Differential effects of 5-HT₄ receptor agonists at gastric versus cardiac receptors: an operational framework to explain and quantify organ specific behaviour


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A framework for tissue-dependent 5-HT₄ receptor agonism

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number of text pages: 35
number of tables: 3
number of figures: 5
number of references: 39
number of words in the Abstract: 224

Introduction: 597

Discussion: 1625

Abbreviations: C-R, concentration-response; GI, gastrointestinal; IBMX, 3-isobutyl-1-methylxanthine; IBS, irritable bowel syndrome; L-NAME, N⁵-nitro-L-arginine-methylester; ML, maximum likelihood; OMOA, operational model of agonism; PDE, phosphodiesterase; REML, restricted maximum likelihood; TTX, tetrodotoxin

Recommended section: Gastrointestinal, Hepatic, Pulmonary, and Renal
Abstract

Quantification of different levels of 5-HT$_4$ receptor agonism expression across animal species as well as across organs within the same animal species, offers substantial potential for the separation of desired gastrointestinal versus undesired cardiac pharmacological activity of compounds in development. Since a detailed investigation of such properties is lacking to date, we set out to quantify gastric and cardiac effects of 5-HT$_4$ receptor ligands in the pig, a model considered to be representative for the human situation. An in vitro test was developed to study the potentiating effect of 5-HT, prucalopride, tegaserod, R149402 and R199715 on electrically induced cholinergic contractions in longitudinal muscle strips of the proximal stomach. The results were compared to inotropic and chronotropic effects of these compounds in the electrically paced left atrium and spontaneously beating right atrium respectively. To quantify the observed tissue-dependent responses, a non-linear mixed effects model based on the operational model of agonism (OMOA) was developed and successfully fitted to the data. The model quantified the tissue-dependent partial agonism of the selective 5-HT$_4$ receptor agonists prucalopride, R149402 and R199715, while tegaserod and 5-HT were equi-efficacious. The model was further extended to incorporate the responses to prucalopride in the presence of the 5-HT$_4$ receptor antagonist GR113808. The results indicate that these interactions do not follow a simple competitive pattern, and that they differ between stomach and left atrium.


**Introduction**

Gastrointestinal (GI) prokinetic drugs activating 5-HT \(_4\) receptors are commonly used to facilitate GI transit. They have proven their value in the treatment of gastro-oesophageal reflux disease, and are still used to treat symptoms in patients suffering from the constipation predominant form of irritable bowel syndrome (IBS) or from gastroparesis; both conditions can cause considerable impairment of quality of life (Bouras et al., 1999; Galligan and Vanner, 2005). However, the presence of functional 5-HT\(_4\) receptors in the human heart might be a concern when using these drugs to treat functional GI disorders (Tonini et al., 1999).

5-HT\(_4\) receptors are seven transmembrane domain receptors, positively linked to adenylyl cyclase, resulting in the production of cAMP, which is the first step in a cell type-specific cascade of events leading to effect (Martin and Humphrey, 1994). In the human GI tract, different effects have been related to 5-HT\(_4\) receptor activation. Relaxant 5-HT\(_4\) receptors are present on human colonic circular smooth muscle cells (McLean and Coupar, 1996; Prins et al., 2000b). Mucosal 5-HT\(_4\) receptors in human small intestine stimulate secretory processes (Borman and Burleigh, 1993). Moreover, 5-HT\(_4\) receptors have been shown to be involved in the initiation of the peristaltic reflex in human jejunum (Foxx-Orenstein et al., 1996). In addition, in the human stomach and colon, activation of 5-HT\(_4\) receptors located on myenteric excitatory cholinergic neurones can lead to an increased release of acetylcholine from these neurones (Prins et al., 2000a; Leclere and Lefebvre, 2002; Leclere et al., 2005). Therefore 5-HT\(_4\) receptors are a compelling target for promotility agents, since the facilitation of fast synaptic transmission in myenteric ganglia will increase motor reflexes, while the facilitation of excitatory input to the muscles will enhance motility directly (Galligan and Vanner, 2005).

The cardiac location of 5-HT\(_4\) receptors acquired a lot of attention because of the cardiac side effects observed with cisapride. Although the observed cardiotoxicity of cisapride is not 5-HT\(_4\) receptor mediated (Mohammad et al., 1997), 5-HT\(_4\) receptor agonists do have inotropic and chronotropic effects in human atria and inotropic effects in human
ventricles (Kaumann et al., 1991; Brattelid et al., 2004). In spite of the clear tissue dependent effects of benzamidic 5-HT$_4$ receptor agonists, a detailed comparison of GI versus cardiac effects of 5-HT$_4$ receptor agonists within the same species has not yet been performed. Because the interesting potential of 5-HT$_4$ receptor agonists in the treatment of GI disorders cannot be seen separately from the possible cardiac effects of these prokinetics, we thought it timely to study cardiac and GI effects of 5-HT$_4$ receptor agonists in an integrated approach.

The pig has been demonstrated to be a relevant species to study human atrial 5-HT$_4$ receptor interactions (Kaumann, 1990). It is also a good species for studying human digestive function given the similarities between the morphology and physiology of their GI tracts (Miller and Ullrey, 1987) and it has been shown that porcine proximal stomach can be used to investigate presynaptic modulation of myenteric acetylcholine release (Leclere and Lefebvre, 2001).

We now developed a functional assay in porcine proximal stomach to study gastric neuronal 5-HT$_4$ receptors. By means of a “coupled” in vitro assay we compared the cardiac versus gastric behaviour of the 5-HT$_4$ receptor agonists prucalopride, tegaserod, R149402 and R199715, and 5-HT. Furthermore, we implemented the framework provided by the operational model of agonism (OMOA) to analyse and classify the agonists, using a mixed effects modelling approach. This model allows for simultaneous agonist- as well as antagonist-related parameter estimation, and provides a tool to predict the expression of partial agonism of new ligands in the heart, and hence their potential side effects.
Methods

Tissue preparation

Female pigs (10-11 weeks, 22-27 kg), obtained from local farms, were anaesthetized with an intravenous (50 mg/kg) sodium pentobarbital (Kela N.V., Hoogstraten, Belgium) injection. After exsanguination, the heart and the entire stomach were dissected and placed in Krebs-Henseleit solution (composition in mM: glucose 11.1, CaCl₂ 2.51, NaHCO₃ 25, MgSO₄ 1.18, KH₂PO₄ 1.18, KCl 4.69, EDTA 0.033 and NaCl 118). Right and left atrium preparations were obtained as described before (De Maeyer et al., 2006). Briefly, for chronotropic effect studies, the right atrium was removed and mounted in toto. Left atrial pectinate muscles (9-12 per left atrium) with a thickness < 1mm and a length varying between 3 and 7 mm were dissected away from the endothelial surface and mounted between platinum wire electrodes, aligned perpendicularly to the tissue.

The stomach was opened along the lesser curvature and the contents were rinsed out. After removal of the mucosa, the tissue was placed at 37°C and muscle strips of approximately 1.5 cm in length were prepared from the ventral side of the proximal stomach in the direction of the longitudinal muscle layer. Most of the circular muscle layer was removed, taking care not to damage the myenteric plexus. The longitudinal muscle strips were vertically attached onto tissue holders, equipped with two coaxially aligned platinum wire electrodes. The tissue was kept at 37°C and continuously gassed with 95% O₂ and 5% CO₂ in an organ bath set-up containing 20 ml of Krebs-Henseleit solution.

Changes in isometric force of the tissues were recorded via Statham UC2 force transducers (Gould, Cleveland, USA) and DBA 18 digital bridge amplifiers (Anerma, Belgium) on a Powerlab data acquisition system and recorded using Chart v5.1.1 software. Electrical field stimulation (EFS) was performed with a constant voltage stimulator (Janssen Pharmaceutica, Belgium). All tissues were used on the day of preparation. The study was approved by the
ethical committee from Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V., Beerse, Belgium.

**Experimental protocols**

**Left atrial pectinate muscles:**

The protocol used for the pectinate muscles was essentially as described before (De Maeyer et al., 2006). Briefly, pectinate muscles were electrically stimulated (0.5 Hz, 5 ms, just above threshold voltage, resting length being half of $L_{\text{max}}$, the length at which maximal active tension was developed upon EFS). For this study, a cumulative concentration-response (C-R) curve to the agonists with semi-log unit concentration increments was established in the paced pectinate muscles, in the presence of propranolol (0.2 µM), to avoid indirect β-adrenoreceptor interactions due to the release of noradrenaline, cocaine (6 µM), to reduce tissue capture of 5-HT, and 3-isobutyl-1-methylxanthine (IBMX; 20 µM), to block the action of phosphodiesterase enzymes (PDEs). Experiments were terminated by the administration of a saturating concentration of isoprenaline (0.1 mM).

**Right atrium:**

Spontaneously beating right atria were studied as described before (De Maeyer et al., 2006). Briefly, a cumulative C-R curve was established for all the agonists under study (one curve per preparation) in the presence of 0.2 µM propranolol and 6 µM cocaine. In the end, isoprenaline (0.1 mM) was administered to the solution.

**Gastric longitudinal muscle strips:**

Since preliminary experiments showed an inhibitory effect of endogenous nitric oxide (NO) on EFS-induced contractions, experiments with gastric muscle strips were carried out in the presence of 0.1 mM of the NO synthase inhibitor N$^G$-nitro-L-arginine-methylester (L-NAME). Indomethacine (1 µM) was added to avoid spontaneous contractions due to the synthesis of prostaglandins. The tissues were allowed to equilibrate for 90 min with rinsing every 15 min under a resting tension of 20 mN. Following this stabilisation period, the strips...
were contracted with carbachol (3 µM) to ensure their viability and responsiveness. After wash out, this step was repeated at 30 min intervals until two similar successive responses to carbachol were obtained (usually three times). Resting tone was reset at 20 mN between each step. After the last washing step, organ baths with muscle strips that were to receive 5-HT as agonist were supplied with methysergide (1 µM), to block interactions of 5-HT at 5-HT₁, 5-HT₂, 5-HT₅, 5-HT₆ and 5-HT₇ receptors, and granisetron (0.3 µM) to exclude 5-HT₃ receptor activation. Cocaine (6 µM) was also added to these organ baths since preliminary experiments had shown that the presence of cocaine produced a leftward shift (one half log unit; P < 0.01) of the C-R curve to 5-HT (cumulative as well as non-cumulative). Thirty min after the last washing step, the gastric muscle strips were electrically stimulated (every 3 min a 10 s pulse train at 4Hz, 0.5 ms and 20V, corresponding current 0.4A-1A). Preliminary experiments showed that under these conditions a stimulation voltage of 20V resulted in maximal contractions. Once successive electrically evoked contractions became reproducible, the applied voltage was adjusted to reduce the contraction force to 50% of the force developed at 20V (corresponding current 0.11A-0.5A), after which the electrical stimulation was set on hold for 20 min. EFS was then reinstated at this adjusted voltage and after at least 15 min the agonist was added in a single concentration (7 increasing concentrations in 7 parallel strips) or in a semi-log unit increment cumulative manner (1 strip) from 0.01-1 nM onwards, depending on the agonist; when the agonist was added cumulatively, the time interval between successive concentration increments was agonist dependent, ranging from 10 to 15 min (corresponding to 3 to 5 EFS trains) for 5-HT to 30 min (10 EFS trains) for tegaserod. From 3 µM prucalopride onwards, a supramaximal sharp increase in electrically-induced gastric contraction occurred, probably mediated through inhibition of cholinesterase enzymes (Johnson & Johnson, unpublished results). The involvement of this non-5-HT₄ receptor related interaction in the generation of the response at lower concentrations of prucalopride is unlikely, considering the binding profile of prucalopride. Therefore, the effects of prucalopride up to this concentration were used for monophasic fitting procedures. The strips
that received one concentration of agonist were followed for 70 min (5-HT, prucalopride and R149402) or 120 min (tegaserod and R199715).

Assessment of the antagonising effect of GR113808 versus prucalopride and 5-HT:

Both in pectinate muscles as well as in gastric muscle strips, the effect of the 5-HT₄ receptor antagonist GR 113808 on the cumulative C-R curve of prucalopride was evaluated. In left atrial pectinate muscle preparations, GR 113808 (1, 3, 10, 30, 100 or 300 nM) was administered to parallel tissues 30 min before constructing a cumulative C-R curve. In the gastric preparations, GR 113808 (1, 3, 10, 30, 300, 1000 nM) was added 60 min before starting the cumulative administration of prucalopride. The effect of GR113808 (stomach, 1 and 10 µM; left atrium, 0.3 µM) was also evaluated on the responses evoked by cumulatively administered 5-HT in the stomach and the left atrium.

Data analysis:

The average contraction to 3 EFS trains (stomach) or during 2 min (left atrium), or the average beating rate during 2 min (right atrium) before the addition of agonist was taken as the initial value. All responses were expressed relative to this initial value per se for gastric experiments or relative to the increase above the initial value caused by 0.1mM isoprenaline for atrial experiments. Increases in contraction force were quantified using the maximal response. When fitting C-R curves, the concentrations where the response declined more than 10 percent under the response obtained at the previously administered agonist concentration, were excluded.

- **Hill equation curve parameters**

To obtain curve parameter estimates for mid-point location ($EC_{50}$, estimated as $-\log(EC_{50})$), upper asymptote of the observed maximal effect ($\alpha$) and Hill slope ($n_H$), C-R curves to the agonists were fitted to the Hill equation by non-linear regression using a mixed-effects
approach (i.e. inter-individual variation in the parameters was accounted for; PROC
NLMIXED; SAS v9.1).

\[ E = \frac{A^{n_t} \cdot \alpha}{EC_{50}^{n_t} + A^{n_t}} \]  

(1)

- **The operational model of agonism (OMOA):**

To quantify the differences in the expression of agonism of the 5-HT4 receptor agonists across
the different tissues, the OMOA was applied (Black and Leff, 1983).

\[ E = \frac{E_{max} \cdot \tau^{n_t} \cdot A^{n_t}}{(K_A + A)^{n_t} + \tau^{n_t} \cdot A^{n_t}} \]  

(2)

where \( E_{max} \) is the maximal pharmacological effect achievable in the system, \( K_A \) is the agonist-receptor complex dissociation constant (the reciprocal of which defines agonist affinity), \( n_t \) is the slope index for the transducer relation (the occupancy - effect function) and therefore is a measure of the sensitivity with which the system transduces AR into E, and \( \tau \) is the efficacy parameter, which is defined by the ratio of total functional receptor concentration (\( R_0 \)) and \( K_E \) which is the value for half-saturation of the transducer function. Therefore, the reciprocal of \( \tau \) represents the fraction of receptors that needs to be occupied to achieve half-maximal tissue response.

- **Modelling**

The experimental data were fitted to the operational model of agonism (2) using a non-linear mixed-effects (nlme) model (nlme package as implemented in S-PLUS v.6.2, Insightful Corporation, Seattle, WA, USA; the S-PLUS code can be obtained with the authors). The efficacy parameter \( \tau \) is estimated for each compound and for each tissue. Since within the same tissue the tissue-dependent aspects of \( K_E \) are cancelled and only the drug-specific aspects of efficacy for the agonists are relevant (relative values of the activated receptor – G-protein complex dissociation constant, \( K_{AR} \)), the ratio of the \( \tau \) values between the different
compounds is kept constant across the different tissues (or vice versa, the ratio of \( \tau \) values between the different tissues is compound independent). For all models, inter-individual variability on the \( \tau \) parameter for all compounds was modelled by an exponential equation. In general:

\[
P_i = P_{pop} \cdot \exp(\eta)
\]  

(3)

where \( P_i \) is the individual value for the parameter (\( \tau_{i, \text{compound}} \)), \( P_{pop} \) is the population value for the parameter (\( \tau \) for that compound), i.e. the value for a typical individual, and \( \eta_i \) is the random deviation of \( P_i \) from \( P_{pop} \). The values of \( \eta_i \) are assumed to be independently normally distributed with mean zero and variance \( \omega^2 \). For each tissue, a different \( E_{\text{max}} \) and \( n_t \) is estimated since these parameters are assumed to be tissue dependent (Leff et al., 1990). In the stomach, responses are expressed as percent increase, without normalisation. Therefore, since Van Der Graaf and Danhof (Van der Graaf and Danhof, 1997) have shown that ignoring inter-individual variation in \( E_{\text{max}} \) may result in erroneous estimates of affinity and efficacy, inter-individual variability of \( E_{\text{max}} \) in the stomach was accounted for, and modelled by an exponential equation (equation 3). In the left and right atrium on the other hand, inter-individual variability of \( E_{\text{max}} \) was assumed to be insignificant since in these tissues, expression of the response relative to that evoked by isoprenaline reduces the variation in the maximal achievable contractile force between the individual animals. It was not possible to account for inter-individual variability of \( n_t \) since this resulted in overparameterization of the model. However, the effect of inter-individual variation of the slope parameter on affinity and efficacy parameter estimates has been shown to be relatively small (Van der Graaf and Danhof, 1997). For each compound, \( K_A \) was assumed to be common for all tissues, since receptor affinity is generally considered to be constant across different tissues of the same individual. Moreover, since \( K_A \) is assumed to be constant across individuals of the same strain, inter-individual variability of \( \rho K_A \) was assumed to be insignificant. \( K_A \) and \( \tau \) were estimated as \(-\log(K_A)\) and \( \log \tau \) respectively, because these parameters are assumed to be log-
normally distributed (Leff et al., 1990; Van der Graaf and Danhof, 1997). Heteroscedasticity in the intra-individual variance (residual errors) was modelled by a power variance function:

\[ y_{mij} = yp_{ij}^c + y_{p_{ij}}^{power} \cdot \epsilon_{ij} \]  

(4)

where \( yp_{ij} \) is the \( j^{th} \) response for the \( i^{th} \) animal predicted by the model, \( y_{mij} \) is the measurement, and \( \epsilon \) accounts for the residual deviation of the model predicted value from the observed response. This results in an additive or proportional residual error model when the power factor is 0 or 1 respectively (and \( c=1 \)). For each tissue, a different value of \( c \) is estimated. The values for \( \epsilon \) are assumed to be independently normally distributed with mean zero and variance \( \sigma^2 \). The residuals were analysed to verify this assumption. For all compounds in all tissues, the mean of the residuals was near zero. Therefore, the residuals distributed evenly around the fitted function, and the model does not deviate in a systematic manner from the data. The deviation of the measured data from the fitted response can therefore be attributed to random deviation. In order to compare models with a different fixed effects structure, a maximum likelihood (ML) estimation method was used during the optimization and model building process. Criteria for selecting the final model included improvement in the residual plots, increased precision in the parameter estimates, reduced residual variances for the parameter estimates and a reduction of the objective function value (-2 x log likelihood).

Population values for the final model parameters and variances \( \omega \) and \( \sigma \) are estimated using a restricted maximum likelihood function (ReML).

The model was also applied to the near full data set, including the data with prucalopride in the presence of increasing concentrations of GR113808 (see results). The same modelling procedure was followed as described above. \( K_B \) (see results section) was estimated as – \( \log(K_B) \) since it has a log-normal distribution. To account for the presence of antagonist, residual error was modelled by equation (4) in which the value of the constant \( c \) is allowed to vary with the presence or absence of antagonist.

**Drugs:**
The following drugs were used (abbreviations and respective suppliers in parentheses): 3-isobutyl-1-methyl-xanthine (IBMX) and L-N\textsuperscript{G}-nitro-arginine methyl ester (L-NAME; Fluka, Switzerland); propranolol HCl (Sigma, Belgium); methysergide maleate (Research Biochemical INS, USA); indomethacin (Merck Belgolabo N.V.); [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate (GR 113808; Tocris Cookson, UK); atropine sulphate, 5-hydroxytryptamine creatinine sulphate (5-HT; Acros chimica, Belgium); tetrodotoxin (TTX; Serva, Germany); cocaine HCl, granisetron HCl, isoprenaline HCl, tegaserod, prucalopride HCl, 4-amino-5-chloro-2,2-dimethyl-2,3-dihydro-benzofuran-7-carboxylic acid [3-hydroxy-1-(3-methoxy-propyl)-piperidin-4-ylmethyl]-amide (R149402 HCl), 4-amino-5-chloro-2,3-dihydro-benzofuran-7-carboxylic acid [3-hydroxy-1-(3-methoxy-propyl)-piperidin-4-ylmethyl]-amide (R199715 HCl) and carbachol (Johnson & Johnson Research and Development, Beerse, Belgium). All compounds were dissolved and diluted in distilled water, except for GR113808, tegaserod and indomethacin. GR113808 was freshly dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution of 10 mM; dilutions were made with distilled water. A stock solution of tegaserod (1 mM) was made in distilled water containing 20% cyclodextrin and diluted with distilled water; indomethacin was dissolved in 9.1 ml distilled water supplemented with 0.9 ml 2% Na\textsubscript{2}CO\textsubscript{3}. These solutions were stored at -20°C.
Results

We previously reported an in depth analysis of the $5\text{-HT}_4$ receptor-mediated effects in the porcine left and right atrium (De Maeyer et al., 2006). The curve location parameters of the cumulative C-R curves for the inotropic effect in left atrium and the chronotropic effect in right atrium used for comparison with the gastric responses in the current study are given in table 1. No previous description of functional gastric $5\text{-HT}_4$ receptors in the pig was made. Therefore, the gastric results will be presented in detail before we describe the comparison between atrial and gastric tissues.

1. Effects of $5\text{-HT}_4$ receptor agonists on porcine gastric muscle strips

Under isometric conditions, the muscle strips displayed very little to no spontaneous contractility throughout the experiment. In preliminary experiments, it was shown that the EFS-induced contractions were blocked by both tetrodotoxin (0.3 $\mu$M) and atropine (0.3 $\mu$M), indicating a neuronal mechanism involving acetylcholine.

a. Effects of 5-HT on the proximal stomach

5-HT induced a concentration-dependent increase of the resting tension (figure 1B) starting from 0.03 $\mu$M 5-HT onwards; the highest concentration tested (10 $\mu$M) resulted in an increase in tone of 22.4 ± 7.3 %, expressed as percentage of the response to carbachol; the maximal effect was not yet reached. When constructing a cumulative C-R curve to 5-HT (up to 100 $\mu$M 5-HT) in the presence of GR113808 (1 and 10 $\mu$M), the increase in basal tone by 5-HT was not prevented and basal tone continued to increase up to the concentration of 100 $\mu$M 5-HT. The amplitude of the EFS-induced contractions was always measured from the newly prevailing basal tone.

5-HT non-transiently enhanced EFS-induced contractions, both in the cumulative (figure 1B) and the non-cumulative administration procedure. The increase in EFS-induced contraction
force reached a maximum after 9-12 min (3 to 4 electrical pulse trains). In approximately 50% of the animals, the response to the higher single concentrations spontaneously decayed. No difference in the mean location parameters between the C-R curves obtained with the two administration methods was found. The location parameters for the cumulative C-R curve of 5-HT in gastric muscle are given in table 1.

b. Effects of prucalopride, tegaserod, R149402 and R199715 on the proximal stomach
Prucalopride, tegaserod, R149402 and R199715 had no influence on resting tension but all non-transiently enhanced EFS-induced contractions (as shown for a single concentration of prucalopride in figure 1A). The kinetics of these increases in EFS-induced contraction force were agonist specific reaching a maximum after 15-50 min (the higher the concentration of agonist, the quicker a maximum was reached) for prucalopride and after 40 to more than 100 min for tegaserod. No difference between cumulative and non-cumulative administration was found for any of the compounds; the location parameters for the cumulative C-R curves are given in table 1.
Since methysergide (1 µM) was present in experiments with 5-HT, and methysergide itself enhanced the EFS-induced contractions, it was tested whether the presence of this antagonist influenced the response to the selective 5-HT₄-receptor agonist prucalopride, in order to justify the comparison with 5-HT. In these experiments, basal EFS-induced contractions were 30.8 ± 0.9 %, while in the absence of methysergide a significantly lower basal response of 24.2 ± 1.1 % was measured (both values, n= 48 tissues from 6 animals; as percentage of the response to carbachol; P<0.001, unpaired t-test between basal values in experiments with prucalopride in the presence vs. those in the absence of methysergide). We have no explanation for the effect of methysergide per se on EFS-induced contractions. However, the maximal effect and pEC₅₀ of the cumulative C-R curve of prucalopride in the presence of methysergide were not different from those in the absence of methysergide. Similarly, no difference in response to prucalopride in the absence or presence of methysergide was observed for any concentration tested in the non-cumulative setup.
2. **Antagonistic effect of GR113808 vs. prucalopride and 5-HT in stomach and left atrium**

The effect of pre-incubation with increasing concentrations of GR113808 on the cumulative concentration-effect curves to prucalopride in the gastric muscle preparations and in the left atrial trabeculae is shown in figure 2A and D respectively. The effect of GR113808 on the 5-HT induced contractions in stomach and atrium is shown in figure 3A and B respectively. The maximal effect of the concentration-effect curves shows a downward tendency with increasing GR113808 concentrations (figure 2A and D; figure 3A).

3. **Development of a framework to compare gastric and atrial responses**

To compare the behaviour of the agonists at atrial and gastric 5-HT1 receptors and to explain the differences observed between the location parameters of the cumulative C-R curves in the 3 tissues (table 1), we applied the OMOA to the data. Table 2 shows the resulting population estimates for the different tissues together with the inter-individual variability (only allowed for log \( \tau \), and for the \( E_{\text{max}} \) in the stomach) expressed as a coefficient of variation.

In order to fit the data simultaneously, including those with prucalopride in the presence of GR113808, the OMOA was adapted to account for the presence of a competitive reversible antagonist.

\[
E = \frac{E_{\text{max}} \tau^n \cdot A^n}{K_A \left(1 + \frac{B}{K_B} \right)^n + \tau^n \cdot A^n} \tag{5}
\]

In this equation \( B \) represents the antagonist concentration and \( K_B \) is the antagonist dissociation constant. The variability of the Hill slope in the right atrium seems to be greater than in the other tissues (table 1). Therefore, inter-individual variability of the slope factor in the right atrium was accounted for (which was not possible in the basic model because this resulted in over parameterization of the model). In the stomach, in the presence of the higher concentrations of GR113808 (above 30nM), the contractile force measured just before the
first concentration of prucalopride that evoked a clear response, was often increased relative to the basal response (Figure 2A). We therefore introduced the parameter $E_{basal}$, to account for the increased baseline for these data. This largely improved the model fit (135 points change in the objective function value). The resulting model fit is shown in figure 2B and E. This model results in a $pK_B$ estimate of 9.43±0.04 and 9.63±0.05 in atrium and stomach respectively. In the atrium, the model predicted response in the presence of increasing concentrations of GR113808, is systematically overestimating the observations at high agonist concentrations (figure 2E). This is less clear in the stomach since the maximal effect is often not yet reached (figure 2B). However, the maximal effect in the presence of 3 and 10 nM GR113808 seems overestimating the observed responses. Furthermore, also for 5-HT an influence of 1 and 10 µM GR113808 on the upper asymptote was observed in the stomach (figure 3A).

Since equation (5) cannot account for a change in the upper asymptote, the model was further adapted by allowing for possible partially insurmountable antagonism of GR113808. The parameter $q$ was introduced, with $q$ being the reduction of $\tau$ in the presence of GR113808 (i.e., allowing for a change in the operational receptor number or in the signal transduction efficiency by GR113808). If $q$ is interpreted as the fraction of receptors available for interaction with an agonist after ‘pseudo-irreversible’ blockade with an antagonist (Zernig et al., 1996), it corresponds to the q-value of Furchgott (Zernig et al., 1996; Black et al., 1985). $q$ depends on the concentration of antagonist used and is modelled by an hyperbolic equation, to allow for the saturation of the relation between log $[B]$ and $\alpha$.

$$q = \frac{\tau_{antagonist}}{\tau_{control}} = 1 - \frac{q_{max}B}{B_{50} + B}$$  \hspace{1cm} (6)

In this equation $q_{max}$ is the maximal reduction of $\tau$ by GR113808, while $B_{50}$ denotes the antagonist concentration at which the reduction is 50% of the value of $q_{max}$. The complete data set was therefore fitted using the following model:
Since no assumptions are made about the underlying mechanism(s) of the insurmountable antagonism, \( q_{\text{max}} \) can well possess (partially) tissue-related properties. Therefore, inter-individual variability of this parameter was accounted for. \( K_B \) and \( B_{50} \) are antagonist related properties and inter-individual variability was not assumed to be insignificant.

The model, adapted to accommodate for partially insurmountable antagonism by GR113808 in the atrium predicts the observations very well and causes a change in the objective function value of 45 points. An additional change by 30 points was obtained when gastric data were also modelled using equation 7. This model resulted in a \( pK_B \) and \( pB_{50} \) estimate in the atrium of 9.02±0.07 and 9.16±0.07 respectively, while in the stomach a \( pK_B \) and \( B_{50} \) of 7.91±0.14 and 9.48±0.13 was found (table 3). This means that in the atrium (figure 2F) and the stomach (figure 2C), the data can be fitted if we assume a concurrent insurmountable and competitive action of GR113808. In the stomach, these 2 actions of GR113808 appear with a different potency. Table 3 summarizes the population predictions obtained with this final model. The parameters are well defined with the inter-individual variability in the atrium being slightly higher than that in the stomach. Population predicted curves for all agonists in each tissue or for each agonist in the 3 tissues from this final OMOA fit are given in figure 4A-C and figure 5A-E respectively. The apparent difference in relative efficacy between R149402 and R199715 in left and right atrium (Figure 4), R149402 being more efficient than R199715 in the left atrium and vice versa in the right atrium, is caused by the rather large inter-individual variability for R149402 and especially R199715 in the atria.
Discussion

Differences in benzamide-induced 5-HT\textsubscript{4} receptor-mediated responses between different tissues or species mainly originate from the either partial or full agonistic behaviour of these compounds in these different test systems (Bockaert et al., 2004; Langlois and Fischmeister, 2003). In fact, because of the difference with mouse colliculi neurones (Dumuis et al., 1989), 5-HT\textsubscript{4} receptors in the heart were first designated as 5-HT\textsubscript{4}-like (Kaumann et al., 1991). Most of these studies use the empirical Hill equation to characterize the effects of benzamides at 5-HT\textsubscript{4} receptors. However, the parameters of the empirical Hill equation contain mixed information on drug-specific properties and characteristics of the biological system which makes it difficult to appreciate the functional potency of a given 5-HT\textsubscript{4} receptor ligand. This complicates the use of this equation for extrapolation and prediction and renders it inappropriate as a model to predict the expression of agonism, and thus possible undesirable side effects of a given agonist (Zuideveld et al., 2004). The OMOA on the other hand, has already been demonstrated to be very useful to quantify and describe tissue related efficacy differences (Leff et al., 1990; Janssen et al., 2004). We now report the establishment of a framework, based on the OMOA, that allows for quantitative assessment of the tissue dependent differences in expressed efficacy of 5-HT\textsubscript{4} receptor agonists in the pig, as a model for the human situation.

For the pig to be a representative animal to study tissue specific 5-HT\textsubscript{4} receptor agonism in man, a relevant GI model needed to be developed in this animal since no proper bioassay was available to analyse the GI motility promoting potency of 5-HT\textsubscript{4} receptor agonists. The data presented in this study now clearly demonstrate that 5-HT, as well as the selective 5-HT\textsubscript{4} receptor agonists prucalopride, tegaserod, R149402 and R199715 concentration-dependently enhanced EFS-induced contractions in the porcine proximal stomach. The involvement of other than 5-HT\textsubscript{4} receptors in the 5-HT induced effects was ruled out by the presence of methysergide and granisetron (Prins et al., 2000a). Since both TTX and the muscarinergic receptor antagonist atropine blocked the contractions, the 5-HT\textsubscript{4} receptors are probably located on cholinergic neurones. We postulate that the 5-HT\textsubscript{4} receptors are operating pre-
synaptically, facilitating the release of the neurotransmitter. Indeed, the same 5-HT$_4$-mediated effect on neurotransmission has been described in the human GI tract, on neurones projecting to both muscle layers in the colon, as well as to the circular muscle layer in the stomach, while no contractile post-synaptically operating 5-HT$_4$ receptor has been described in the GI tract (Leclere and Lefebvre, 2002; Prins et al., 2000a; Leclere et al., 2005).

Despite the blockade of every 5-HT receptor except the 5-HT$_4$ receptor, 5-HT, but not the other agonists, induced a TTX-insensitive (results not shown) tonic contraction, that could not be inhibited by GR113808. This is in line with a previous study of Janssen et al. (2002) who showed, in the same preparation as used in this study, the involvement of an uncharacterised high affinity receptor population and of the 5-HT$_2A$ receptor in the basal contractile response to 5-HT; in their study, the basal response to 5-HT in the presence of 1µM methysergide was the same as in our study (Janssen et al., 2002).

We now applied the OMOA to quantify the differences in the expression of 5-HT$_4$ receptor-mediated agonism in the newly described gastric bioassay and in the porcine left and right atrium, which we previously described in great detail (De Maeyer et al., 2006). We used a mixed effects approach, which allows to fit all data simultaneously and which provides, by incorporating inter-individual variability, the necessary flexibility for the model to be practicable with decreased need for a priori assumptions. As a noteworthy example, there is no need for the assumption that the maximal response to a full agonist equals $E_{\text{max}}$, which is undermined by the inherent difficulty in defining what a full agonist is (Black and Shankley, 1990).

Our data show that the application of the OMOA allows to describe and predict the tissue dependent efficacy of 5-HT$_4$ receptor agonism. The results show that tegaserod has the same $\tau$ value as 5-HT, while prucalopride, R149402 and R199715 are less efficacious than 5-HT. Operationally, this implies that in a low efficacy system like the atrium, the latter compounds show less effect than 5-HT and hence that the intrinsic power of prucalopride to produce cardiac side effects is lower than this of tegaserod.
The estimated affinity values for 5-HT, prucalopride, R149402 and R199715 (the latter two being prucalopride-derived experimental 5-HT$_4$ receptor agonists, developed by Johnson & Johnson Pharmaceutical Research and Development, Beerse) are in good agreement with results found in receptor binding experiments (pIC$_{50}$ against GR113808 in HEK cells transfected with the human 5-HT$_4$(b) receptor: 6.6, 7.0, 8.0 and 8.8 respectively; Johnson & Johnson, unpublished data). The estimated affinity for tegaserod on the other hand (pK$_A$: 5.83) is lower than found in receptor binding experiments (pIC$_{50}$ of 7.4). The estimated slope of the transducer relation in all tissues is smaller than one, implicating a decrease of the Hill slope with increasing values of $\tau$ (Black et al., 1985). However, in case of tegaserod, the Hill slope was smaller than can be expected from its $\tau$ value in the different tissues. Difficult penetration of tegaserod into the tissue or possible partial precipitation because of its low solubility could be explanations for the underestimated K$_A$ and the low Hill slope. This idea is supported by the very slow kinetics of the response following the administration of tegaserod compared to the other agonists.

In the left and right atrium, the maximal tissue response is estimated to be approximately 100%, i.e. identical to the response to a supramaximal isoprenaline concentration. This implies that 5-HT$_4$ receptors and $\beta$-receptors, which are both positively coupled to cAMP, allow for the same maximal achievable tissue response. The left atrial experiments were performed in the presence of IBMX to exclude the actions of phosphodiesterase enzymes (PDE), a condition which greatly enhanced the 5-HT$_4$ receptor-mediated responses (De Maeyer et al., 2006). It was not possible to include the data in the absence of IBMX in our model since, at equilibrium conditions, none of the agonists showed a positive inotropic effect. 5-HT actually behaved as a partial agonist in the non IBMX-treated left atrium, and the predicted efficacy for the left atrium in the present study may be overrating the significance for the in vivo situation. In the right atrium, where chronotropic responses to the agonists can be obtained in the absence of IBMX, the $E_{\text{max}}$ estimate greatly exceeded the maximum of the C-R curve for 5-HT, implying that also in this tissue 5-HT behaved as a partial agonist. A similar finding was reported, when applying the OMOA, for the contractile effect of 5-HT in
rings of rabbit aorta (Leff et al., 1990). The inclusion of cardiovascular in vivo data with 5-HT4 receptor agonists in the model would extend its predictive properties and would allow for the prediction of tissue-dependent efficacy in vivo (Van der Graaf et al., 1999).

The flexibility of our framework is clear from the fact that it can accommodate antagonist data. Liu et al. (1992) adapted the OMOA in a similar way to explain their observations with Angiotensin II receptor antagonists. From a modelling point of view, our results suggest that GR113808 behaves as a partial insurmountable antagonist in the atrium and in the stomach (GR113808-dependent change of $\tau$, and hence of the operational receptor number or the coupling constant). These observations do not stand alone since deviations from competitive antagonism, including insurmountable antagonism and deviation from linear Schild slopes, have been described for 5-HT4 receptor antagonists, e.g. GR113808, in different tissues, including the piglet right atrium (Medhurst and Kaumann, 1993; Tam et al., 1995; Gale et al., 1994). Furthermore, we have previously shown that in the left atrium, in the presence of IBMX, GR113808 reverted the increased contraction by a single concentration of prucalopride or tegaserod to a level below basal (De Maeyer et al., 2006). This might fit into the recent theories on insurmountable antagonism in which the antagonist bound receptor may adopt multiple conformations and/or states (Vauquelin et al., 2002). Claeyssen et al. (2003) have shown that a single mutation in the 5-HT4 receptor generates a receptor that can not be bound and activated by 5-HT or tegaserod. Numerous synthetic ligands on the other hand, as well as GR113808 that behaves as a partial agonist on this receptor, can still stimulate this receptor. This suggests an agonist (or antagonist)-dependent requirement of certain binding sites or conformations. Furthermore, this could explain the different efficacy associated with 5-HT and tegaserod compared to the other agonists (see above). GR1138808 has previously been shown to behave as a partial agonist on the 5-HT4(h) splice variant, a variant that has been described in porcine tissue (Bender et al., 2000; Ullmer et al., 1995). It could thus well be that GR113808 acts as a very weak partial agonist, resulting in the loss of some receptors.
for restimulation with an agonist. However, finding an explanation for the complex behaviour of GR113808 was beyond the scope of this study.

In conclusion, we established a framework that allows quantitative assessment of the tissue dependent differences in expressed efficacy associated with agonists at 5-HT₄ receptors. Our results clearly demonstrate that this provides a practical test for quantifying the expression of partial agonism in the heart by new ligands. This can provide a useful tool for selecting drugs based on their property to exert a balanced GI effect without relevant cardiac effects. Furthermore, our model allows incorporation of antagonist data. The use of mixed-effects modelling allows to fit all data simultaneously and provides the necessary flexibility to be used without restricting assumptions and opens perspectives to extend the model to in vivo data.

Acknowledgements

The authors wish to thank Roel Straetemans for his help with the SAS analysis.
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Zernig G, Issaevitch T and Woods JH (1996) Calculation of agonist efficacy, apparent affinity, and
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M (2004) Mechanism-based pharmacokinetic-pharmacodynamic modeling of 5-HT_{1A} receptor
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Pharmacol Exp Ther* 308:1012-1020.
Footnotes

a).

The study was financially supported by Interuniversity Attraction Poles Programme P5/20, Belgian Science Policy.

b).

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Legends for Figures

Figure 1
Representative tracings showing the response to prucalopride and 5-HT in electrically stimulated longitudinal muscle strips from the porcine proximal stomach, in the presence of L-NAME (0.1 mM) and indomethacine (1 µM). The upper tracing (A) represents the effect caused by the administration of a single concentration of prucalopride (0.1 µM). In this experiment, GR113808 (0.1 µM) was administered on top of prucalopride. The recording in (B) shows the response following the cumulative administration of 5-HT with half log unit concentration increments, indicated by the arrows, in the additional presence of methysergide (1 µM), granisetron (0.3 µM) and cocaine (6 µM).

Figure 2
Antagonism by GR113808 of the prucalopride-induced contraction of porcine electrically stimulated proximal stomach muscle preparations (A, B, C) or left atrial pectinate muscles (D, E, F). The negative logarithm of the concentrations of GR113808 used are given in the upper left corner of each panel. The effect of pre-incubation of GR113808 on the observed, cumulatively administered C-R curve of prucalopride is shown in (A) for the stomach and (D) for the left atrium. Vertically averaged data points are shown, and connected by a line. The S.E.M. is not shown on all mean data points for clarity reasons. The other panels show the prediction of the prucalopride-induced effect in the stomach (B and C) and the left atrium (E and F). In B and E, the curves superimposed on the mean observed data points used for fitting represent population predictions based on population parameter estimates from the non-linear mixed effect model fit of the observed data to the operational model of agonism, adapted for the presence of a competitive antagonist. C and F show the same for the model adapted for partially insurmountable antagonism.
Figure 3

Antagonism by GR113808 of the 5HT-induced contraction of porcine electrically stimulated proximal stomach muscle preparations (A) or left atrial pectinate muscles (B). The negative logarithm of the concentrations of GR113808 used are given in the upper left corner of each panel. The effect of pre-incubation of GR113808 on the observed, cumulatively administered C-R curve of 5-HT is shown in (A) for the stomach and (D) for the left atrium. Vertically averaged data points with S.E.M. are shown, and connected by a line.

Figure 4

Prediction of the responses induced by 5-HT and the 5-HT4 receptor agonists prucalopride, tegaserod, R149402 and R199715 in the proximal stomach (A), left atrium (B) and right atrium (C) based on population parameter estimates obtained from the non-linear mixed effect model fit of the observed data to the operational model of agonism. The population predicted curve is shown superimposed on the mean observed data points used for fitting.

Figure 5

Prediction of the responses induced by 5-HT (A) and the 5-HT4 receptor agonists prucalopride (B), tegaserod (C), R149402 (D) and R199715 (E) in the proximal stomach, and both the left and the right atrium based on population parameter estimates obtained from the non-linear mixed effect model fit of the observed data to the operational model of agonism. The population predicted curve is shown superimposed on the mean observed data points used for fitting.
Table 1: Hill equation curve fit parameters of the concentration-effect curves in the stomach and the left and right atrium

<table>
<thead>
<tr>
<th>agonist</th>
<th>pEC_{50}</th>
<th>α</th>
<th>n_{H}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stomach: inotropic effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>8.41 ± 0.09</td>
<td>87.1 ± 8.1</td>
<td>0.70 ± 0.04</td>
</tr>
<tr>
<td>prucalopride</td>
<td>8.25 ± 0.07</td>
<td>91.8 ± 7.1</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>tegaserod</td>
<td>8.15 ± 0.10</td>
<td>92.8 ± 7.8</td>
<td>0.61 ± 0.04</td>
</tr>
<tr>
<td>R149402</td>
<td>9.59 ± 0.09 ***</td>
<td>60.6 ± 8.0 ***</td>
<td>1.10 ± 0.07</td>
</tr>
<tr>
<td>R199715</td>
<td>9.91 ± 0.08 ***</td>
<td>54.64 ± 7.2 ***</td>
<td>1.07 ± 0.07</td>
</tr>
<tr>
<td><strong>Left atrium: inotropic effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>7.71 ± 0.10</td>
<td>88.7 ± 5.2</td>
<td>0.93 ± 0.07</td>
</tr>
<tr>
<td>prucalopride</td>
<td>7.70 ± 0.09</td>
<td>64.3 ± 6.7 ***</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td>tegaserod</td>
<td>6.85 ± 0.17 ***</td>
<td>93.5 ± 6.8</td>
<td>0.58 ± 0.02</td>
</tr>
<tr>
<td>R149402</td>
<td>8.97 ± 0.08 ***</td>
<td>51.6 ± 5.5 ***</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td>R199715</td>
<td>8.94 ± 0.09 ***</td>
<td>32.8 ± 6.7 ***</td>
<td>0.91 ± 0.06</td>
</tr>
<tr>
<td><strong>Right atrium: chronotropic effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>6.53 ± 0.14</td>
<td>54.4 ± 4.8</td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td>prucalopride</td>
<td>7.01 ± 0.14 *</td>
<td>27.6 ± 4.6 ***</td>
<td>1.16 ± 0.11</td>
</tr>
<tr>
<td>tegaserod</td>
<td>6.58 ± 0.17</td>
<td>57.6 ± 5.7</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>R149402</td>
<td>8.53 ± 0.15 ***</td>
<td>16.3 ± 4.2 ***</td>
<td>1.30 ± 0.16</td>
</tr>
<tr>
<td>R199715</td>
<td>8.70 ± 0.17 ***</td>
<td>25.6 ± 5.4 ***</td>
<td>1.23 ± 0.15</td>
</tr>
</tbody>
</table>

α: from De Maeyer, et al. (2006); *: P<0.05; ***: P<0.001: significantly different versus 5-HT.
Table 2: Population and individual parameters of the operational model of agonism fit of the inotropic effects observed in the stomach and left atrium and the chronotropic effect in the right atrium.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stomach</th>
<th>Left Atrium</th>
<th>Right Atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population</td>
<td>Inter-individual</td>
<td>Population</td>
</tr>
<tr>
<td></td>
<td>predicted mean</td>
<td>variability CV (95% CI)</td>
<td>predicted mean</td>
</tr>
<tr>
<td>pKₐ 5-HT</td>
<td>5.92 (0.10)</td>
<td>n.d.</td>
<td>5.92 (0.10)</td>
</tr>
<tr>
<td>pKₐ prucalopride</td>
<td>6.53 (0.05)</td>
<td>n.d.</td>
<td>6.53 (0.05)</td>
</tr>
<tr>
<td>pKₐ tegaserod</td>
<td>5.94 (0.10)</td>
<td>n.d.</td>
<td>5.94 (0.10)</td>
</tr>
<tr>
<td>pKₐ R149402</td>
<td>8.15 (0.06)</td>
<td>n.d.</td>
<td>8.15 (0.06)</td>
</tr>
<tr>
<td>pKₐ R199715</td>
<td>8.34 (0.07)</td>
<td>n.d.</td>
<td>8.34 (0.07)</td>
</tr>
<tr>
<td>Eₘₐₓ</td>
<td>78.2 (4.3)</td>
<td>32 (25-41)</td>
<td>103.2 (2.0)</td>
</tr>
<tr>
<td>log τ₅-HT</td>
<td>2.65 (0.13)</td>
<td>11 (7-18)</td>
<td>1.35 (0.15)</td>
</tr>
<tr>
<td>log τprucalopride</td>
<td>1.72 (0.09)</td>
<td>14 (9-23)</td>
<td>0.42 (0.11)</td>
</tr>
<tr>
<td>log τtegaserod</td>
<td>2.45 (0.14)</td>
<td>9 (5-18)</td>
<td>1.15 (0.16)</td>
</tr>
<tr>
<td>log τ_{R149402}</td>
<td>1.27 (0.09)</td>
<td>11 (3-31)</td>
<td>-0.03 (0.11)</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
<tr>
<td>log τ_{R199715}</td>
<td>1.33 (0.13)</td>
<td>19 (8-44)</td>
<td>0.03 (0.15)</td>
</tr>
<tr>
<td>n_i</td>
<td>0.83 (0.02)</td>
<td>n.d.</td>
<td>0.74 (0.02)</td>
</tr>
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</table>

CV: coefficient of variation
Table 3: Population and individual parameters of the operational model of agonism fit of the data set, of inotropic effects in the stomach and left atrium and the chronotropic effect in the right atrium, including the data with prucalopride in the presence of GR113808.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stomach</th>
<th></th>
<th>Left Atrium</th>
<th></th>
<th>Right Atrium</th>
<th></th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Population</td>
<td>Inter-individual</td>
<td>Population</td>
<td>Inter-individual</td>
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<td>Inter-individual</td>
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<tr>
<td></td>
<td>predicted mean</td>
<td>variability CV</td>
<td>predicted mean</td>
<td>variability CV</td>
<td>predicted mean</td>
<td>variability CV</td>
</tr>
<tr>
<td></td>
<td>(s.e.)</td>
<td>(95% CI)</td>
<td>(s.e.)</td>
<td>(95% CI)</td>
<td>(s.e.)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>pK_A 5-HT</td>
<td>6.32 (0.08)</td>
<td>n.d.</td>
<td>6.32 (0.08)</td>
<td>n.d.</td>
<td>6.32 (0.08)</td>
<td>n.d.</td>
</tr>
<tr>
<td>pK_A prucalopride</td>
<td>6.68 (0.04)</td>
<td>n.d.</td>
<td>6.68 (0.04)</td>
<td>n.d.</td>
<td>6.68 (0.04)</td>
<td>n.d.</td>
</tr>
<tr>
<td>pK_A tegaserod</td>
<td>5.83 (0.10)</td>
<td>n.d.</td>
<td>5.83 (0.10)</td>
<td>n.d.</td>
<td>5.83 (0.10)</td>
<td>n.d.</td>
</tr>
<tr>
<td>pK_A R149402</td>
<td>8.27 (0.05)</td>
<td>n.d.</td>
<td>8.27 (0.05)</td>
<td>n.d.</td>
<td>8.27 (0.05)</td>
<td>n.d.</td>
</tr>
<tr>
<td>pK_A R199715</td>
<td>8.52 (0.06)</td>
<td>n.d.</td>
<td>8.52 (0.06)</td>
<td>n.d.</td>
<td>8.52 (0.06)</td>
<td>n.d.</td>
</tr>
<tr>
<td>E_max</td>
<td>82.0 (4.2)</td>
<td>30 (23-38)</td>
<td>106.7 (2.2)</td>
<td>n.d.</td>
<td>116.0 (21.1)</td>
<td>n.d.</td>
</tr>
<tr>
<td>log τ 5-HT</td>
<td>2.21 (0.12)</td>
<td>13 (8-21)</td>
<td>0.99 (0.13)</td>
<td>27 (16-43)</td>
<td>0.01 (0.20)</td>
<td>27 (16-43)</td>
</tr>
<tr>
<td>log τ prucalopride</td>
<td>1.53 (0.08)</td>
<td>14 (9-23)</td>
<td>0.32 (0.10)</td>
<td>29 (18-49)</td>
<td>-0.67 (0.18)</td>
<td>29 (18-49)</td>
</tr>
<tr>
<td>log τ tegaserod</td>
<td>2.44 (0.15)</td>
<td>10 (5-20)</td>
<td>1.23 (0.16)</td>
<td>41 (23-72)</td>
<td>0.25 (0.22)</td>
<td>41 (23-72)</td>
</tr>
<tr>
<td></td>
<td>( \log \tau_{R149402} )</td>
<td>n.t.</td>
<td>( \log \tau_{R199715} )</td>
<td>n.t.</td>
<td>( n_t )</td>
<td>CV: coefficient of variation.</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------</td>
<td>------</td>
<td>---------------------------</td>
<td>------</td>
<td>-----------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>R149402</td>
<td>1.11 (0.09)</td>
<td>13 (3-50)</td>
<td>-0.10 (0.11)</td>
<td>44 (21-106)</td>
<td>-1.09 (0.18)</td>
<td>44 (21-106)</td>
</tr>
<tr>
<td>R199715</td>
<td>1.12 (0.16)</td>
<td>41 (22-79)</td>
<td>-0.09 (0.17)</td>
<td>60 (25-211)</td>
<td>-1.07 (0.23)</td>
<td>60 (25-211)</td>
</tr>
<tr>
<td>( n_t )</td>
<td>0.78 (0.02)</td>
<td>n.d.</td>
<td>0.77 (0.02)</td>
<td>n.d.</td>
<td>0.88 (0.07)</td>
<td>36 (26-49)</td>
</tr>
<tr>
<td>( pK_B )</td>
<td>7.91 (0.14)</td>
<td>n.d.</td>
<td>9.02 (0.07)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>( pB_{50} )</td>
<td>9.48 (0.13)</td>
<td>n.d.</td>
<td>9.16 (0.07)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>( q_{max} )</td>
<td>0.70 (0.03)</td>
<td>6 (2-18)</td>
<td>0.39 (0.08)</td>
<td>42 (14-124)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
Figure 1

A
Prucalopride 0.1 µM  GR113808 0.1 µM

50 mN
5 min

B
5-HT (µM)

0.0001 0.0003 0.001 0.003 0.01 0.03 0.1 0.3 1 3 10
Figure 2
Figure 3
Figure 4
Figure 5

A

B

C

D

E