a C-3RA Chromatopac integrator (Shimadzu Corporation). The mobile phase consisted of a mixture of 0.1 M potassium phosphate buffer (pH 5) and acetonitrile (60:40, v/v), to which 0.005 M KCl was added. The extracts were extracted from blood using an extensive liquid-liquid extraction to allow for the simultaneous analysis of both buspirone and 1-PP. To 50 μl of blood hemolyzed in 400 μl of water, 100 μl of the internal standard solution (5000 ng/ml 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine) was added. The mixture was deproteinized by adding 2 ml of acetonitrile and centrifuged. The supernatant was transferred to a clean tube and volumes of 50 μl of hydrochloric acid (1 M), 1 ml of water and 3 ml of distilled ethyl acetate were added. After separation, 2 ml of borate buffer (0.2 M, pH 10) and 3 ml of dichloromethane was added to the aqueous solution. The organic phase was transferred to a clean tube and evaporated to dryness under vacuum. The residue was reconstituted in 100 μl of 0.1 M sodium hydroxide, of which 50 μl was injected into the HPLC system. On the day of analysis, a 9-point calibration curve was prepared for both buspirone and 1-PP by spiking 50 μl of blood hemolyzed in 400 μl of water with 50 μl of buspirone and 1-PP. This resulted in a blood concentration range of 10 to 10,000 ng/ml for buspirone and 1 to 10,000 ng/ml for 1-PP. Samples were processed as described, and the peak-area ratio of either buspirone or 1-PP over the internal standard were calculated. Calibration curves were constructed by weighted linear regression ([weight factor = 1/peak-area ratio]²). Recovery, intra- and inter-assay variation were determined using spiked blood of 500 and 1500 ng/ml for buspirone and 50, 500, and 1500 ng/ml for 1-PP. For buspirone, recovery was (n = 3, mean ± S.E.M., corrected for volume loss) 41 ± 3.6% and 45 ± 8.6% for 500 and 1500 ng/ml, respectively. For 1-PP, recovery was (n = 3, mean ± S.E.M., corrected for volume loss) 88 ± 9.8%, 82 ± 3.8%, and 88 ± 16.5% for 50, 500, and 1500 ng/ml, respectively. Inter-assay variation (n = 10) was 9.9% and 19.2% (accuracy: -8.9% and 1.2%) for 500 and 1500 ng/ml buspirone and 9.3, 14.4, and 16.3% (accuracy: -12.6, -0.4, and -0.8%) for 50, 500, and 1500 ng/ml 1-PP. Intra-day assay variation (n = 4) was 4.4 and 3.4% (accuracy: -2.5 and 1.4%) for 500 and 1500 ng/ml buspirone and 7.4, 8.9, and 7.9% (accuracy: 12.6, -1.7, and -17.3%) for 50, 500, and 1500 ng/ml 1-PP. Detection limit (signal-to-noise ratio 3) using 50 μl of blood was 22.2 and 5.2 ng/ml for buspirone and 1-PP, respectively.

Data Acquisition

Temperature Measurements. To measure the body temperature of the rat a telemetric system (Physiotel Telemetry system; Data Sciences International) was used. A telemetric transmitter (Physiotel implant TA10TA-F40; Data Sciences International) was implanted in the abdominal cavity of the rat. The transmitter measured the body temperature every 30 s for a 2-s period and signaled the data to a receiver (Physiotel receiver, model RPC-1; Data Sciences International). The receiver was connected to the computer through a BCM 100 consolidation matrix (Data Sciences International). The temperature profiles and the room temperature data (C10T temperature adapter; Data Sciences International) were processed and visualized using the Dataquest LabPro software (Data Sciences International, running under OS/2 Warp, IBM).

Dosage Regimen. The experiments were performed 8 days after surgery. Rats received infusions of buspirone and 1-PP. For buspirone, infusions of 5 and 15 mg/kg in 15 min (12.96 and 38.91 μmol/kg for n = 6 and n = 7, respectively) were given. For 1-PP, an infusion of 10 ng/kg (60.90 μmol/kg for n = 6) in 15 min was administered. Six rats received vehicle treatments in which an equivalent amount of saline was infused. For the infusions, an external cannula with a specific volume was filled with a solution of the drug in an amount of saline, which was calculated according to the weight of the rat and connected to the infusion pump (BAS BeeHive, BAS Bioanalytical Systems Inc., West Lafayette, IN). All the experiments started between 9:00 and 9:30 AM.

Blood Sampling. Approximately 15 to 18 serial blood samples of 50 μl were taken according to a fixed time schedule to determine the pharmacokinetics of the drug. The exact amount was measured with a 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.

Experimental Protocol

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Chemicals. Buspirone and 1-PP were generously donated by Bristol-Myers Squibb (Princeton, NJ), 4-[3-(Benzoazol-1-yl)propyl]-1/2-methoxyphenyl]-piperazine maleate was purchased from Tocris (Lanford, Bristol, UK). Dichloromethane was purchased from Riedel-de Haën (Seelze, Germany). All other chemicals used were of analytical grade (Baker, Deventer, The Netherlands).

Data Analysis

A population approach was used to quantify both the pharmacokinetics and pharmacodynamics of buspirone and 1-PP. Using this approach, the population is taken as the unit of analysis while taking into account both intra-individual variability in the model parameters as well as inter-individual residual error. Modeling was performed using the nonlinear mixed effects modeling software NONMEM developed by Sheiner & Beal (version V 1.1, NONMEM project Group, University of California, San Francisco, CA). Individual predictions were obtained in a Bayesian post hoc step.

Pharmacokinetic Analysis. The concentration-time profiles of the buspirone administration, the 1-PP formed during the buspirone administration, and the 1-PP following direct administration were best described using a two-compartment model with metabolite formation. In this model, the input function for the metabolite following administration of the parent drug is the fraction of buspirone that is converted into 1-PP. This model is mathematically described as follows (Houston, 1985)

\[
\begin{align*}
V_1 & \dot{C}_{1,B} = Dose_{inf} \cdot \delta(t) - CL_B \cdot C_{1,B} - CL_d \cdot C_{1,B} + CL_d \cdot C_{1,1-PP} - C_1 \\
V_2 & \dot{C}_{2,B} = CL_d \cdot C_{1,B} - CL_d \cdot C_{2,B} + CL_d \cdot C_{2,1-PP} - C_2 \\
V_1 & \dot{C}_{1,1-PP} = CL_d \cdot C_{1,1-PP} - CL_d \cdot C_{1,B} - C_{1,1-PP} \\
V_2 & \dot{C}_{2,1-PP} = CL_d \cdot C_{1,1-PP} - CL_d \cdot C_{2,1-PP} - C_{2,1-PP}
\end{align*}
\]

where \( C_1 \) represents the concentration in the central compartment and \( C_2 \) the concentration in the distribution compartment. \( V_1 \) and \( V_2 \) are the compartments’ volumes of distribution. \( CL \) represents the clearance and \( CL_d \) the inter-compartmental clearance (see Fig. 1 for a schematic overview of the model). The subscripts B and 1-PP denote buspirone and the metabolite 1-PP respectively. For an infusion, \( \delta(t) \) is defined as

\[
in = \frac{Dose_{inf}}{T_{inf}} \quad \text{when} \quad t \leq T_{inf},
\]

\[
in = 0 \quad \text{when} \quad t > T_{inf},
\]

where \( T_{inf} \) is the duration of the infusion and \( Dose_{inf} \) is the total amount of drug infused. \( CL_{B,1-PP} \) in eq. 1 represents the clearance for the conversion of buspirone into 1-PP. The value of this clearance can be used to determine the fraction of buspirone that is converted into 1-PP, according to

\[
f_{B \rightarrow 1-PP} = \frac{CL_{B,1-PP}}{CL_{B,1-PP} + CL_d}
\]

The fraction calculated based on this equation does not take into account the amount of 1-PP metabolized directly after being formed and is therefore smaller than the actual amount formed (Houston, 1985). The model (eq. 1) was implemented in NONMEM using ADVAN6. The concentration-time profiles of the separate buspirone and 1-PP infusions were also modeled using a standard two-compartment pharmacokinetic model. Concentrations were all entered in molar units. The model as implemented in NONMEM’s ADVAN3 TRANS4 was used for this purpose. For all models, inter-individual 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 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 variances \( \sigma^2 \). Residual error was characterized by a proportional error model,

\[
CM_i = CP_i \cdot (1 + \varepsilon_i),
\]

where \( CP_i \) is the \( i-th \) plasma concentration for the \( i-th \) individual predicted by the model, \( CM_i \) is the measured concentration, and \( \varepsilon \) accounts for the residual deviance of the model predicted value from the observed concentration. Different \( \varepsilon \) values were assigned for the observations of buspirone, 1-PP during buspirone infusions, and 1-PP when modeled simultaneously. The values for \( \varepsilon \) are assumed to be independently normally distributed with mean zero and variance \( \sigma^2 \). The values for the population \( \theta \), \( \sigma^2 \), and \( \sigma^2 \) are estimated using the first order method in NONMEM.

Pharmacodynamics Analysis. Recently we have proposed a physiological model, to describe the complex time course of the hypothermic response in vivo following the administration of 5-HT1A receptor agonists (Zuideveld et al., 2001). The model contains a set-point control that can be attenuated by the drug receptor interaction and utilizes the concept of an indirect physiological response model (Dayneka et al., 1993). This model considers 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 model is further characterized by the phenomenological system parameter A and a parameter \( y \), which determines the amplification in the system (for details, see Zuideveld et al., 2001). In this model, the stimulus generated by the drug-receptor interaction, which drives the physiological processes that lower temperature and characterizes the drug-receptor interaction in terms of the drugs’ potency and intrinsic activity, is described by the function \( f(C) \),

\[
f(C) = S \cdot \frac{C}{SC_50 + C},
\]

where \( S \) is the stimulus, \( SC_50 \) is 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 the slope factor, which determines the steepness of the curve. The parameters were estimated for both 1-PP and buspirone.

However because 1-PP is formed during the buspirone administration, both compounds will be present simultaneously. Hence a pharmacodynamic interaction model may be needed to quantify the pharmacodynamics in this situation. The contribution of 1-PP to the effect of buspirone was evaluated considering both a non-competitive and a competitive interaction model. To characterize the non-comp.
petitive pharmacodynamic interaction between the two drugs, the following equation has been proposed (Ariens and Simonis, 1964),

\[ S_{B+1-PP} = \frac{S_B \cdot C^{100}_{B}}{SC^{50}_{B} + C^{100}_{B}} + \frac{S_{1-PP} \cdot C^{100}_{1-PP}}{SC^{50}_{1-PP} + C^{100}_{1-PP}} \]

where \( S_{B+1-PP} \) is the combined stimulus of both drugs, \( S_B \) and \( S_{1-PP} \) are the single drug stimuli and \( S_m \) is the maximum achievable stimulus of the system. As the maximum achievable stimulus in the system studied equals 1, substituting eq. 6 for both drugs in eq. 7, yields

\[ S_{B+1-PP} = \frac{S_B \cdot C^{100}_{B}}{SC^{50}_{B} + C^{100}_{B}} + \frac{S_{1-PP} \cdot C^{100}_{1-PP}}{SC^{50}_{1-PP} + C^{100}_{1-PP}} \]

The competitive pharmacodynamic interaction between the two drugs acting at the same receptor was characterized by the equation originally proposed by Holford and Sheiner (1981),

\[ S_{B+1-PP} = \frac{S_{\text{max}} \cdot (C_{B} / SC_{B})^{\theta} + S_{\text{max},1-PP} \cdot (C_{1-PP} / SC_{1-PP})^{\theta}}{1 + (C_{B} / SC_{B})^{\theta} + (C_{1-PP} / SC_{1-PP})^{\theta}} \]

where the stimulus of both drugs is combined. Recently, a reduced version of this model has been applied successfully to characterize the interaction between R-8-OH-DPAT and the competitive antagonist WAY-100,635 (Zuideveld et al., 2002a). The pharmacodynamic parameters for buspirone with eqs. 8 and 9 were compared with parameters obtained when considering each drug separately on the basis of eq. 6. This allows estimation of the relative contribution of 1-PP to the observed effect during and after the buspirone infusion.

The pharmacodynamic model was implemented in NONMEM using ADVAN6. The maximum stimulus \( S_{\text{max}} \) was assumed to be 1 and therefore fixed to this value (Zuideveld et al., 2001). Inter-individual variability on the parameters was modeled to an exponential equation, as described in eq. 4. Residual error was characterized by a proportional error model:

\[ y_{mi} = y_{Pi} \cdot (1 + e_i) \]

where \( y_{mi} \) is the \( j \)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 \( \theta \), \( \omega^2 \), and \( \sigma^2 \) are estimated using the centering first-order conditional estimation method with the first-order model in NONMEM. A conditional estimation method 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 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. 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 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.

**Statistical Analysis.** 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 the accuracy and precision of parameter estimates and correlations between the parameter estimates. Comparisons of maximal decreases in body temperature were performed using an unpaired t test, where a P value <0.05 was considered significant.

**Results**

**Pharmacokinetics.** During the experiments blood samples were taken to construct individual concentration-time profiles for both buspirone and 1-PP. Individual concentration profiles were predicted by fitting a population pharmacokinetic model to the data (eq. 1). On the basis of the concentration curves, the goodness-of-fit plots and the minimum value of the objective function, two-compartment models were selected for buspirone, 1-PP, and 1-PP following administration of buspirone. The concentration-time profiles of the 1-PP administration were fitted both separately and simultaneously with the “1-PP” during the buspirone administration profiles. Ultimately the values of the pharmacokinetic parameters of 1-PP were fixed to those obtained upon the separate administration. These values were subsequently entered as constants in the analysis of the concentration profiles of buspirone and 1-PP on the basis of the two-compartment metabolite model (eq. 1), which resulted in a significant improvement of the objective function. The obtained pharmacokinetic parameter estimates were used as input in the pharmacodynamic analysis because it is believed that these concentrations are closest to the “true” concentrations at each observation of the body temperature. Both the individually measured concentrations and the population predicted profiles following administration of buspirone and 1-PP are depicted in Fig. 2. All parameters were estimated in their mixed effect form, the random effect being incorporated in exponential error models. The results of the analysis are represented in Table 1. The clearance found for buspirone was 13 ml/min resulting in a half-life of 25 min. For 1-PP, a clearance of 8.2 ml/min was found, which corresponds to a half-life of 79 min. Based on the pharmacokinetic analysis (eq. 3), 26% of the administered dose of buspirone is metabolized to 1-PP. Pharmacokinetic parameter estimates were
TABLE 1
Population pharmacokinetic parameters and inter- and intra-individual variabilities of buspirone and 1-PP

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameter</th>
<th>Value</th>
<th>CV Inter-Individual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-PP Fixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Buspirone</td>
<td>CL_{t,1}</td>
<td>13.1</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>CL_{t,2}</td>
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<td>35</td>
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<tr>
<td></td>
<td>V_{f,1}</td>
<td>8.94</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>V_{f,2}</td>
<td>626</td>
<td>38</td>
</tr>
<tr>
<td>1-PP</td>
<td>CL_{t,1,PP}</td>
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<td>51</td>
</tr>
<tr>
<td></td>
<td>CL_{f,1,PP}</td>
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<td>31</td>
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<tr>
<td></td>
<td>CL_{d,1,PP}</td>
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<td>35</td>
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<tr>
<td></td>
<td>V_{f,1,PP}</td>
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</tr>
<tr>
<td></td>
<td>V_{f,2,1,PP}</td>
<td>924</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>f_{p,1-PP}</td>
<td>25.7</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

Buspirone CV intra-individual: 28%
1-PP (metabolite) CV intra-individual: 51%
1-PP (administered) CV intra-individual: 26%

CV, coefficient of variation; N.A., not applicable.

* 95% CI is the 95% confidence interval over the precision of the estimated parameter.

independent of administered dose and no statistically significant correlations between the parameter estimates and between the parameters from the different dosing groups were detected. Table 1 further displays the inter-individual variation, and the intra-individual variation, which are both found to be reasonable for both buspirone and 1-PP. For the different drugs and administrations, different intra-individual variation was predicted, which significantly improved the fit. The precision of the parameters prediction, as represented by their 95% confidence intervals, is good.

Pharmacodynamics. The average time-effect profiles for the effect on body temperature following administration of vehicle, buspirone, and 1-PP are depicted in Fig. 3. The average baseline temperature (S.E.M., n = 33) was 38.0 ± 0.005°C. Following administration of 5 and 15 mg/kg buspirone in a 15-min infusion, a maximal decrease of 2.8 ± 0.3°C was observed after approximately 55 min. Administration of 10 mg/kg 1-PP in a 15-min infusion resulted in a maximum temperature decrease of 1.6 ± 0.2°C after approximately 36 min. The temperature decreases were all statistically significantly (P < 0.05) different from the vehicle treatment, consisting of saline. After reaching a maximal decrease, a rapid recovery was observed followed by a plateau phase before the body temperature returned to baseline.

The time-effect profiles for buspirone and 1-PP were analyzed on the basis of the set-point model combined with 1) the sigmoidal pharmacodynamic model, 2) the noncompetitive, and 3) the competitive interaction model. This analysis results in two types of pharmacodynamic parameters: drug-specific parameters (SC_{50}, S_{max} and n) and system-specific parameters (k_{m}, A, and γ). These parameters were estimated using the centering first-order conditional estimation method with a first-order model. The average values for all η values were not significantly different from 0. Population parameter estimates are depicted in Table 2. Individual post hoc predictions of the parameters were not biased with respect to the different treatment groups. Interestingly, no difference was found in the pharmacodynamic parameter estimates for buspirone regardless of whether the sigmoidal pharmacodynamic model (eq. 6) or the noncompetitive interaction model (eq. 8) were used. The parameters obtained with the competitive interaction model (eq. 9) differ substantially, in particular with respect to the values of SC_{50} and S_{max}. No difference was found in the system-specific parameters between the various models or various compounds. The inter-individual variations of the pharmacodynamic parameter estimates for 1-PP were larger than for buspirone. Figure 4 depicts the individual observations, predictions, and the population predictions for each model and two typical individual rats.

Discussion

The present study provides novel information on the pharmacokinetic and pharmacodynamic relationship of buspirone and its metabolite 1-PP in vivo. Specifically, the present investigation has also focused on the relative potency and intrinsic activity of the metabolite 1-PP and the extent to which 1-PP contributes to the effect of the buspirone. This study shows that with values of the relative intrinsic activity of 0.465 and 0.312 both buspirone and 1-PP behave as partial agonists relative to R-8-OH-DPAT. Furthermore it is shown that the potency of 1-PP is 20-fold lower compared with...
buspirone and that following intravenous administration of the parent drug, formation of 1-PP does not contribute to the observed effect.

In comparative pharmacodynamic investigations in vivo, it is important to take differences in pharmacokinetics between the compounds into account. Therefore in the present investigation, the effects of buspirone and 1-PP were determined by an integrated PK-PD approach. In the present study, the value of the clearance was 13 ml/min resulting in a half-life of 25 min for buspirone and a clearance of 8.2 ml/min with a corresponding half-life of 79 min for the metabolite 1-PP. Both values are in close agreement with the values reported by Caccia et al. (1985). In the present study, it was estimated on the basis of the two-compartment model with metabolite formation that at least 26% of the administered dose of buspirone is metabolized into 1-PP. Again, this appears to be consistent with findings of Caccia et al. (1986) and Jajoo et al. (1989a, 1989b) who showed that 25% of the administered dose is excreted into urine as 1-PP. Overall the pharmacokinetics of both buspirone and 1-PP could be characterized successfully using the metabolite model.

To quantify the pharmacodynamic response of both buspirone and 1-PP the hypothermic response was chosen as a pharmacodynamic endpoint. Recently, we have developed an integrated pharmacokinetic-pharmacodynamic model to characterize 5-HT₁A receptor-mediated hypothermia, in terms of potency and intrinsic activity of the agonists used (Zuideveld et al., 2001). The 5-HT₁A agonist-induced hypothermic response was chosen, because it is considered to be a robust marker for 5-HT₁A activity (Millan et al., 1993; Cryan et al., 1999) and behaves as an ideal biomarker, which is continuous, reproducible, and sensitive enough to discriminate between a full and a partial agonist (Hadrava et al., 1996) and is selective (Millan et al., 1993; Cryan et al., 1999). The PK-PD model itself combines an indirect physiological response model (Dayneka et al., 1993) with a set-point control. It is believed that the 5-HT₁A receptors play a role in maintaining the body’s set-point temperature (Lin et al., 1988; Schwartz et al., 1998; Zeisberger, 1998). In addition numerous reports suggest that this set-point is regulated through an interplay between the 5-HT₁A (hypothermia) and the 5-HT₂₅C (hyperthermia) receptor system (Gudelsky et al., 1986; Salmi and Ahlenius, 1998). Therefore the proposed model is considered to reflect physiology. This is confirmed by the observation that a competitive interaction model accurately characterizes the interaction between R- and S-8-OH-DPAT and WAY-100,635 (Zuideveld et al., 2002a). In addition, this model has been applied successfully to characterize the in vivo pharmacodynamics of a range of 5-HT₁A ligands including R- and S-8-OH-DPAT, WAY-100,635 and flesinoxan (Zuideveld et al., 2001; 2002b) showing differences in both in vivo potency as well as intrinsic activity.

Regarding the hypothermic response induced by buspirone, much evidence has been presented indicating that it is mediated through the 5-HT₁A receptor (Higgins et al., 1988; Young et al., 1993). Furthermore its effect can be antagonized by selective 5-HT₁A receptor antagonists (Koenig et al., 1988; Cryan et al., 1999) and its selectivity over other receptors that may also mediate a hypothermic response, e.g., the α₂-AR (Dilsaver et al., 1989; MacDonald et al., 1989), µ-opioid (OP3) (Spencer et al., 1988), dopamine D₁ (Barik and de Beaufreapie, 1998) and adenosine A₁ receptors (Malhotra and Gupta, 1997) is high (New, 1990; Gobert et al., 1995). 1-PP on the other hand has affinity for both the 5-HT₁A (Kᵢ = 1035 nM = 94 ng/ml) and the α₂-AR (Kᵢ = 40 nM = 3.6 ng/ml) (Gobert et al., 1995). The mechanism of the hypothermic response of 1-PP is therefore subject to debate. Despite the fact that 1-PP behaves as an antagonist (rather than an agonist) at the α₂-AR, evidence has been presented that suggests that its hypothermic response may not be mediated by 5-HT₁A receptors (Komissarev et al., 1990). In this respect it is of interest that in a separate set of experiments it has

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<th>System-Specific</th>
<th>Drug-Specific</th>
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![Fig. 4](https://example.com/image.png)

Selected representative fits of different treatments of buspirone and 1-PP. Closed circles represent measured body temperature, the solid line represents individual prediction, and the dashed line the population prediction for 10 mg/kg infusion of 1-PP in 15 min (C), 5 (A) and 15 (B) mg/kg buspirone in 15-min infusions. Infusions all started at t = 60.
been shown that the selective 5-HT₁₅ receptor agonist WAY-100,635, when administered in doses that inhibit the effect of 8-OH-DPAT in a competitive manner, does not influence the hypothermic response of 1-PP (Zuideveld et al. unpublished observations). This shows that 1-PP apparently induces hypothermia via a mechanism that is different from that of buspirone and other selective 5-HT₁₅ agonists.

In the present study, the PK-PD relationships of the hypothermic effect for both buspirone and 1-PP are characterized in a quantitative manner using the set-point model developed for characterization of 5-HT₁₅ receptor agonists. Although the mechanism of the 1-PP-induced hypothermic response is unclear, the set-point model could be applied successfully to describe the 1-PP-induced hypothermic response. This seems justified since the observed complex time-effect profile bears a great similarity to that following administration of a selective 5-HT₁₅ agonist. Furthermore, it is well established that the mechanism of α₂-AR-mediated hypothermia is very similar to that of 5-HT₁₅-mediated hypothermia. Both receptors are connected to the regulation of the body’s set-point and utilize the same effector mechanisms (Frank et al., 1997; Salillien et al., 1997). Pharmacodynamic analysis revealed that the hypothermic response of 1-PP could be well characterized using this model, showing that 1-PP behaves as a partial agonist with a value of the parameter Sₘax of 0.312 relative to R-8-OH-DPAT. Furthermore the potency is roughly 20 times lower than that of buspirone itself.

In the pharmacodynamic analysis, we have utilized both a non- and a competitive interaction model to describe the possible interaction between buspirone and 1-PP. The non-competitive interaction model as proposed by Ariens and Simonis (1964) was chosen because of its simplicity, and since the maximal stimulus of the system is known, as defined by the maximal response of R-8-OH-DPAT, the parameters are identifiable. A specific feature of this model is that it assumes the interaction to be additive, with the consequence that neither synergism nor antagonism can be estimated. Therefore, the pharmacodynamics of buspirone has also been characterized with a regular sigmoidal model, disregarding 1-PP. As can be concluded from the pharmacodynamic analysis, the parameter estimates for potency, intrinsic activity, and the slope factor are very similar between the interaction model and the regular sigmoidal model, suggesting that 1-PP hardly plays a significant role in buspirone’s hypothermic response, essentially adding zero to the effect. The data was also analyzed using a competitive interaction model as proposed by Holford and Sheiner (1981). The major disadvantage of this model is that essentially the slope factor n determines whether either synergism or antagonism is predicted. Despite the fact that the parameters obtained with the competitive interaction model deviate the most from the ones found with the noncompetitive and sigmoidal model, the slope factors are relatively close to 1, and it is concluded that the competitive interaction model is more sensitive to a relatively small amount of 1-PP in the body. The peak concentration of 1-PP during the 15 mg/kg dose of buspirone is 210 ± 34 ng/ml after 60 min, which only represents a mere 69% of its SC₅₀ and therefore too low to contribute significantly. However, as buspirone is subjected to large scale presystemic metabolism (Caccia et al., 1985), which is expected given its high clearance (present investigation), it should be anticipated that in humans higher concentrations of 1-PP will be found after an oral administration, which could ultimately interfere with buspirone effect in a significant manner. In this respect it is interesting to note that based on the potency and its ratio with its affinity for the 5-HT₁₅ receptor [potency, 304 ng/ml]/[affinity, 94 ng/ml] = 3.2 for 1-PP versus [potency, 17.6 ng/ml]/[affinity, 2.7 ng/ml] = 6.5 for buspirone, it is conceivable that 1-PP mediates its hypothermic effect through the 5-HT₁₅ receptor.

In the present study, the PK-PD relationship of the hypothermic effect for both buspirone and 1-PP is characterized in a quantitative manner using the set-point model. The results show that buspirone and 1-PP behave as partial 5-HT₁₅ agonists in vivo relative to 5-HT₁₅ agonists such as R-8-OH-DPAT and fleinoxan. Pharmacodynamic analysis using both a sigmoidal E₅₀, a non- and a competitive interaction model revealed that in the rat, the peak 1-PP concentrations reached are too low to contribute significantly to the buspirone effect after intravenous administration.

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References


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