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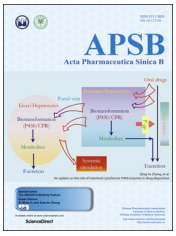

ORIGINAL ARTICLE

Development of a mechanism-based pharmacokinetic/pharmacodynamic model to characterize the thermoregulatory effects of serotonergic drugs in mice

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Abstract We have shown recently that concurrent harmaline, a monoamine oxidase-A inhibitor (MAOI), potentiates serotonin (5-HT) receptor agonist 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT)-induced hyperthermia. The objective of this study was to develop an integrated pharmacokinetic/pharmacodynamic (PK/PD) model to characterize and predict the thermoregulatory effects of such serotonergic drugs in mice. Physiological thermoregulation was described by a mechanism-based indirect-response model with adaptive feedback control. Harmaline-induced hypothermia and 5-MeO-DMT–elicited hyperthermia were attributable to the loss of heat through the activation of 5-HT_{1A} receptor and thermogenesis *via* the stimulation of 5-HT_{2A} receptor, respectively. Thus serotonergic 5-MeO-DMT–induced hyperthermia was readily distinguished from handling/injection stress-provoked hyperthermic effects. This PK/PD model was able to simultaneously describe all experimental data including the impact of drug-metabolizing enzyme status on 5-MeO-DMT and harmaline PK properties, and drug- and stress-induced simple hypo/hyperthermic and complex biphasic effects. Furthermore, the modeling results revealed a 4-fold decrease of

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apparent SC_{50} value (1.88–0.496 μmol/L) for 5-MeO-DMT when harmaline was co-administered, providing a quantitative assessment for the impact of concurrent MAOI harmaline on 5-MeO-DMT–induced hyperthermia. In addition, the hyperpyrexia caused by toxic dose combinations of harmaline and 5-MeO-DMT were linked to the increased systemic exposure to harmaline rather than 5-MeO-DMT, although the body temperature profiles were mispredicted by the model. The results indicate that current PK/PD model may be used as a new conceptual framework to define the impact of serotonergic agents and stress factors on thermoregulation.

1. Introduction

Thermoregulation is a critical process in all mammals that requires a fine balance between heat production and heat loss[1]. Heat production is achieved by contracting skeletal muscles (shivering thermogenesis) and metabolic activities (non-shivering thermogenesis), such as metabolism of interscapular brown adipose tissue (iBAT). Heat loss, on the other hand, is modulated by vasoconstriction and vasodilation[2,3]. As a result, body temperature is controlled by a number of systems including central, energetic, metabolic and endocrine systems. Other factors including circadian rhythm, gender or aging as well as ambient temperature may affect thermoregulatory adjustments[4–6]. Central thermoregulation is primarily controlled by the hypothalamus, where serotonergic system plays an important role[5,7]. Stimulation of different 5-hydroxytryptamine (5-HT or serotonin) receptors mechanistically causes divergent effects on thermoregulation. Hypothermia can be induced by 5-HT precursor tryptophan, monoamine oxidase-A (MAO-A) inhibitor (MAOI) harmaline, 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), and lower doses of 5-HT releaser 3,4-methylenedioxy-*N*-methylamphetamine (MDMA), which may be suppressed by 5-HT_{1A} receptor antagonist or small interfering RNA (siRNA)[8–10]. On the other hand, 5-HT_2 receptor agonists such as 2,5-dimethoxy-4-iodoamphetamine (DOI) and high doses of MDMA induce hyperthermia in rats, which may be attenuated by 5-HT_{2A} receptor antagonists[11–14]. In addition, pretreatment with 5-HT_{1A} agonist 8-OH-DPAT abolishes the hyperthermia induced by 5-HT_{2A} receptor agonist DOI, suggesting a functionally antagonistic interaction between 5-HT_{1A} and 5-HT_{2A} receptors in thermoregulation[15].

In addition to those approved and potential therapeutic applications[16–19], some serotonergic agents are also used for recreational purposes, including 5-HT receptor agonists (*e.g.*, 5-methyoxy-*N,N*-dimethyltryptamine or 5-MeO-DMT, a non-selective 5-HT receptor agonist acting on 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors with moderate to high affinities) and MAOIs (*e.g.*, harmaline)[19–22]. Co-abuse or overdose of serotonergic drugs results in excessive serotonergic signaling and, subsequently, in life-threatening serotonin toxicity or syndrome that has become an important clinical problem[16,17,23]. Indeed several cases of serotonin toxicity related to harmaline and 5-MeO-DMT drug-drug interactions (DDI) have been documented in recent years[24–26]. Serotonin toxicity/syndrome is featured by neuromuscular (*e.g.*, tremor and hyperreflexia), autonomic (*e.g.*, hyperthermia) and mental (*e.g.*, confusion and hyperactivity) effects in humans and animal models, among which excessive hyperthermia (*e.g.*, over 41.0 °C) may lead to a variety of abnormalities (*e.g.*, metabolic acidosis and renal failure) and even fatality.

Our recent studies on 5-MeO-DMT and harmaline DDI using wild-type and cytochrome P450 2D6 (CYP2D6) humanized (Tg-*CYP2D6*) mouse models have demonstrated that co-administration of MAOI harmaline largely alters the pharmacokinetics (PK) of 5-MeO-DMT[27–29]. The status of CYP2D6, which metabolizes both harmaline and 5-MeO-DMT, may further influence the PK interactions between harmaline and 5-MeO-DMT[29]. In addition, pretreatment with harmaline, which itself induces hypothermia[30], greatly enhances 5-MeO-DMT–elicited pharmacodynamics (PD) as indicated by the hyperthermic effects[31]. To better understand and quantitatively describe the PK/PD interplay between harmaline and 5-MeO-DMT in thermomodulation, we developed a mechanism-based mathematical model to characterize the thermomodulatory effects of serotonergic drugs in mice. This PK/PD model consisted of an indirect response (IDR) model with mechanistic actions of serotonergic harmaline and 5-MeO-DMT in central thermoregulation, the pharmacokinetic DDI between harmaline and 5-MeO-DMT, and the influence of CYP2D6 status. The developed and qualified model not only captured simple hypo/hyperthermia and complex biphasic effects induced by serotonergic drugs and stress factors, but also identified harmaline as the major cause of hyperpyrexia induced by toxic doses of harmaline and 5-MeO-DMT.

2. Material and methods

2.1. Chemicals and materials

Harmaline hydrochloride dihydrate and 5-MeO-DMT oxalate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Isoflurane (AErrane) was bought from Baxter Healthcare (Deerfield, IL, USA).

2.2. Animals

Age-matched male wild-type FVB/N and Tg-*CYP2D6* mice (25–35 g) were used in the study. Animals were housed in an animal care facility maintained at 20 ± 2.0 °C on a 12-h light/dark cycle (lights on 6 a.m.–6 p.m.) with food and water provided *ad libitum*. All animal procedures were approved by the Institutional Animal Care and Use Committee at University at Buffalo, The State University of New York, USA.

Implantation of sterile telemetric transmitter (Physiotel implant TA10TA-F20 system; Data Sciences International, St. Paul, MN, USA) and intraperitoneal (i.p.) administration of harmaline,

5-MeO-DMT or control vehicle were conducted as previously described[31]. Drugs were dissolved in saline and all doses were calculated as free bases and administered in the same volume (10 mL/kg).

2.3. *Core body temperature measurement*

All measurements were performed in an isolated and quiet room with an ambient temperature maintained at 20±2.0 °C[31]. Core body temperatures (CBTs) of animals kept in home cages were continuously measured (10 times per minute) using the DSI PhysioTel telemetry system (Data Sciences International, St. Paul, MN, USA). Average CBT values every 5 or 10 min were calculated for PK/PD modeling. Basal level of CBT of each animal involved in this study was also determined before the test/drug administration.

2.4. *Pharmacokinetic/pharmacodynamic modeling*

The PK interaction between harmaline and 5-MeO-DMT was described by our group recently[27] and was adopted in the current study to characterize the PK profiles of 5-MeO-DMT when administered alone or in combination with harmaline. Briefly, a two compartment-model, with first order absorption and linear elimination by CYP2D6 and inherited murine elimination pathways, was used to describe the PK properties of harmaline in both genotypes of mice, where $C_{C–H}$ represents harmaline concentration in the central compartment and $X_{P–H}$ represents the mass of harmaline in the peripheral compartment. $V_{C–H}$ (2.43 L/kg) and $V_{P–H}$ (2.86 L/kg) are the volumes of central and peripheral compartment, respectively. $CL_{D–H}$ (0.879 L/min/kg) is the distribution clearance between the central and peripheral compartments. $k_{a–H}$ (0.307 min^{-1}) represents the absorption rate constant, and F_H represents the bioavailability of harmaline. F_H values for i.p.-dosed 2, 5 and 15 mg/kg of harmaline were 34.6%, 74.2% and 90.3%, respectively, in wild-type mice; the values were 36.8%, 68.8% and 80.1%, respectively, in Tg-*CYP2D6* mice. $CL_{other–H}$ (0.0962 L/min) represents the intrinsic murine elimination pathways, which is shared by both wild-type and Tg-*CYP2D6* mice, whereas $CL_{CYP2D6–H}$ (0.0608 L/min) represents CYP2D6-mediated elimination that is only present in Tg-*CYP2D6* mice. Likewise, a two compartment model with first order absorption and capacity-limited elimination from the central compartment has been established to describe the PK properties of 5-MeO-DMT, where $C_{C–M}$ represents the concentration of 5-MeO-DMT in central compartment and $X_{P–M}$ represents the mass of 5-MeO-DMT in peripheral compartment. $V_{C–M}$ (0.460 L/kg) and $V_{P–M}$ (2.29 L/kg) are the volumes of central and peripheral compartment, respectively. $CL_{D–M}$ (0.301 L/min/kg) is the distribution clearance between the central and peripheral compartments. $k_{a–M}$ (0.0748 min^{-1}) represents the absorption rate constant while F_M (74.8%) represents the bioavailability of 5-MeO-DMT. $V_{max(M)–M}$ (2.69 μmol/min/kg), $V_{max(O)–M}$ (0.0610 μmol/min/kg) and $V_{max(D)–M}$ (0.0334 μmol/min/kg) represent the maximum metabolic rates of 5-MeO-DMT by MAO-A, other murine elimination pathway and *O*-demethylation pathway, respectively. $K_{m(M)–M}$ (16.7 μmol/L), $K_{m(O)–M}$ (0.446 μmol/L) and $K_{m(D)–M}$ (1.27 μmol/L) represent the concentration of 5-MeO-DMT at 50% of the maximum metabolism by MAO-A, other murine elimination pathway and *O*-demethylation pathway, respectively. A $f_{mCYP2D6(D)–M}$ value (30.1%), which only presents in Tg-*CYP2D6* mice, was used to describe the fraction of 5-MeO-DMT *O*-demethylation by CYP2D6 in addition to murine 5-MeO-DMT *O*-demethylase capacity. $K_{i(M)–H}$ (0.048 μmol/L) was obtained from literature[32], which represents inhibition constant for harmaline against MAO-A. $K_{i(D)–H}$ (7.13 μmol/L) was obtained from our PK model fitting, which represents inhibition constant for harmaline against 5-MeO-DMT *O*-demethylase activity. Values of respective PK parameters were taken from our recent PK interaction study[27], and the resulting PK profiles were used as the PK input for PD modeling to drive thermoregulation (Fig. 1). The final integrated PK/PD model for thermoregulatory effects of harmaline and 5-MeO-DMT is shown in Fig. 1.

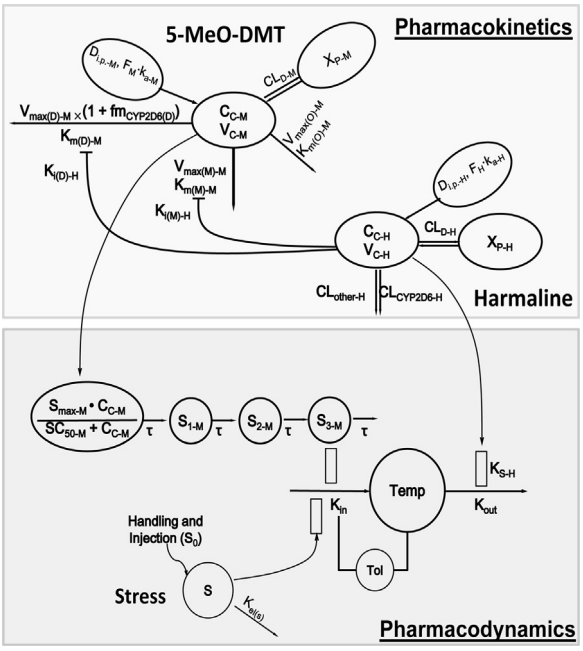

Figure 1 The PK/PD model for thermoregulation by serotonergic drugs harmaline and 5-MeO-DMT in wild-type and Tg-*CYP2D6* mice. Abbreviations of Harmaline and 5-MeO-DMT PK parameters as well as thermoregulation PD parameters are defined under Materials and Methods. Model estimates are shown in Table 1.

The CBT baseline ($Temp_{basal}$) was described by a linear function Eq. (1), in which $Temp_{basal(0)}$ represents the basal level CBT and slope λ defines the influence of time on baseline CBT.

$$Temp_{basal} = Temp_{basal(0)} + \lambda \cdot t \quad (1)$$

An indirect response model with a feedback mechanism[33] was employed to characterize the turnover of CBT as well as the adaptive feedback mechanism of the body to the change in body temperature, as shown in the following differential Eq. (2):

$$\frac{dTemp}{dt} = k_{in} \cdot \left(\frac{Temp_{basal}}{Temp}\right) - k_{out} \cdot Temp \quad (2)$$

where k_{in} and k_{out} represent the production and loss of heat, respectively; $Temp_{(0)}$ equals to $Temp_{basal(0)}$. The adaptive function of the body to thermoregulation is described as a ratio of baseline ($Temp_{basal}$) to response (Temp) that affects thermogenesis (k_{in})[33]. At time zero of the observation period, the system was assumed to be at its physiological steady state that yields the following baseline Eq. (3):

$$Temp_{basal(0)} = \frac{k_{in}}{k_{out}} \quad (3)$$

The stress to animal caused by handling and injection affects thermoregulation by stimulating thermogenesis (k_{in})[5,34,35]. This placebo effect was best described by a hypothetical signal ($S_S(t)$)

that wears off at a rate k_{el-S} as time progresses in Eq. (4):

$$S_S(t) = S_0 \cdot e^{-k_{el-S} \cdot t} \quad (4)$$

The same S_0 value was used for the hypothetical magnitude of stress on thermoregulation by any vehicle/saline, because our data already revealed that experimentally-induced stress level was vehicle-independent. A first-order rate constant k_{el-S} was used to describe the loss of this stress over time. This equation is similar to the stress function reported elsewhere[6].

The thermoregulatory effects of harmaline and 5-MeO-DMT are described by the following equations, with an assumption that harmaline and 5-MeO-DMT concentrations in the biophase (effect site) are in rapid equilibration with those in serum (central compartment). 5-HT_{1A} receptor acts as a central vasodepressor, which facilitates heat loss[36]. Therefore, activation of 5-HT_{1A} receptor on hypothermia $S_H(t)$ was expressed as stimulation of k_{out}, as shown in Eq. (5).

$$S_H(t) = k_{S-H} \cdot C_{C-H}(t) \quad (5)$$

where k_{S-H} represents a sensitivity constant of harmaline on k_{out}, which is different between single dose treatment ($k_{S-H\text{-}single}$) and combination treatment ($k_{S-H\text{-}DDI}$), given the observation that DDI interrupted the hypothermic effect of harmaline (5-MeO-DMT or saline was dosed 15 min after the administration of harmaline). Although our previous study employed an E_{max} model to characterize the rectal temperature change in mice induced by harmaline alone[30], a linear function was found sufficient to appropriately describe the change of core body temperature in mice in the present study.

The stimulation effect of 5-MeO-DMT (S_nM, $n = 1, 2, 3$) on thermogenesis (k_{in}) was best described using a transduction model with three transit compartments (6–8)[37], which accurately characterized the delay between 5-MeO-DMT-induced 5-HT_{2A} receptor activation and change in CBT.

$$\frac{dS_{1M}}{dt} = \frac{1}{\tau}\left(\frac{S_{max-M} \cdot C_{C-M}(t)}{SC_{50-M} + C_{C-M}(t)} - S_{1M}\right); (S_{1M(0)} = 0) \quad (6)$$

$$\frac{dS_{2M}}{dt} = \frac{1}{\tau}(S_{1M} - S_{2M}); (S_{2M(0)} = 0) \quad (7)$$

$$\frac{dS_{3M}}{dt} = \frac{1}{\tau}(S_{2M} - S_{3M}); (S_{3M(0)} = 0) \quad (8)$$

where τ represents the mean transit time in each transit compartment, and S_{max-M} represents the maximum stimulation effect of 5-MeO-DMT on thermogenesis. SC_{50-M} represents the concentration of 5-MeO-DMT that produces 50% of the maximum stimulatory effect, which is different when 5-MeO-DMT was dosed with ($SC_{50-M\text{-}DDI}$) or without ($SC_{50-M\text{-}non\text{-}DDI}$) the presence of harmaline. The final thermoregulatory model is shown in Eq. (9), which is derived from Eq. (2):

$$\frac{dTemp}{dt} = k_{in} \cdot \left(\frac{Temp_{basal}}{Temp}\right) \cdot (1 + S_S(t)) \cdot (1 + S_{3M}(t)) - k_{out} \cdot (1 + S_H(t)) \cdot Temp \quad (9)$$

where $Temp_{(0)}$ equals to $Temp_{basal(0)}$. All model fittings were performed in ADAPT V (Biomedical Simulations Resource, University of Southern California, Los Angeles, CA, USA) with a naïve-pooled population analysis. All PK parameters[27] were fixed and linked to PD model. PD model parameters were obtained by simultaneous estimation of CBT profiles at physiological conditions with the maximum-likelihood method[38], which included no treatment (baseline), control vehicles, harmaline alone (2, 5 and 15 mg/kg), 5-MeO-DMT alone (2, 10 and 20 mg/kg), harmaline (2, 5 and 15 mg/kg) plus vehicle, vehicle plus 5-MeO-DMT (2, 10 and 20 mg/kg), and harmaline (2, 5 and 15 mg/kg) plus 5-MeO-DMT (2 mg/kg) treatments.

Different PD model structures were tested during model development, which included an IDR model with or without transit compartments, an IDR model with different numbers of transit compartments, DDI and non-DDI conditions with different or the same parameters (k_{S-H}, S_{max-M}, SC_{50-M}), and the influence of concurrent harmaline on 5-MeO-DMT (S_{max-M} or SC_{50-M}) when the PD interaction was considered. Model selection was based on goodness-of-fit criteria, which included model convergence, Akaike Information Criterion (AIC), Schwarz Criterion (SC), precision of parameter estimations, estimation criterion value for the maximum likelihood method, visual inspection of predicted *versus* observed values, and residual plots as well as physiological plausibility and statistical significance of model parameter estimates. Different variance models were also tested during model development and the final variance model was defined as

$$VAR_i = (\sigma_1 + \sigma_1 \cdot Y(\theta, t_i))^2 \quad (10)$$

where σ_1 and σ_2 are the variance model parameters and $Y(\theta, t_i)$ is the ith predicted value from the PK/PD model.

This developed PK/PD model was externally validated by comparing the simulated and experimentally-determined CBT profiles in mice treated with 10 mg/kg of harmaline alone, 5 mg/kg of 5-MeO-DMT alone, and 5 mg/kg of harmaline plus 5 mg/kg of 5-MeO-DMT that were all proven non-toxic by our previous[27] and pilot studies. The qualified model was further utilized to investigate the interactions between harmaline (2, 5 or 15 mg/kg) and 5-MeO-DMT (10 mg/kg), which was shown to be toxic to both genotypes of mice[27,31,39]. Simulations of harmaline and 5-MeO-DMT PK profiles were also conducted using our PK DDI model described recently[27].

3. Results

3.1. PK/PD model development and estimation

Our final model (Fig. 1) was able to characterize all experimental effect-time profiles in the presence or absence of single or combination drug treatment reasonably well (Figs. 2–4). Respective models parameters were estimated with good precision that was indicated by the low CV (%) values (Table 1). A simple linear function Eq. (1) was found to be sufficient to appropriately characterize the baseline temperatures in both Tg-*CYP2D6* and wild-type mice during the observation time frame (10:30 a.m.–3:30 p.m., Fig. 2). The estimated $Temp_{basal(0)}$ was 35.8 °C and λ was 0.00322 °C/min, which indicates a slow but steady increase of baseline CBT during the light phase of the light/dark circle.

The modified IDR model consisting of a feedback mechanism (Fig. 1) adequately described the thermoregulation in response to injection/handling stress and serotonergic drug perturbation, estimating a k_{out} value of 0.0291 min^{-1} (Table 1). Vehicle treatment consistently caused a mild transient hyperthermia that was initiated rapidly, whereas it was independent of drug treatment (Figs. 2–4). The estimated S_0 and k_{el-S} values were 0.265 and 0.103 min^{-1}, respectively (Table 1), which reasonably described the hyperthermia of animals induced by single or repeated handling and injection (Fig. 2). Nevertheless, animal handling could alter harmaline-induced hypothermia (Fig. 4A and 4B *versus* Fig. 3A and 3B), which resulted in a k_{S-H} value of 0.0347 L/μmol for single treatment ($k_{S-H\text{-}single}$) and 0.0136 L/μmol for repeated treatments ($k_{S-H\text{-}DDI}$) (Table 1).

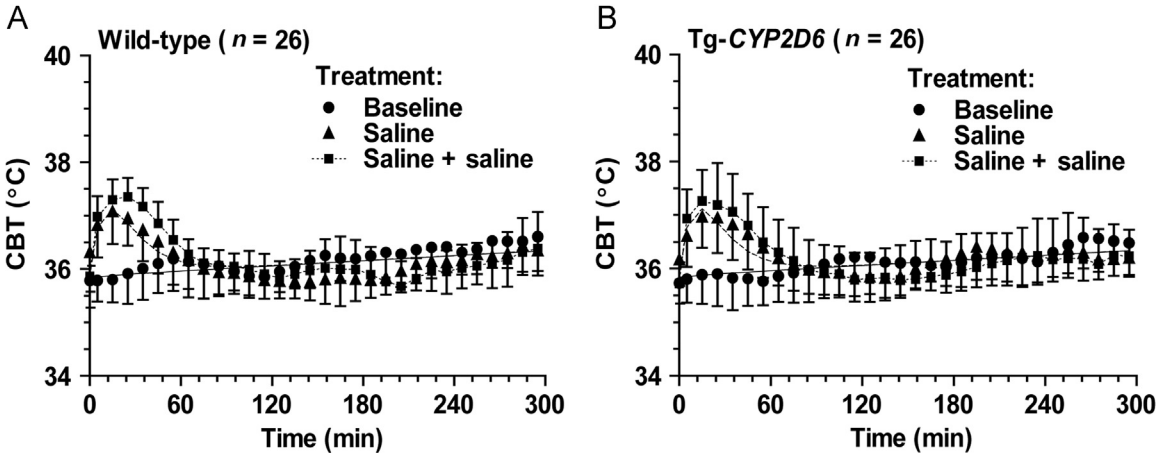

Figure 2 Model estimation of CBT profiles in wild-type (A) and Tg-*CYP2D6* (B) mice ($n=26$ in each group) at the baseline level (circle) or after single (triangle) or double (square) injections of saline. The solid (—), dashed (- -) and dotted (· ·) lines represent the fitted data of baseline, single and double injections of saline, respectively, which were obtained from simultaneous estimation using the PK/PD model shown in Fig. 1. CBT, core body temperature.

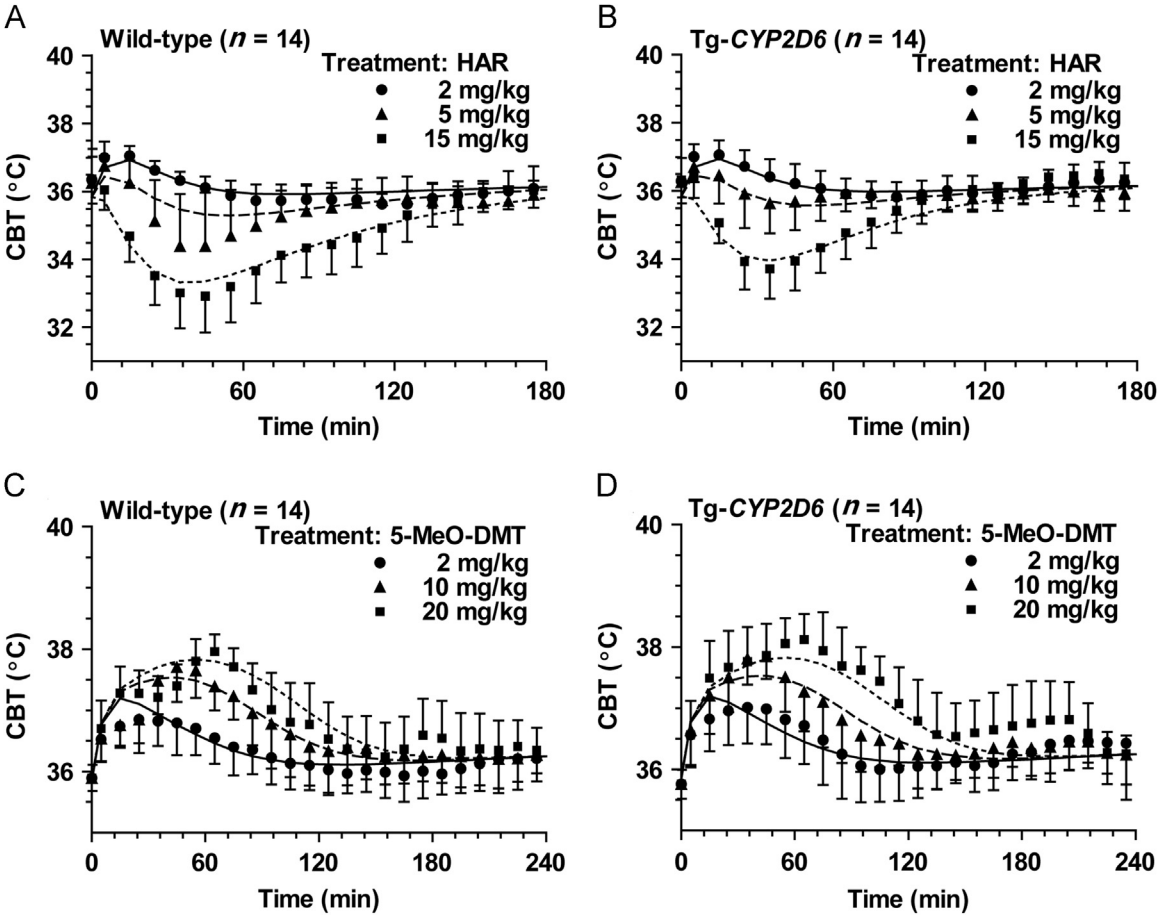

Figure 3 Model estimation of the hypothermia (A and B) and hyperthermia (C and D) in mice induced by harmaline and 5-MeO-DMT alone, respectively. Harmaline or 5-MeO-DMT was administered i.p. to wild-type and Tg-*CYP2D6* mice ($n=14$ in each group) at 0 min. The solid (—), dashed (- -) and dotted (· ·) lines represent the fitted data with the ascending doses of respective drugs, which were obtained from simultaneous estimation using the PK/PD model shown in Fig. 1.

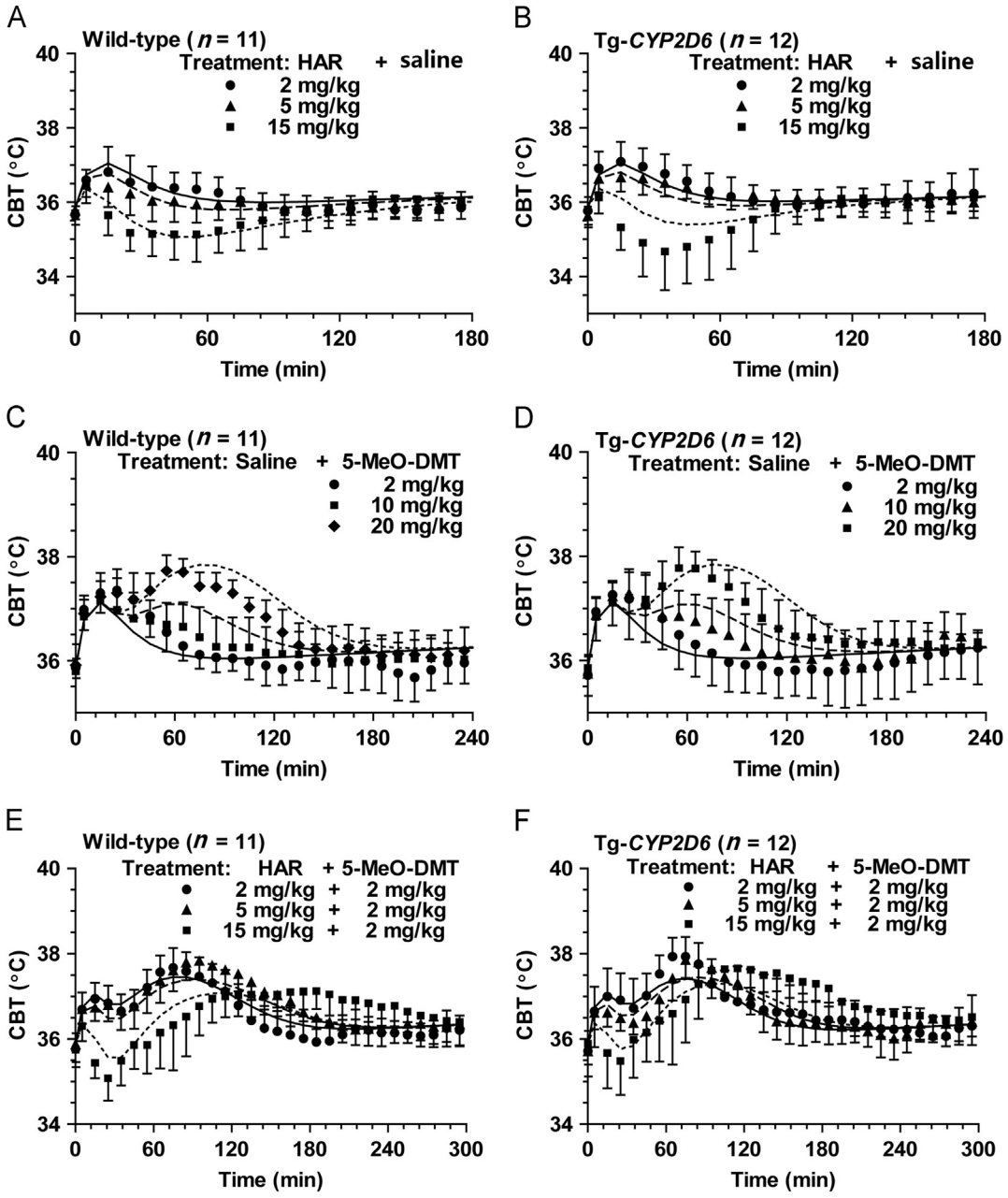

Figure 4 Model estimation of the thermomodulatory effects in mice co-administered with harmaline and 5-MeO-DMT, including harmaline plus vehicle (A and B), vehicle plus 5-MeO-DMT (C and D), and various doses of harmaline plus 2 mg/kg 5-MeO-DMT (E and F) in wild-type ($n = 11$) and Tg-*CYP2D6* ($n = 12$) mice. Harmaline and 5-MeO-DMT or corresponding vehicle were administered i.p. at 0 and 15 min, respectively. The solid (—), dashed (- -) and dotted (· ·) lines represent the fitted data with the ascending doses of respective drugs, which were obtained from simultaneous estimation using the PK/PD model shown in Fig. 1.

5-MeO-DMT alone stimulated hyperthermia (Figs. 3 and 4) with a transit time of 6.20 min for each of the 3 transit compartments, an $S_{max–M}$ value of 0.134, and an $SC_{50–M–non-DDI}$ value of 1.88 μmol/L (Table 1). Co-administration of harmaline with 2 mg/kg 5-MeO-DMT provoked biphasic effects, hypothermia at early phase (0–45 min) and hyperthermia at late phase (45–120 min), which was captured well by current PK/PD model (Fig. 4). Furthermore, co-administered harmaline significantly enhanced 5-MeO-DMT–induced late-phase hyperthermia that was adequately described by a decreased $SC_{50–M-DDI}$ value (0.496 μmol/L; Table 1) in this model. As expected, the influence of CYP2D6 status on harmaline PK and harmaline-5-MeO-DMT PK interaction[27] might be translated into significant difference in PD, which was manifested by the greater and prolonged hypothermia in wild-type mice following harmaline treatment (Figs. 3A, 3B, 4A and 4B) or co-administration of 15 mg/kg harmaline and 2 mg/kg 5-MeO-DMT (Fig. 4E and F), in contrast to the similar hyperthermic effects in two genotypes of mice treated with 5-MeO-DMT alone (Figs. 3C, 3D, 4C and 4D).

3.2. *Model validation*

The CBT profiles of animals treated with 10 mg/kg harmaline, 5 mg/kg 5-MeO-DMT, and 5 mg/kg harmaline plus 5 mg/kg

Table 1 Pharmacodynamic parameters of harmaline and 5-MeO-DMT in thermoregulation.

Parameter	Unit	Definition	Estimated value	CV (%)
$Temp_{basal(0)}$	°C	Baseline core body temperature at the beginning of the experiment	35.8	0.0454
λ	°C/min	The slope of change of baseline core body temperature	0.00322	4.79
τ	min	Transit time in teach compartment	6.20	5.86
k_{out}	min^{-1}	Rate constant of loss of heat	0.0291	4.44
S_0	–	Hypothetic magnitudes of stress on thermoregulation	0.265	5.75
$k_{el–S}$	min^{-1}	Rate constant of elimination of stress	0.103	6.46
$k_{S–H-single}$	L/μmol	Sensitivity constant of harmaline on thermogenesis after single dosing regimen	0.0347	1.80
$k_{S–H-DDI}$	L/μmol	Sensitivity constant of harmaline on thermogenesis after DDI dosing regimen	0.0136	2.86
$S_{max–M}$	–	The maximum stimulation effect of 5-MeO-DMT on thermogenesis	0.134	4.28
$SC_{50–M-non-DDI}$	μmol/L	The concentration of 5-MeO-DMT producing 50% of the maximum stimulation effect when treated alone or pretreated with vehicle	1.88	12.8
$SC_{50–M-DDI}$	μmol/L	The concentration of 5-MeO-DMT producing 50% of the maximum stimulation effect when pretreated with harmaline	0.496	12.2

5–MeO-DMT that did not induce any significant toxicity in mice, were used to validate the developed PK/PD model under nontoxic physiological conditions. The simulated CBT profiles (Fig. 5) using the PK/PD model (Fig. 1) and estimated PD parameters (Table 1) reasonably captured the experimental data obtained in mice. Similar to the dose levels of harmaline utilized for model development, a single dose of harmaline (10 mg/kg) alone induced more severe hypothermia in wild-type mice than Tg-*CYP2D6* mice (Fig. 5A and B), whereas 5-MeO-DMT (5 mg/kg) alone had similar thermomodulatory effects in the two genotypes of mice (Fig. 5C and D). On the other hand, co-administration of harmaline (5 mg/kg) with 5-MeO-DMT (5 mg/kg) led to a prolonged hyperthermia in wild-type mice (Fig. 5E and F). The results indicate that the present PK/PD model is able to quantitatively characterize the thermomodulatory effects of serotonergic harmaline and 5-MeO-DMT under physiological conditions.

3.3. Application of the developed PK/PD model for evaluating hyperpyrexia induced by toxic dose combinations of 5-MeO-DMT and harmaline

Higher dose combinations of harmaline (5 or 15 mg/kg) and 5-MeO-DMT (10 mg/kg) are toxic to both genotypes of mice[27,31,39] (and unpublished data). Such dose combinations caused a rapid increase of CBT (15–75 min) to an extremely high level (*e.g.*, >39 °C) in mice (Fig. 6), indicating a transition to toxicokinetic/toxicodynamic (TK/TD) interactions between harmaline and 5-MeO-DMT. In contrast, the treatment with 2 mg/kg harmaline plus 10 mg/kg 5-MeO-DMT led to a maximal CBT around 38 °C, suggesting that DDI of harmaline and 5-MeO-DMT at this dose combination remained as nontoxic to mice. Consistently, the validated PK/PD model described herein (Fig. 1) reasonably predicted the changes of CBT in both genotypes of mice following the treatment with 2 mg/kg harmaline plus 10 mg/kg 5-MeO-DMT (Fig. 6A and B), whereas the PK/PD model was unable to describe the CBT profiles in mice treated with toxic dose combinations of harmaline (5 or 15 mg/kg) and 5-MeO-DMT (10 mg/kg), including the rapid increase of CBT at early times (15–75 min) and quick decline during the late stage (Fig. 6C–F). These results suggest that the physiological thermoregulation in mice may be disrupted by toxic doses of serotonergic harmaline and 5-MeO-DMT (Fig. 6).

4. Discussion and conclusions

It has been shown that MAOI harmaline significantly enhances 5-MeO-DMT–induced hallucinogenic effects and toxicity[21,24–26]. Recent studies also demonstrate the influence of harmaline on 5-MeO-DMT–elicited neuropharmacological effects in animal models including thermoregulation and behaviors[31,39–43] as well as 5-MeO-DMT pharmacokinetics[27,29,43]. In the present study, a mathematical PK/PD model, which consists of an IDR model with baseline behavior, the adaptive feedback mechanism of the biological system, the impact of stress caused by handling and injection, and mechanistic actions of serotonergic drugs in thermomodulation, was developed and validated to characterize the relationship between drug concentrations and modulation of CBT by harmaline and 5-MeO-DMT, administered alone or in combination. This PK/PD model provides a quantitative explanation for the PK and PD DDIs between harmaline and 5-MeO-DMT, and it should have broad applications to the prediction of thermoregulation by serotonergic drugs.

A set-point model has been developed previously to describe the oscillatory behavior of CBT in rats by a series of hypothermic 5-HT_{1A} agents[44,45]. Another set-point model was developed to characterize interleukin-21–induced hyperthermia in cynomolgus monkeys[46]. While both compounds examined in the present study were serotonergic drugs, they did not cause any significant oscillation in mice at tested doses. Therefore, a simple mechanism-based IDR model was employed to define thermoregulation, which nicely characterized drug-induced changes of CBT in mice *via* the modulation of either thermogenesis (k_{in}) or heat loss (k_{out}). 5-MeO-DMT acts mainly as a 5-HT_{2A} agonist under pharmacological conditions despite that it can bind to multiple 5-HT receptors[47,48]. In addition, the effect of tested doses of 5-MeO-DMT on 5-HT_{1A} receptor may be minimal, given previous finding that 5-MeO-DMT is less potent than 5-HT in terms of binding to 5-HT_{1A} receptor[49], and murine cerebral 5-MeO-DMT concentrations were also remarkably lower than 5-HT following the administration of 5-MeO-DMT (2 or 10 mg/kg) alone or combined with harmaline (5–20 mg/kg)[27,50]. Therefore, only the activation of 5-HT_{2A} receptor was considered in the present PK/PD model for thermogenesis (Fig. 1). On the other hand, the heat loss was simply attributed to 5-HT_{1A} activation by harmaline-induced increase of 5-HT levels[50]. The simplification was made as harmaline-induced hypothermia was only attenuated by 5-HT_{1A} receptor antagonist[31].

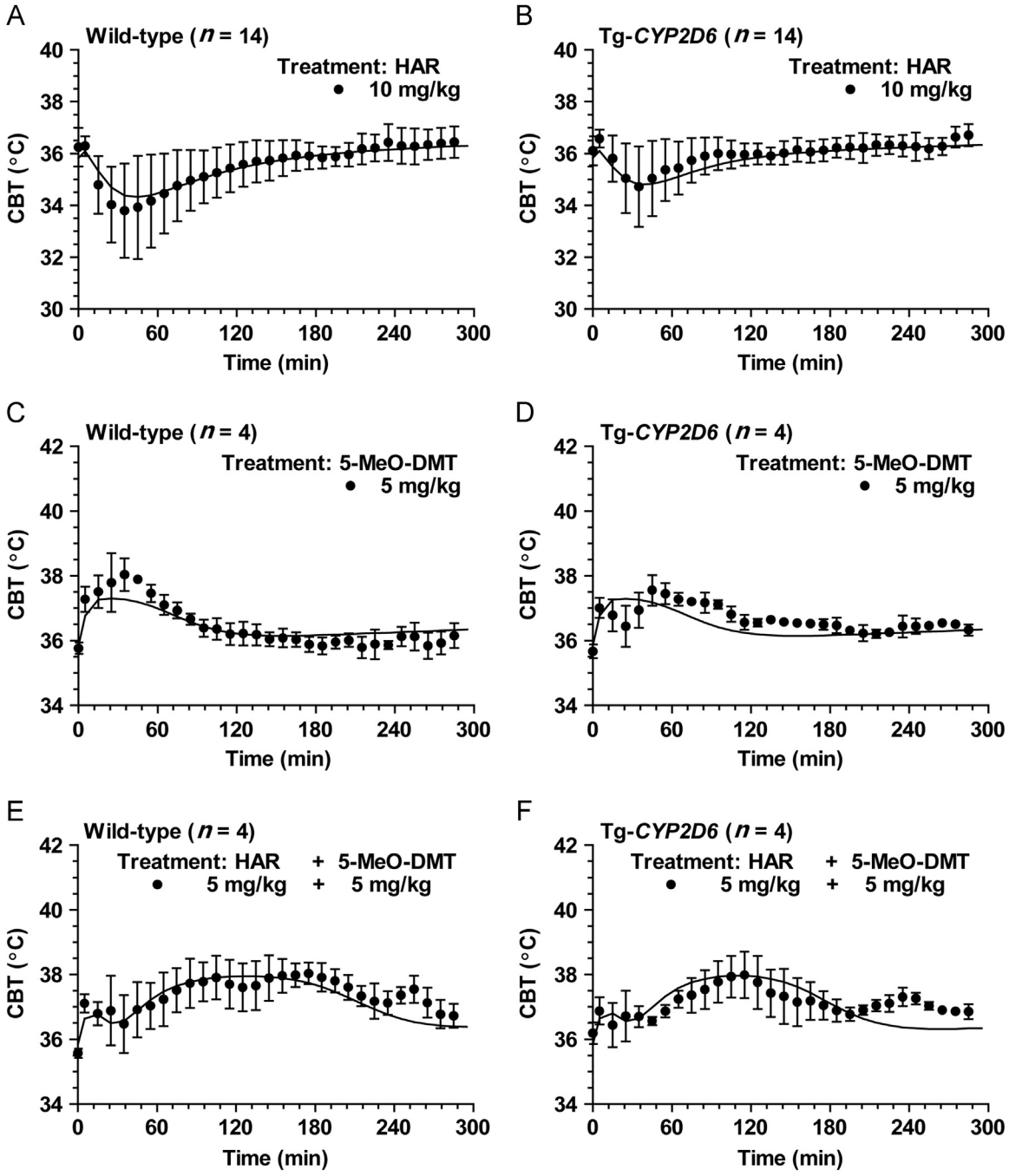

Figure 5 Comparison of experimental and model predicted CBT profiles in wild-type and Tg-*CYP2D6* mice treated with 10 mg/kg harmaline alone (A and B; $n=14$ in each group), 5 mg/kg 5-MeO-DMT alone (C and D; $n=4$ in each group), and 5 mg/kg harmaline plus 5 mg/kg 5-MeO-DMT (E and F; $n=4$ in each group). For single dose study, drug was dosed i.p. at 0 min. For DDI study, harmaline and 5-MeO-DMT were dosed i.p. at 0 and 15 min, respectively. The solid (—) lines represent the predicted data that were obtained from the developed PK/PD model shown in Fig. 1.

An adaptive negative feedback mechanism[33], which was described as the ratio of baseline ($Temp_{basal}$) to response (Temp) that regulates thermogenesis (k_{in}), was incorporated into this IDR model to capture the influence of serotonergic drugs on the homeostasis of CBT. Because wild-type and Tg-*CYP2D6* mice share the same genetic background[51] and the only difference is the expression of human drug-metabolizing enzyme CYP2D6 in the Tg-*CYP2D6* mice, the variation in PD response is simply attributable to CYP2D6-caused differences in harmaline-5-MeO-DMT PK that drives the IDR. Therefore, the same set of PD parameters was used for wild-type and Tg-*CYP2D6* mice. Indeed, the present PK/PD model sufficiently described the thermoregulatory effects of harmaline and 5-MeO-DMT in both genotypes of mice. Single or multiple injections of control vehicle initiated similar transient hyperthermic effects in mice, and there were no significant differences in the change of CBT profiles (Fig. 2) and area under effect curve values (about 7% difference). Therefore, the same post-injection hyperthermic component (S_0 and k_{el-s}) was

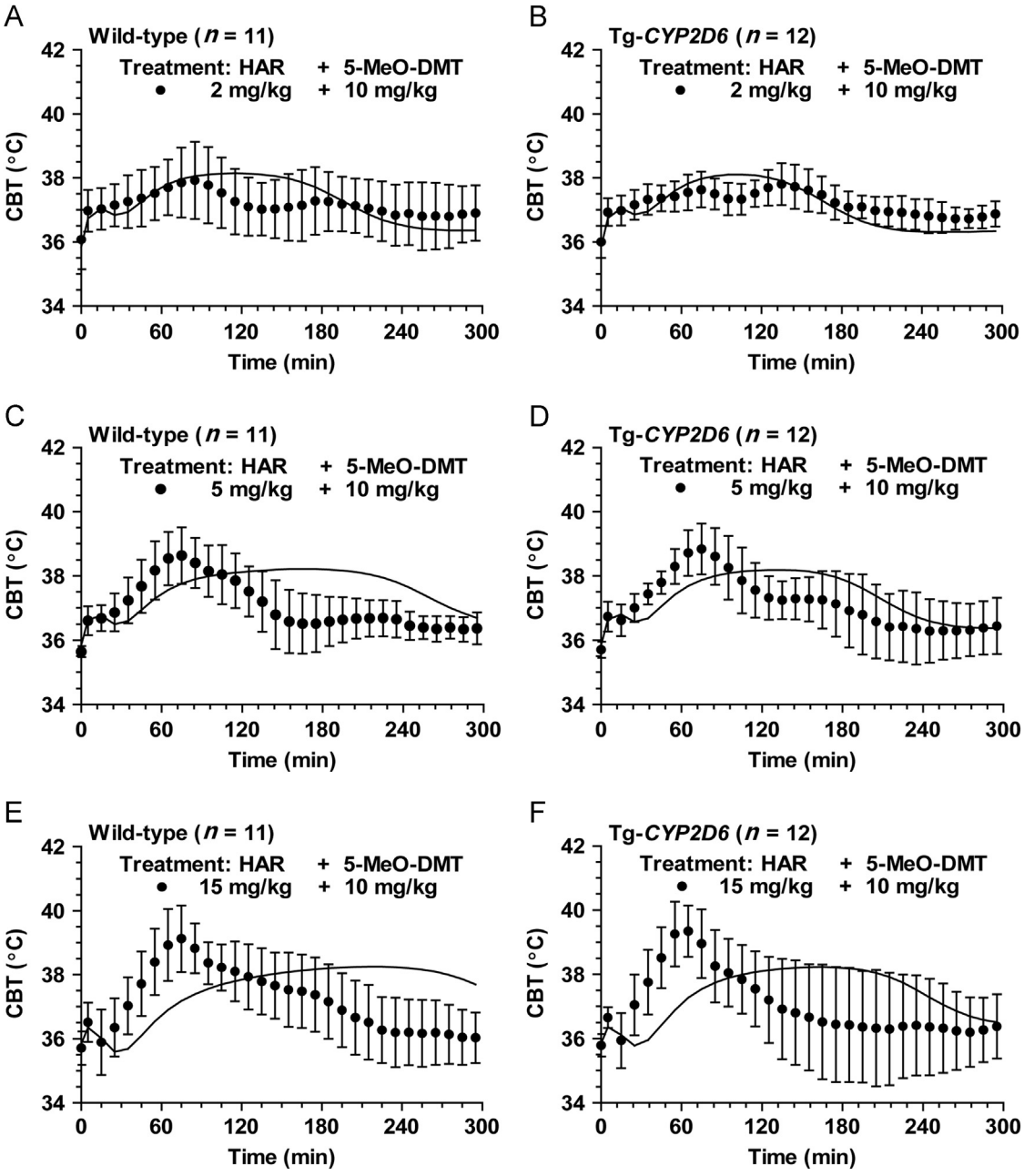

Figure 6 Comparison of experimental and model simulated CBT profiles in wild-type ($n=11$) and Tg-*CYP2D6* ($n=12$) mice treated with 2 (A and B), 5 (C and D) or 15 mg/kg (E and F) harmaline plus 10 mg/kg 5-MeO-DMT. Harmaline and 5-MeO-DMT were dosed i.p. at 0 and 15 min, respectively. The solid (—) lines represent the predicted data that were obtained from the developed PK/PD model shown in Fig. 1.

applied to describe such stress-induced hyperthermia (Fig. 2). Nevertheless, the handling/injection in the DDI studies (vehicle or 5-MeO-DMT administered 15 min after harmaline) significantly affected harmaline-induced hypothermia (Figs. 3A and B, 4A and B) by reducing the k_{S-H} more than 50% (0.0347–0.0136 L/μmol, Table 1) in mice.

Given the observation that there was a delay in the appearance of hyperthermia through the activation of 5-HT_{2A} receptor, in contrast to an immediate onset of hyperthermia induced by handling/injection, a separate component was utilized to characterize 5-HT_{2A} agonist–stimulated thermogenesis (k_{in}) (Fig. 1). The delay was described by three transit compartments that may be associated with the signal transduction processes from central 5-HT_{2A} receptor activation to peripheral systems, heat generation in iBAT and skeletal muscle as well as the thermoregulatory response[5,7]. The mean transit time (τ) in each compartment was estimated to be around 6.2 min. The activation of 5-HT_{2A} receptor was shown to stimulate thermogenesis with an $SC_{50\text{–M–non-DDI}}$ value of 1.88 μmol/L, which is close to the binding affinity (0.9–1.5 μmol/L) of 5-MeO-DMT to human 5-HT_{2A} receptor reported previously[49,52].

Current PK/PD model adequately captured and predicted the CBT profiles including biphasic effects in all mice treated with nontoxic doses of 5-MeO-DMT in the absence and presence of

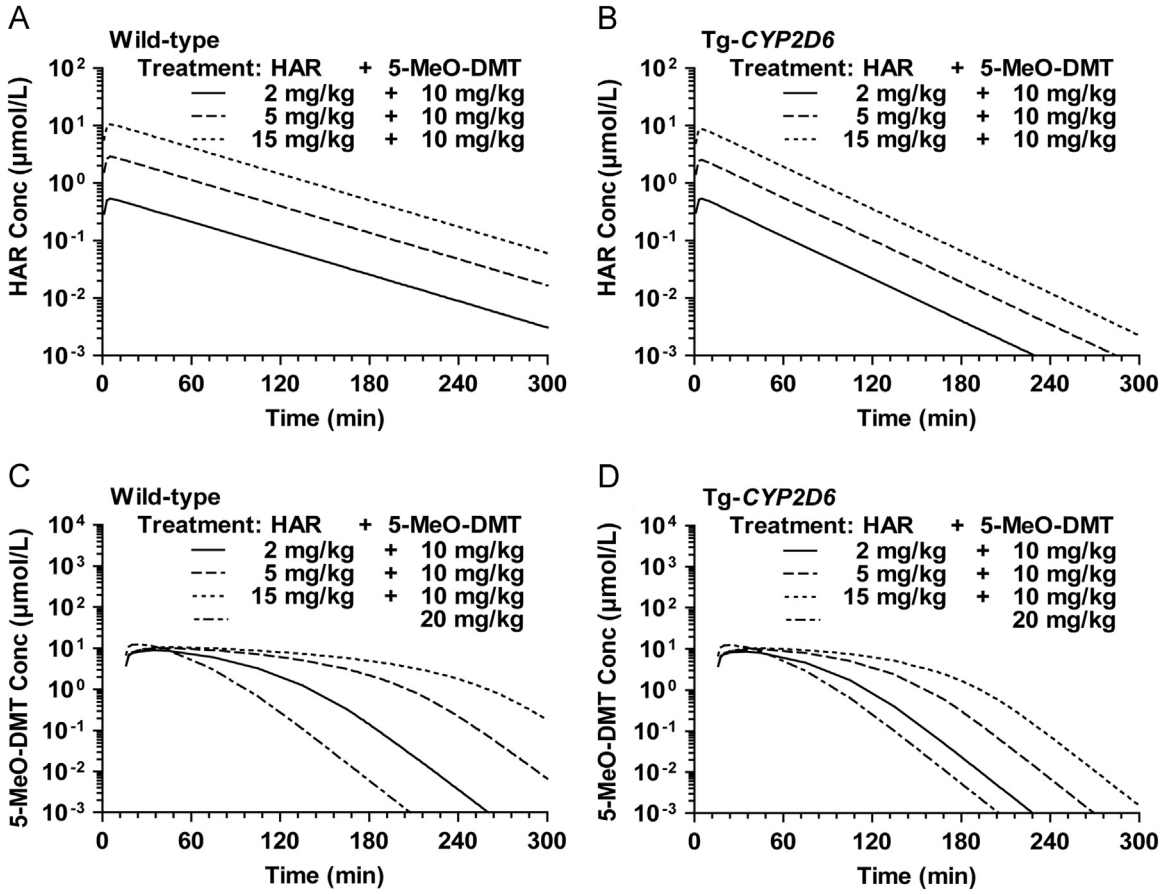

Figure 7 Model predicted serum harmaline (A and B) and 5-MeO-DMT (C and D) concentration *versus* time profiles following the administration of 2, 5 or 15 mg/kg harmaline plus 10 mg/kg 5-MeO-DMT, or 20 mg/kg 5-MeO-DMT alone in wild-type and Tg-*CYP2D6* mice. Harmaline and 5-MeO-DMT were dosed i.p. at 0 and 15 min, respectively. The solid (—), dashed (- -) and dotted (· ·) lines represent the simulated data of harmaline and 5-MeO-DMT in mice treated with ascending doses of harmaline (2–15 mg/kg) plus 10 mg/kg 5-MeO-DMT, while the dashed plus dotted (- · -) lines represent 5-MeO-DMT profiles in mice treated with 20 mg/kg 5-MeO-DMT alone. The PK profiles were obtained using our previously established PK DDI model[27] that is also shown in Fig. 1.

harmaline (Figs. 4 and 5), supporting the utility of mathematical modeling in quantitative neuropharmacology. The PK/PD modeling also revealed that 5-MeO-DMT–induced hyperthermia enhanced by MAOI harmaline was partly driven by the increased exposure to 5-MeO-DMT (Fig. 7). The potentiation of hyperthermia was indicated by the lower $SC_{50\text{–M-DDI}}$ value (0.496 μmol/L) estimated by the current PK/PD model, which was almost a 4-fold enhancement of hyperthermic potency than 5-MeO-DMT alone ($SC_{50\text{–m-non-DDI}}$, 1.88 μmol/L). The interaction at PD level may be associated with the elevation of 5-HT by MAOI harmaline[50]. Additionally, several studies have shown that harmaline may directly bind to multiple neurotransmitter receptors including 5-HT, norepinephrine (NE), dopamine D_2/D_3 and *N*-methyl-D-aspartate (NMDA) glutamate receptors and/or modulate cerebellar glutamatergic transmission[53–57].

The validated PK/PD model was further employed to evaluate the hyperpyrexia caused by toxic doses of harmaline and 5-MeO-DMT (Fig. 6). Comparison of simulated and experimental CBT profiles revealed that the present PK/PD model reasonably predicted the thermoregulatory effects of 2 mg/kg harmaline plus 10 mg/kg 5-MeO-DMT (Fig. 6A and B). However, this PK/PD model underestimated the early-stage sharp increase of CBT and overestimated the late-stage decrease of CBT in mice treated with toxic dose combinations of harmaline (5 or 15 mg/kg) and 5-MeO-DMT (10 mg/kg) (Fig. 6C–F), suggesting that severe serotonin toxicity might significantly disrupt physiological thermoregulation. Interestingly, the early-time (0–60 min) systemic exposure to 10 mg/kg 5-MeO-DMT only increased by 20% when harmaline dose was increased from 2 to 15 mg/kg, according to the observed[27] and simulated PK profiles (Fig. 7C and D). Given the estimated EC_{50} values for 5-MeO-DMT (1.88 and 0.496 μmol/L under 5-MeO-DMT alone or DDI condition, respectively) in inducing hyperthermia and a much higher serum drug concentration (> 8 μmol/L, Fig. 6), a full stimulation of the 5-HT_{2A} receptor would be expected. Therefore, the potentiation of 5-MeO-DMT–induced hyperthermia was presumably related to harmaline actions. Indeed, systemic exposure to harmaline was elevated almost 20-fold when the doses of harmaline were increased from 2 to 15 mg/kg (Fig. 7A and B). Under such toxic drug combination conditions, the highly elevated levels of 5-HT and other monoamine neurotransmitters following harmaline-dictated inhibition of MAO, as well as the excessive stimulation of multiple neurotransmitter receptors by harmaline itself[50,53–56], would be the major causes of serotonin toxicity including the hyperpyrexia underestimated by current model.

In summary, an integrated PK/PD model was developed in this study and it was able to quantitatively characterize and predict the

impact of serotonergic drugs on thermoregulation in mice. This model consisted of mechanistic activation of 5-HT receptors in the control of thermogenesis and heat loss. The current model also described the complex biphasic effects including the potentiation of 5-MeO-DMT–induced late-phase hyperthermia by co-administered MAOI harmaline after considering their interactions at both PD and PK levels, *i.e.*, acting on the same serotonergic system and enhancing systemic exposure to 5-MeO-DMT and 5-HT. In addition, our findings indicated that the hyperpyrexia caused by high dose combination of harmaline and 5-MeO-DMT was mainly attributed by the exposure to harmaline. This mechanism-based PK/PD model may serve as a new framework for the investigation of thermoregulatory or other neuropharmacological effects of serotonergic agents.

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References

1. Johnson JM, Minson CT, Kellogg Jr. DL. Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Compr Physiol* 2014;**4**:33–89.
2. Nakamura K, Morrison SF. Central efferent pathways for cold-defensive and febrile shivering. *J Physiol* 2011;**589**:3641–58.
3. Contreras C, Gonzalez F, Fernø J, Diéguez C, Rahmouni K, Nogueiras R, et al. The brain and brown fat. *Ann Med* 2015;**47**:150–68.
4. Terrien J, Perret M, Aujard F. Behavioral thermoregulation in mammals: a review. *Front Biosci* 2011;**16**:1428–44.
5. Rusyniak DE, Sprague JE. Hyperthermic syndromes induced by toxins. *Clin Lab Med* 2006;**26**:165–84.
6. Sällström B, Visser SAG, Forsberg T, Peletier LA, Ericson AC, Gabrielsson J. A pharmacodynamic turnover model capturing asymmetric circadian baselines of body temperature, heart rate and blood pressure in rats: challenges in terms of tolerance and animal-handling effects. *J Pharmacokinet Pharmacodyn* 2005;**32**:835–59.
7. Ootsuka Y, Blessing WW. Thermogenesis in brown adipose tissue: increase by 5-HT_{2A} receptor activation and decrease by 5-HT_{1A} receptor activation in conscious rats. *Neurosci Lett* 2006;**395**:170–4.
8. Rusyniak DE, Zaretskaia MV, Zaretsky DV, DiMicco JA. 3,4-Methylenedioxymethamphetamine- and 8-hydroxy-2-di-*n*-propylamino-tetralin-induced hypothermia: role and location of 5-hydroxytryptamine 1A receptors. *J Pharmacol Exp Ther* 2007;**323**:477–87.
9. Hedlund PB, Kelly L, Mazur C, Lovenberg T, Sutcliffe JG, Bonaventure P. 8-OH-DPAT acts on both 5-HT_{1A} and 5-HT_7 receptors to induce hypothermia in rodents. *Eur J Pharmacol* 2004;**487**:125–32.
10. Ferrés-Coy A, Santana N, Castañé A, Cortés R, Carmona MC, Toth M, et al. Acute 5-HT_{1A} autoreceptor knockdown increases antidepressant responses and serotonin release in stressful conditions. *Psychopharmacology* 2013;**225**:61–74.
11. Mazzola-Pomietto P, Aulakh CS, Wozniak KM, Hill JL, Murphy DL. Evidence that 1-(2,5-dimethoxy-4-iodophenyl)−2-aminopropane (DOI)-induced hyperthermia in rats is mediated by stimulation of 5-HT_{2A} receptors. *Psychopharmacology* 1995;**117**:193–9.
12. Herin DV, Liu SJ, Ullrich T, Rice KC, Cunningham KA. Role of the serotonin 5-HT_{2A} receptor in the hyperlocomotive and hyperthermic effects of (+)−3,4-methylenedioxymethamphetamine. *Psychopharmacology* 2005;**178**:505–13.
13. Löscher W, Witte U, Fredow G, Ganter M, Bickhardt K. Pharmacodynamic effects of serotonin (5-HT) receptor ligands in pigs: stimulation of 5-HT_2 receptors induces malignant hyperthermia. *Naunyn Schmiedebergs Arch Pharmacol* 1990;**341**:483–93.
14. Beig MI, Baumert M, Walker FR, Day TA, Nalivaiko E. Blockade of 5-HT_{2A} receptors suppresses hyperthermic but not cardiovascular responses to psychosocial stress in rats. *Neuroscience* 2009;**159**:1185–91.
15. Salmi P, Ahlenius S. Evidence for functional interactions between 5-HT_{1A} and 5-HT_{2A} receptors in rat thermoregulatory mechanisms. *Pharmacol Toxicol* 1998;**82**:122–7.
16. Gillman PK. Triptans, serotonin agonists, and serotonin syndrome (serotonin toxicity): a review. *Headache* 2010;**50**:264–72.
17. Kant S, Liebelt E. Recognizing serotonin toxicity in the pediatric emergency department. *Pediatr Emerg Care* 2012;**28**:817–21.
18. Jiang CH, Xu QL, Wen XA, Sun HB. Current developments in pharmacological therapeutics for chronic constipation. *Acta Pharm Sin B* 2015;**5**:300–9.
19. Yu AM. Indolealkylamines: biotransformations and potential drug–drug interactions. *AAPS J* 2008;**10**:242–53.
20. Dennehy CE, Tsourounis C, Miller AE. Evaluation of herbal dietary supplements marketed on the internet for recreational use. *Ann Pharmacother* 2005;**39**:1634–9.
21. Ott J. Pharmepéna-Psychonautics: human intranasal, sublingual and oral pharmacology of 5-methoxy-*N*,*N*-dimethyl-tryptamine. *J Psychoact Drugs* 2001;**33**:403–7.
22. Bruno R, Matthews AJ, Dunn M, Alati R, Mcllwraith F, Hickey S, et al. Emerging psychoactive substance use among regular ecstasy users in Australia. *Drug Alcohol Depend* 2012;**124**:19–25.
23. Boyer EW, Shannon M. The serotonin syndrome. *N Engl J Med* 2005;**352**:1112–20.
24. Brush DE, Bird SB, Boyer EW. Monoamine oxidase inhibitor poisoning resulting from Internet misinformation on illicit substances. *J Toxicol Clin Toxicol* 2004;**42**:191–5.
25. Sklerov J, Levine B, Moore KA, King T, Fowler D. A fatal intoxication following the ingestion of 5-methoxy-*N*,*N*-dimethyltryptamine in an ayahuasca preparation. *J Anal Toxicol* 2005;**29**:838–41.
26. Yuruktumen A, Karaduman S, Bengi F, Fowler J. Syrian rue tea: a recipe for disaster. *Clin Toxicol* 2008;**46**:749–52.
27. Jiang XL, Shen HW, Mager DE, Yu AM. Pharmacokinetic interactions between monoamine oxidase A inhibitor harmaline and 5-methoxy-*N*,*N*-dimethyltryptamine, and the impact of CYP2D6 status. *Drug Metab Dispos* 2013;**41**:975–86.
28. Shen HW, Jiang XL, Yu AM. Nonlinear pharmacokinetics of 5-methoxy-*N*,*N*-dimethyltryptamine in mice. *Drug Metab Dispos* 2011;**39**:1227–34.
29. Shen HW, Wu C, Jiang XL, Yu AM. Effects of monoamine oxidase inhibitor and cytochrome P450 2D6 status on 5-methoxy-*N*,*N*-dimethyltryptamine metabolism and pharmacokinetics. *Biochem Pharmacol* 2010;**80**:122–8.
30. Wu C, Jiang XL, Shen HW, Yu AM. Effects of CYP2D6 status on harmaline metabolism, pharmacokinetics and pharmacodynamics, and a pharmacogenetics-based pharmacokinetic model. *Biochem Pharmacol* 2009;**78**:617–24.
31. Jiang XL, Shen HW, Yu AM. Potentiation of 5-methoxy-*N*,*N*-dimethyltryptamine-induced hyperthermia by harmaline and the involvement of activation of 5-HT_{1A} and 5-HT_{2A} receptors. *Neuropharmacology* 2015;**89**:342–51.
32. Kim H, Sablin SO, Ramsay RR. Inhibition of monoamine oxidase A by β-carboline derivatives. *Arch Biochem Biophys* 1997;**337**:137–42.
33. Karlsson MO, Anehall T, Friberg LE, Henningsson A, Kloft C, Sandström M, et al. Pharmacokinetic/pharmacodynamic modelling in oncological drug development. *Basic Clin Pharmacol Toxicol* 2005;**96**:206–11.
34. Olivier B, Zethof T, Pattij T, van Boogaert M, van Oorschot R, Leahy C, et al. Stress-induced hyperthermia and anxiety: pharmacological validation. *Eur J Pharmacol* 2003;**463**:117–32.
35. Goldstein DS. Stress-induced activation of the sympathetic nervous system. *Baillières Clin Endocrinol Metab* 1987;**1**:253–78.

36. Villalón CM, Centurión D. Cardiovascular responses produced by 5-hydroxytriptamine: a pharmacological update on the receptors/mechanisms involved and therapeutic implications. *Naunyn Schmiedebergs Arch Pharmacol* 2007;**376**:45–63.
37. Mager DE, Wyska E, Jusko WJ. Diversity of mechanism-based pharmacodynamic models. *Drug Metab Dispos* 2003;**31**:510–8.
38. Wang XN, Schumitzky A, D'Argenio DZ. Nonlinear random effects mixture models: maximum likelihood estimation *via* the EM algorithm. *Comput Stat Data Anal* 2007;**51**:6614–23.
39. Jiang XL, Shen HW, Yu AM. Modification of 5-methoxy-*N*,*N*-dimethyltryptamine–induced hyperactivity by monoamine oxidase A inhibitor harmaline in mice and the underlying serotonergic mechanisms. *Pharmacol Rep* 2016;**68**:608–15.
40. Halberstadt AL, Buell MR, Masten VL, Risbrough VB, Geyer MA. Modification of the effects of 5-methoxy-*N*,*N*-dimethyltryptamine on exploratory behavior in rats by monoamine oxidase inhibitors. *Psychopharmacology* 2008;**201**:55–66.
41. Winter JC, Amorosi DJ, Rice KC, Cheng K, Yu AM. Stimulus control by 5-methoxy-*N*,*N*-dimethyltryptamine in wild-type and *CYP2D6*-humanized mice. *Pharmacol Biochem Behav* 2011;**99**:311–5.
42. Halberstadt AL, Nichols DE, Geyer MA. Behavioral effects of α,α,β,β-tetradeutero-5-MeO-DMT in rats: comparison with 5-MeO-DMT administered in combination with a monoamine oxidase inhibitor. *Psychopharmacology* 2012;**221**:709–18.
43. Halberstadt AL. Behavioral and pharmacokinetic interactions between monoamine oxidase inhibitors and the hallucinogen 5-methoxy-*N*,*N*-dimethyltryptamine. *Pharmacol Biochem Behav* 2016;**143** 1-0.
44. Zuideveld KP, Maas HJ, Treijtel N, Hulshof J, van der Graaf PH, Peletier LA, et al. A set-point model with oscillatory behavior predicts the time course of 8-OH-DPAT-induced hypothermia. *Am J Physiol Regul Integr Comp Physiol* 2001;**281**:R2059–71.
45. Zuideveld KP, Van der Graaf PH, Newgreen D, Thurlow R, Petty N, Jordan P, et al. Mechanism-based pharmacokinetic-pharmacodynamic modeling of 5-HT_{1A} receptor agonists: estimation of *in vivo* affinity and intrinsic efficacy on body temperature in rats. *J Pharmacol Exp Ther* 2004;**308**:1012–20.
46. Overgaard RV, Holford N, Rytved KA, Madsen H. PKPD model of interleukin-21 effects on thermoregulation in monkeys-application and evaluation of stochastic differential equations. *Pharm Res* 2007;**24**:298–309.
47. Halberstadt AL, Geyer MA. Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology* 2011;**61**:364–81.
48. Halberstadt AL, Koedood L, Powell SB, Geyer MA. Differential contributions of serotonin receptors to the behavioral effects of indoleamine hallucinogens in mice. *J Psychopharmacol* 2011;**25**:1548–61.
49. Spencer Jr DG, Glaser T, Traber J. Serotonin receptor subtype mediation of the interoceptive discriminative stimuli induced by 5-methoxy-*N*,*N*-dimethyltryptamine. *Psychopharmacology* 1987;**93**:158–66.
50. Cheng J, Zhen YY, Miksys S, Beyoğlu D, Krausz KW, Tyndale RF, et al. Potential role of CYP2D6 in the central nervous system. *Xenobiotica* 2013;**43**:973–84.
51. Corchero J, Granvil CP, Akiyama TE, Hayhurst GP, Pimprale S, Feigenbaum L, et al. The *CYP2D6* humanized mouse: effect of the human *CYP2D6* transgene and *HNF4α* on the disposition of debrisoquine in the mouse. *Mol Pharmacol* 2001;**60**:1260–7.
52. Roth BL, Choudhary MS, Khan N, Uluer AZ. High-affinity agonist binding is not sufficient for agonist efficacy at 5-hydroxytryptamine$_{2A}$ receptors: evidence in favor of a modified ternary complex model. *J Pharmacol Exp Ther* 1997;**280**:576–83.
53. Abdel-Fattah AFM, Matsumoto K, Murakami Y, Abdel-Khalek Gammaz H, Mohamed MF, Watanabe H. Central serotonin level-dependent changes in body temperature following administration of tryptophan to pargyline- and harmaline-pretreated rats. *Gen Pharmacol* 1997;**28**:405–9.
54. Arshaduddin M, Kadasah S, Al Deeb S, Al Moutaery K, Tariq M. Exacerbation of harmaline-induced tremor by imipramine. *Pharmacol Biochem Behav* 2005;**81**:9–14.
55. Paterson NE, Malekiani SA, Foreman MM, Olivier B, Hanania T. Pharmacological characterization of harmaline-induced tremor activity in mice. *Eur J Pharmacol* 2009;**616**:73–80.
56. Grella B, Teitler M, Smith C, Herrick-Davis K, Glennon RA. Binding of β-carbolines at 5-HT_2 serotonin receptors. *Bioorg Med Chem Lett* 2003;**13**:4421–5.
57. Ossowska K, Wardas J, Berghauzen-Maciejewska K, Glowacka U, Kuter K, Pilc A, et al. Lu AF21934, a positive allosteric modulator of mGlu4 receptors, reduces the harmaline-induced hyperactivity but not tremor in rats. *Neuropharmacology* 2014;**83**:28–35.