BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2009), 158, 252–258

0007-1188/09
www.brjpharmacol.org

THEMED ISSUE: GPCR

RESEARCH PAPER

Inhibition of colonic motility and defecation by RS-127445 suggests an involvement of the 5-HT$_{2B}$ receptor in rodent large bowel physiology

AK Bassil[1], CM Taylor[2], VJN Bolton[1], KM Gray[1], JD Brown[2], L Cutler[1], SG Summerfield[3], G Bruton[1], WJ Winchester[1], K Lee[1] and GJ Sanger[1]

[1]*Immuno-Inflammation CEDD, GlaxoSmithKline, Stevenage, UK,* [2]*Neuroscience CEDD, GlaxoSmithKline, Harlow, UK, and* [3]*Pre-clinical Development, GlaxoSmithKline, Ware, UK*

Background: 5-HT$_{2B}$ receptors are localized within the myenteric nervous system, but their functions on motor/sensory neurons are unclear. To explore the role of these receptors, we further characterized the 5-HT$_{2B}$ receptor antagonist RS-127445 and studied its effects on peristalsis and defecation.
Experimental approach: Although reported as a selective 5-HT$_{2B}$ receptor antagonist, any interactions of RS-127445 with 5-HT$_4$ receptors are unknown; this was examined using the recombinant receptor and Biomolecular Interaction Detection technology. Mouse isolated colon was mounted in tissue baths for isometric recording of neuronal contractions evoked by electrical field stimulation (EFS), or under an intraluminal pressure gradient to induce peristalsis; the effects of RS-127445 on EFS-induced and on peristaltic contractions were measured. Faecal output of rats in grid-bottom cages was measured over 3 h following i.p. RS-127445 and separately, validation of the effective doses was achieved by determining the free, unbound fraction of RS-127445 in blood and brain.
Key results: RS-127445 (up to 1 μmol·L^{-1}) did not interact with the 5-HT$_4$ receptor. RS-127445 (0.001–1 μmol·L^{-1}) did not affect EFS-induced contractions of the colon, although at 10 μmol·L^{-1} the contractions were reduced (to 36 ± 8% of control, n = 4). RS-127445 (0.1–10 μmol·L^{-1}) concentration-dependently reduced peristaltic frequency (n = 4). RS-127445 (1–30 mg·kg^{-1}), dose-dependently reduced faecal output, reaching significance at 10 and 30 mg·kg^{-1} (n = 6–11). In blood and brain, >98% of RS-127445 was protein-bound.
Conclusions and implications: High-protein binding of RS-127445 indicates that relatively high doses are required for efficacy. The results suggest that 5-HT$_{2B}$ receptors tonically regulate colonic motility.
British Journal of Pharmacology (2009) **158,** 252–258; doi:10.1111/j.1476-5381.2009.00155.x; published online 9 April 2009

This article is part of a themed issue on GPCR. To view this issue visit http://www3.interscience.wiley.com/journal/121548564/issueyear?year=2009

Keywords: 5HT$_{2B}$; RS-127445; peristalsis; faecal output; colon

Abbreviations: DMSO, dimethylsulphoxide; EFS, electrical field stimulation; ENS, enteric nervous system

Introduction

5-HT$_{2B}$ receptors (nomenclature follows Alexander *et al.*, 2008) are expressed by the smooth muscle of the adult human gut (Borman *et al.*, 2002), where activation of the receptor can evoke contraction of the longitudinal muscle of various gastrointestinal regions and species, including the rat isolated forestomach (Baxter *et al.*, 1994), human terminal ileum (Borman and Burleigh, 1997) and colon (Borman *et al.*, 2002). 5-HT$_{2B}$ receptor activation is also reported to facilitate neuronally mediated contractions of the human isolated colon (Borman *et al.*, 2002), although it is not clear from these experiments if this activity is due to a non-specific increase in muscle excitability or a direct action on neuronal function. Nevertheless, 5-HT$_{2B}$ receptors have been found within the myenteric nervous system of human (Borman *et al.*, 2002) and mouse (Fiorica-Howells *et al.*, 2000) intestine, where a

Correspondence: Dr GJ Sanger, E-mail: Gareth.J.Sanger@gsk.com; gareth.sanger@tiscali.co.uk
Received 27 October 2008; revised 14 November 2008; accepted 16 December 2008

role in the development of the enteric nervous system (ENS) has been suggested (Fiorica-Howells *et al.*, 2000).

It is not known if the expression of the 5-HT$_{2B}$ receptor within the ENS reflects a localization of the receptor to enteric motor and/or intrinsic primary afferent neurons, or indeed, if such a distribution can affect intestinal motility. To explore this possibility, it is necessary to use preparations where the intact neurocircuitry is allowed to operate. For studies *in vitro*, this can be achieved by investigating the effects of substances on the peristaltic reflex, and then comparing the findings with studies *in vivo*. In the present investigation, we have looked at the effects of the 5-HT$_{2B}$ receptor antagonist RS-127445 (Bonhaus *et al.*, 1999) on electrically stimulated, neuronally mediated, contractions of isolated colon, on peristaltic contractions and on the defecation behaviour of rodents. However, although reported as a selective 5-HT$_{2B}$ receptor agonist, any effects of RS-127445 on the 5-HT$_4$ receptor are unknown. It was important, therefore, to begin by studying the effects of RS-127445 on the 5-HT$_4$ receptor, given the major influence of this receptor on intestinal function. Our results confirm the selectivity of RS-127445 for the 5-HT$_{2B}$ receptor and suggest that 5-HT$_{2B}$ receptors play a tonic role in regulating the motility of the colon. These data may have profound implications in understanding how endogenous 5-HT can regulate colonic function.

Methods

Animals

All animal care and procedures were in accordance with the UK Animals (Scientific Procedures) Act 1986 and all efforts were made to minimize the number of animals used.

Pharmacological characterization of RS-127445 at the 5HT$_4$ receptor

The experiments were performed using a Biomolecular Interaction Detection (BIND) system (SRU Biosystems; Cunningham *et al.*, 2004), a label-free, cell-based platform which employs a biosensor to monitor ligand-induced responses in living cells (Fang *et al.*, 2008); the biosensor utilizes an optical transducer to convert a ligand-induced change in a cell layer into a quantifiable signal. In brief, human embryonic kidney (HEK) macrophage scavenger II (MSRII) cells were cultured in Earle's modified essential medium, 10% foetal bovine serum (FBS), 1% non-essential amino acids, 200 mmol·L^{-1} glutamine and 0.8% geneticin (Invitrogen). Cells were grown until 80% confluent before the human 5HT$_{4(a)}$ receptor (Bockaert *et al.*, 2004) was transiently expressed utilizing a BacMam virus expression system. Cells were removed from the flask by Versene treatment and cells resuspended in the above media containing 10% dialysed FBS. Human 5HT$_{4(a)}$ receptor virus was added to the cell suspension and incubated for 5 min. Cells were then plated at 10^5 cells per well in an SRU TiO 96-well Biosensor plate (SRU Biosystems) and incubated at room temperature for 20 min. The plates were then placed in an atmosphere of 95% O_2 and 5% CO_2 at 37°C and left for 24 h. On the day of the experiment, plates were placed for 30 min at room temperature for equilibration. Cells were then washed with phenol red free Dulbecco's modified Eagle's medium (DMEM) supplemented with 20 mmol·L^{-1} HEPES at pH 7.4. Another 30 min incubation period followed to allow the cells to aclimatize to the new media. Plates were then placed on the reader and baseline measurements recorded for 5 min. All drugs were diluted in the DMEM/HEPES buffer.

Following 30 min incubation, agonist studies were conducted by constructing half log unit concentration-response curves (0.01 nmol·L^{-1}–1 μmol·L^{-1}). After addition of each concentration of the agonist, the cellular response was recorded for 20 min. Each agonist concentration-response curve was constructed using a four-parameter logistic equation from GraphPad Prism software as follows: Y = Bottom + (Top – Bottom)/1 + 10(logEC_{50} – X)^{nH}. The concentration of agonist that produced a half-maximal response was represented by the EC_{50} value, the logarithm of which yielded the *p*EC_{50} value. For antagonist studies, a range of concentrations of antagonist (0.1 nmol·L^{-1}–10 μmol·L^{-1}) were tested against an EC_{80} concentration of 5-HT (previously determined from a 0.1 nmol·L^{-1} to 10 μmol·L^{-1} concentration-response curve) by pre-incubating with the cells for 30 min prior to the addition of 5-HT (for a further 20 min). The IC_{50} value, the concentration producing half the maximum inhibition, was extrapolated and corrected by the Cheng-Prusoff correction: $K_B = IC_{50}/(1 + A/EC_{50})$, where A = the final assay concentration of agonist used (Cheng and Prusoff, 1973). pK_B was subsequently calculated using the equation: pK_B = –log(K_B).

Electrical field stimulation (EFS) of isolated colon

Experiments were performed as previously described by Bassil *et al.* (2005). Briefly, adult male C57BL/6J mice (25–30 g) were killed by a rising concentration of CO_2, followed by cervical dislocation. Following a midline incision, colons were blunt-dissected and placed immediately in Krebs solution (in mmol·L^{-1}; NaCl 121.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 5.6) previously equilibrated with 5% CO_2 in O_2.

Full wall thickness strips of distal colon (~10 × ~5 mm) were cut parallel to the circular muscle and were suspended under 10 mN of tension for isometric recording between two parallel platinum ring electrodes in 5 mL tissue baths containing Krebs solution bubbled with 5% CO_2/95% O_2, maintained at pH ~7.4 and 37°C. Tension was measured using isometric force transducers (MLT0201/D, AD Instruments, Chalgrove, UK). Data acquisition and analysis were performed using MP100 hardware and AcqKnowledge® software (Biopac Systems, Inc., Santa Barbara, CA, USA). Tissues were allowed to equilibrate for at least 45 min during which time bath solutions were changed every 15 min. EFS was achieved using monophasic square-wave pulses of 0.2 ms pulse width, 5 Hz frequency at a maximally effective voltage (50 V; Digitimer, Welwyn Garden City, UK) for 10 s at 2 min intervals for 30 min periods; each period was separated by a 5 min interval in which the bath solutions were changed. These parameters of EFS were selected as those that consistently evoked nerve-mediated responses with a good signal-to-noise ratio over background spontaneous muscle activity. The effects of RS-127445 (1 nmol·L^{-1}–10 μmol·L^{-1}, single concentration per

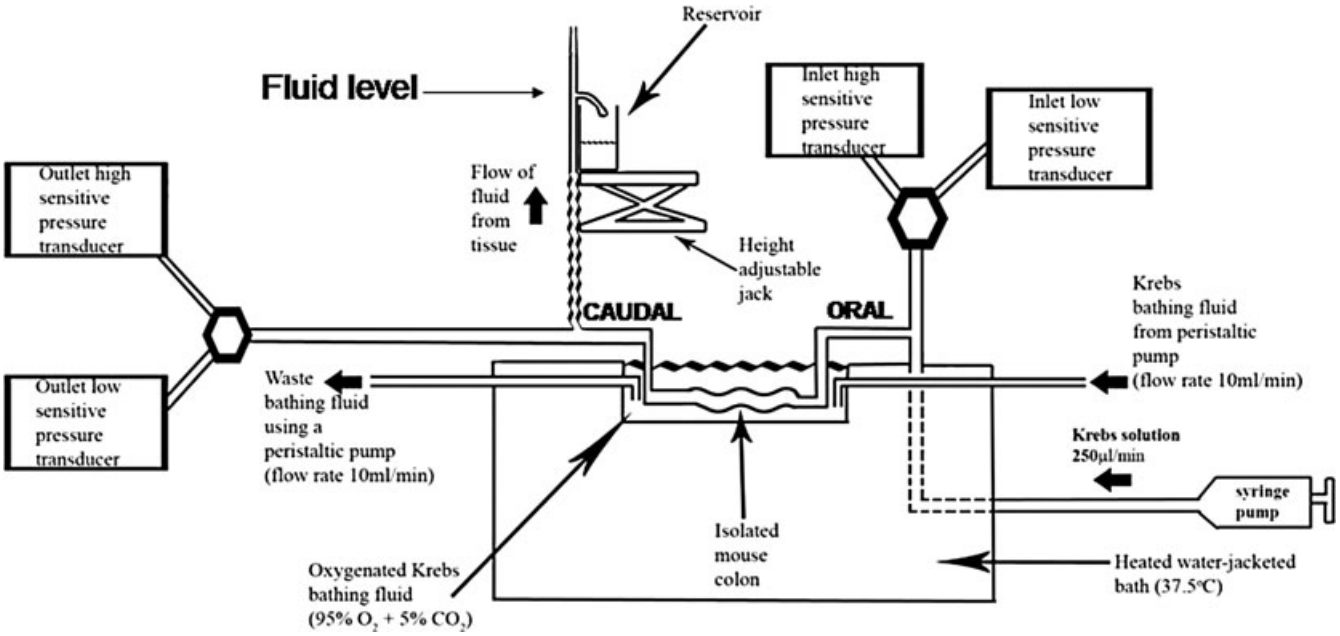


Figure 1 Apparatus for initiating and recording peristalsis in mouse isolated colon. Using this equipment, peristalsis was induced by applying an intraluminal pressure by raising the reservoir to 3.5 cm above the height of the tissue in the bath (see Methods).

tissue, 15 min contact time) or vehicle [5 or 50 µL dimethylsulphoxide (DMSO)] were expressed as the percentage change in amplitude compared with the mean amplitude of four pre-drug, post-EFS contractile responses. The results were analysed using a two-sample equal variance *t*-test.

Peristalsis of isolated colon

Adult male C57BL/6J mice (25–35 g) were killed and colons removed as described above. The tissues were cut free from the caecum and mesentery and placed in a custom-made 80 mL tissue bath (Applied Technologies, GSK, Harlow, UK; see Figure 1 for apparatus setup) containing Krebs solution, bubbled with 5% CO_2/95% O_2, maintained at pH ~7.4 and 37°C. To minimize mucosal degradation, it was important that the entire procedure, from resection to placing in the bath, was completed within approximately 10 min. All efforts were made to refrain from touching or stretching the colon except at the oral and aboral ends. The oral end of the colon was attached via an inlet cannula and tubing to a 50 mL syringe mounted in a syringe pump and filled with Krebs solution. A flow of Krebs solution into the colon was initiated with a flow rate of 450 µL·min^{-1} to allow the luminal contents to empty. Once empty, the flow into the colon was halted and the aboral end of the colon was attached to an outlet cannula. Both the inlet and outlet cannulas were connected to tubing containing a three-way micro stop-tap and a high- and low-sensitivity pressure transducer [2× high sensitive pressure transducers (high sensitivity: P75, Hugo Sachs, Germany; low sensitivity: MLT 844, ADInstruments, UK)]. The aboral end of the colon was also connected via tubing to a vertical glass reservoir with an overflow chute. This glass tube was mounted on a platform which could be lowered or raised to change the back pressure on the colon. Once the colon was mounted, the bath was emptied of any fluid using a vacuum and immediately refilled with fresh oxygenated Krebs solution. After a 5 min period to allow the bath temperature to equilibrate, the tissue was stretched to approximately 0.5 cm over that of its initial length of 3–4 cm. After a further 5 min, the valve of the syringe pump was opened and the syringe pump was started at a lower flow rate of 250 µL·min^{-1}. Tissues were then left for another 15 min before applying an intraluminal pressure by increasing the height of the glass reservoir to 3.5 cm above the height of the tissue in the bath to initiate peristaltic contractions. Tissues were then left for approximately 1 h to enable peristalsis to stabilize. Once a consistent peristaltic activity had been observed (visually confirmed as a movement from the oral to the aboral end of the colon), a single concentration of RS-127445 or vehicle was added to the bath (serosal application) and measurements made for the next eight contractions (further additions of RS-127445 or vehicle to the same tissue were not attempted, to minimize time-dependent variability). The effects of RS-127445 on the frequency of peristaltic contractions were measured and expressed as a percentage change in frequency compared with the mean of the frequency of 10 pre-drug contractions. Where tissues failed to give typical and/or consistent basal peristaltic contractions, they were discarded from further evaluation in the experiment. Data acquisition and analysis of intraluminal pressure changes were performed using MP100 hardware and AcqKnowledge® software (Biopac Systems, Inc., Santa Barbara, CA, USA). Data are expressed as means ± standard error of the mean; *n*-values are numbers of tissues examined. The effect of compounds compared with vehicle upon the percentage change in frequency of peristaltic contractions was analysed using a repeated measures model.

Defecation

Male Sprague-Dawley rats (body weight, 180–300 g) obtained from Charles River Laboratories (Thanet, UK) were used. Rats

were singly housed on wire bottom cages under conditions of controlled humidity, temperature (22°C–24°C) and reversed illumination (12 h light cycle starting at 7:00 p.m.) for at least 3 days before the experiments, with *ad libitum* access to food and water.

Animals were given the test compound (RS-127445 1, 3, 10 or 30 mg·kg^{-1}) or vehicle (10% ethanol, 40% polyethylene glycol, 50% distilled H_2O) intraperitoneally in a final dose volume of 3 mL·kg^{-1}. Following drug administration, animals were returned to the home cage. Three hours following treatment, the faecal output was collected and weighed; pellets were collected onto paper tray liners, to minimize contamination from urine or water. Because of a high variability in the numbers and sizes of faecal pellets excreted by individual rats, the results are expressed as the mean wet weight ± SEM of pellets excreted. The effect of compound administration compared with vehicle upon the faecal wet weight was analysed using an ANOVA with Bonferroni comparison of multiple variables. $P < 0.05$ was considered statistically significant.

Determination of fraction unbound (fu) in blood and brain for RS-127445

The methodology employed in this study was as described by Summerfield *et al.* (2006). In brief, 96-well equilibrium dialysis was used to determine the free fraction of RS-127445 in blood and brain. Fresh rat blood was diluted 1:1 with PBS and fresh rat brain was homogenized 1:2 with PBS. Known amounts of RS-127445 were added to the resulting diluted blood and brain homogenate to produce a 1000 ng·g^{-1} incubation solution. Aliquots (100 µL; six replicates) of each mixture were inserted into the bottom half of the 96-well equilibrium dialysis plate and the equivalent volume of PBS added to the top half of the wells. Dialysis versus PBS was carried out for 5 h in an incubator at 37°C, using an orbital microplate shaker to mix the samples. Following incubation the blood, brain homogenate and PBS aliquots were extracted, mixed and centrifuged. The unbound fraction of RS-127445 was determined, following sample analysis by means of HPLC/MS/MS, as the ratio of the peak area in buffer to that in blood or brain, with correction for dilution factors according to the following equation (Kalvass and Maurer, 2002):

$$\mathrm{fu} = \frac{1/D}{1/\mathrm{fu\,(apparent)} + 1/D}$$

where D = dilution factor in blood or brain homogenate and fu(apparent) is the measured free fraction of diluted blood or brain tissue.

Materials used

5-HT hydrochloride was obtained from Sigma, UK. RS-127445 and the 5-HT$_4$ receptor agonist (tegaserod) and antagonist (SB-207266; Wardle *et al.*, 1996) were each synthesized in-house. RS-127445 was freshly prepared prior to use. For isolated tissue studies, RS-127445 was dissolved in 100% DMSO and these solutions were then added to the bathing solution in amounts which gave a bath concentration of DMSO of 0.1 or 1% (EFS experiments) or 0.01% (peristalsis experiments). For the experiments *in vivo*, RS-127445 was dissolved in 10% ethanol, 40% polyethylene glycol, 50% distilled H_2O at a maximum concentration of 10 mg·mL^{-1}. For the *in vitro* fu studies, RS-127445 was dissolved in 100% DMSO and then added to the biological samples such that the final volume contained no more than 1% DMSO.

Results

Pharmacological characterization of RS-127445 at the 5HT$_4$ receptor

RS-127445, in concentrations up to 1 µmol·L^{-1}, had no effects on the baseline response of the HEK293 MSRII cells expressing the human 5HT$_{4(a)}$ receptor (n = 3); higher concentrations were not tested. By contrast, 5-HT and tegaserod evoked concentration-dependent positive responses (respectively, pEC$_{50}$ values were 7.98 ± 0.09 and 8.10 ± 0.06), whereas SB-207266 gave a concentration-dependent negative BIND response (pIC$_{50}$ = 8.16 ± 0.11); n = 3 each. Further, RS-127445 (up to 1 µmol·L^{-1}) did not antagonize the effects of an EC$_{80}$ concentration of 5-HT in this system, in contrast to the antagonism demonstrated with SB-207266 (pK$_B$ values were, respectively, <6 and 8.79 ± 0.07; n = 3 each).

EFS of isolated colon

RS-127445 (1 nmol·L^{-1}–1 µmol·L^{-1}) had no effect on the amplitude of the EFS-induced contraction in mouse isolated colon circular muscle (n = 4 for each concentration, $P > 0.05$). At 10 µmol·L^{-1} a decrease in the size of the EFS contraction was recorded; this was measured as 36 ± 8% of the contraction amplitude measured before administration of RS-127445, compared with 73 ± 7% of the pre-drug contraction with vehicle (n = 4, $P < 0.01$, Figure 2).

Peristalsis

RS-127445 (1 nmol·L^{-1}–10 µmol·L^{-1}) had no consistent effects on the amplitude of the peristaltic contractions. However, RS-127445 (10 nmol·L^{-1}–10 µmol·L^{-1}) concentration-dependently reduced the frequency of these contractions. This reduction was highly statistically significant ($P < 0.002$) at 100 nmol·L^{-1} or above. The tendency for 10 nmol·L^{-1} RS-127445 to reduce the frequency of peristaltic contractions did not, however, achieve statistical significance (P = 0.09; Figure 3). The magnitude of reduction in frequency induced by 100 nmol·L^{-1} RS-127445 was similar to that induced by an approximately threshold concentration of morphine (300 nmol·L^{-1}). In this preparation, 1 µmol·L^{-1} morphine totally abolished peristaltic contractions (n = 4; data not shown).

Defecation

Control values for faecal output over a 3 h collection period were 1.08 ± 0.12 g wet weight. Animals pretreated with RS-127445 displayed a significant reduction in the faecal output compared with control animals, the difference achieving statistical significance at 10 mg·kg^{-1} and 30 mg·kg^{-1}

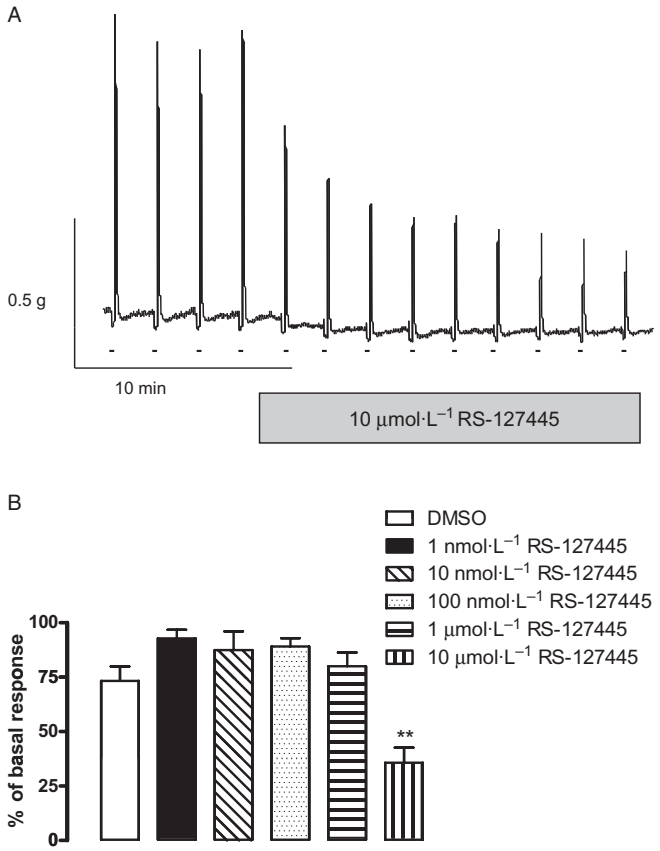


Figure 2 The effects of RS-127445 on EFS-evoked, nerve-mediated contractions in mouse isolated colon circular muscle. (A) Representative trace of the effects of RS-127445 (10 μmol·L^{-1}). The bars indicate the periods of EFS, as described in the Methods. (B) RS-127445 1 nmol·L^{-1}, 10 nmol·L^{-1}, 100 nmol·L^{-1} and 1 μmol·L^{-1} had no effect on EFS-evoked, nerve-mediated contractions in mouse isolated colon circular muscle compared with vehicle controls (0.1 or 1% DMSO; data combined); $n = 4$, $P > 0.05$. RS-127445 10 μmol·L^{-1} significantly reduced the contraction amplitude; $n = 4$, $^{**}P < 0.01$. Data expressed as the mean ± SEM change in pre-drug contractions. EFS, electrical field stimulation.

(n = 6–11, $P < 0.05$, Figure 4). Morphine (3 mg·kg^{-1}) in a separate experiment also produced an inhibition of faecal wet weight (0.27 ± 0.08 g vs. 1.21 ± 0.13 g for vehicle treated animals; $n = 10$; $P < 0.001$).

Fu

RS-127445 had a low fu in blood and brain. The fu(blood) was 0.0175 ± 0.0012. The fu(brain) was 0.0040 ± 0.0003. Therefore, RS-127445 is highly bound in both blood (98.25%) and brain (99.60%).

Discussion

5-HT$_{2B}$ receptor-induced muscular contraction that does not involve neuronal activation has previously been demonstrated in various gastrointestinal regions in rats and humans (Baxter *et al.*, 1994; Borman and Burleigh, 1997; Borman *et al.*, 2002). However, the presence of 5-HT$_{2B}$ receptors in the myenteric nervous system suggests another possible role for the receptor which involves neuronal participation. To fully investigate this potential neuronal role, we used mouse isolated colon models of colonic motility and compared our

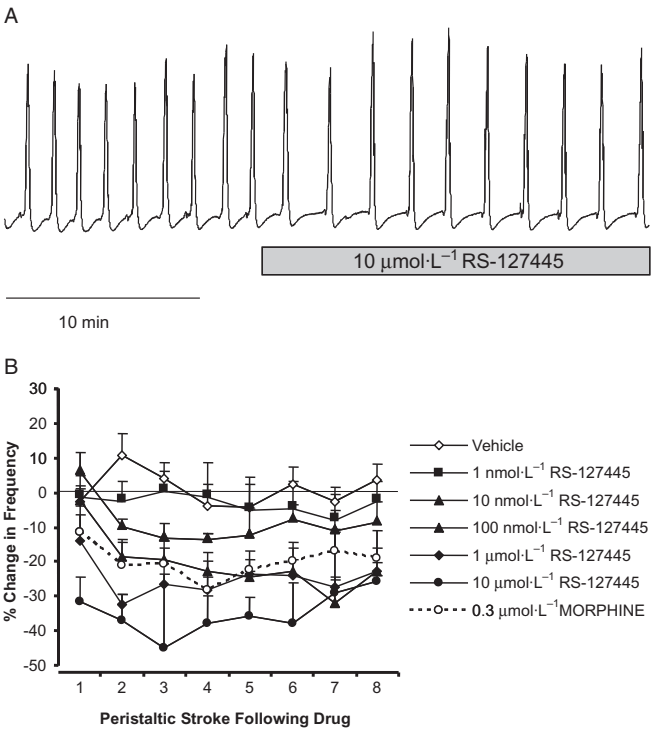
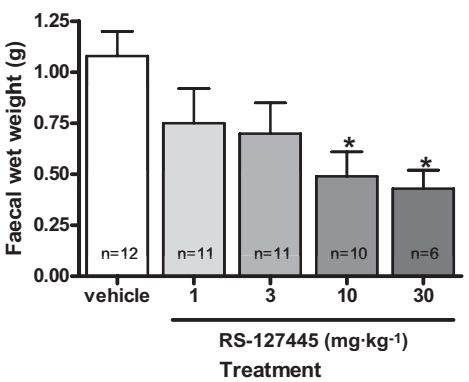


Figure 3 The effects of RS-127445 on the frequency of peristaltic contractions in mouse isolated colon. (A) Representative trace of the effects of RS-127445 (10 μmol·L^{-1}). (B) RS-127445 100 nmol·L^{-1}, 1 μmol·L^{-1} & 10 μmol·L^{-1} concentration-dependently reduced the overall frequency of peristaltic contractions in mouse isolated colon compared with vehicle controls (0.1% DMSO; $P < 0.002$ each); the overall effects of the lower concentrations of RS-127445 were not statistically significant ($P = 0.09$). Morphine 300 nmol·L^{-1} is included as a reference, where the overall reduction in frequency of contractions was again statistically significant ($P < 0.002$). Data expressed as the mean ± SEM change relative to the frequency of pre-drug contractions; n = 2–3 at each concentration.



*p<0.05 ANOVA with Dunnetts post-test analysis for multiple comparisons

Figure 4 The effects of RS-127445 on faecal output in the rat. RS-127445 when given i.p. to free-feeding rats dose-dependently reduced the faecal output over a 3 h period, compared with the vehicle controls. The data are expressed as means ± SEM; n = 6–11. The doses of 10 and 30 mg·kg^{-1} achieved statistical significance ($^{*}P < 0.05$; ANOVA).

findings with faecal output *in vivo*. The 5-HT$_{2B}$ receptor antagonist used in these experiments was RS-127445 (Bonhaus *et al.*, 1999). This compound has previously been described as a selective 5-HT$_{2B}$ receptor antagonist, but although selectivity was demonstrated against most known 5-HT receptors (1000-fold over other receptors studied), information about any potential activity at the 5-HT$_4$ receptor was not included. Given that activation of this receptor can exert profound effects on intestinal motility (Beattie and Smith, 2008), it was necessary to further demonstrate the selectivity of RS-127445 by evaluating its actions at the 5-HT$_4$ receptor. In the present experiments, RS-127445 was found not to activate or antagonize at the human 5HT$_4$ receptor, at concentrations up to 1 μmol·L^{-1}.

RS-127445 (1 nmol·L^{-1}–1 μmol·L^{-1}) had no effect on EFS-induced neuronal contractions but did significantly reduce contraction amplitude at 10 μmol·L^{-1}. It seems unlikely that this effect of 10 μmol·L^{-1} RS-127445 is mediated via the 5-HT$_{2B}$ receptor as a pK$_i$ of 9.5 is reported for this compound in binding studies with h5-HT$_{2B}$ and a pA$_2$ of 9.5 was obtained using rat isolated forestomach preparations contracted with 5-HT (Bonhaus *et al.*, 1999); the mechanism of action is, therefore, unknown. However, lower concentrations of RS-127445 concentration-dependently reduced peristaltic contraction frequency. This reduction was highly statistically significant at 100 nmol·L^{-1} and 1 μmol·L^{-1}, as well as at 10 μmol·L^{-1}; the tendency for 10 nmol·L^{-1} RS-127445 to reduce the overall frequency of contractions did not achieve statistical significance. Together, these data suggest that the effects observed with the lower concentrations of RS-127445 are not due to a non-specific inhibition of motor nerve activity (observed with 10 μmol·L^{-1} RS-127445 in the EFS experiments) but, instead, are caused by a specific effect of the compound on peristalsis, where the neurocircuitry needs to be functionally intact. Nevertheless, the effective concentrations of RS-127445 are higher than those reported to be active at the 5-HT$_{2B}$ receptor (see above), although within the known 1000-fold selectivity range for this compound (Bonhaus *et al.*, 1999). Given that in the present study RS-127445 is required to operate against the effects of endogenously released 5-HT in a dynamic model of intestinal activity, it seems reasonable to suggest that the effects observed are due to 5-HT$_{2B}$ receptor antagonism. However, further work is required with new 5-HT$_{2B}$ receptor antagonists to confirm this possibility, when such compounds become available.

Consistent with the *in vitro* data, RS-127445 also reduced faecal output *in vivo*, suggesting that the peristalsis model is a good predictor of defecation in rodents; in these studies, wet and dry weights of faecal pellets were not determined, as the intention was simply to try and validate the *in vitro* modelling. At first sight, the effective doses (10 and 30 mg kg^{-1} i.p.) seem higher than those which in rats (1–10 mg kg^{-1} i.p.), are reported to give blood plasma concentrations of RS-127445 above those required to antagonize at the 5-HT$_{2B}$ receptor (Bonhaus *et al.*, 1999). However, such a comparison cannot be used to suggest that in the present study inappropriately high doses of RS-127445 were used. Thus, plasma concentrations of compounds consist of both protein-bound and -unbound fractions. As it is the free fraction which is available for binding to the receptor, it is likely that the concentrations of RS-127445 provided by Bonhaus *et al.* (1999) do not reflect the doses of this compound that are required to fully antagonize at the 5-HT$_{2B}$ receptors within the gut or elsewhere. The fact that RS-127445 was shown to be highly protein bound (>98%) in both brain and blood confirms the need to exercise caution when interpreting such data.

The possibility that 5-HT$_{2B}$ receptors regulate colonic motility in a physiological manner, via the ENS, means that this receptor may join the 5-HT$_3$ receptor [where antagonists may cause constipation in healthy volunteers; Gore *et al.* (1990) and Talley *et al.* (1990)], as candidate 5-HT receptors through which endogenous 5-HT may affect colonic function during health. Further, our results could have an impact on interpreting the activity of the gastrointestinal prokinetic agent and non-selective 5-HT receptor ligand, tegaserod. Thus, this compound, which until recently was marketed in the USA for treatment of constipation-predominant irritable bowel syndrome, not only is thought to stimulate intestinal motility via activation of the 5-HT$_4$ receptor, but also acts at a number of other 5-HT receptors (De Maeyer *et al.*, 2008), including an ability to antagonize at the 5-HT$_{2B}$ receptor (Beattie *et al.*, 2004; McCullough *et al.*, 2006).

Our suggestion that endogenous 5-HT can act on 5-HT$_{2B}$ receptors to increase colonic motility now needs to be confirmed using other selective 5-HT$_{2B}$ receptor antagonists, perhaps with a lower protein binding characteristic. If confirmed, however, these finding will have profound implications on our understanding of how endogenous 5-HT can affect colonic function.

Acknowledgements

We would like to thank Steve Addicott (Applied Technologies, GSK, Harlow, UK) for his help in designing and making the tissue baths, Natalie Mitchell (Bradford University, UK) for her diagram of the peristalsis equipment and Kimberley Stephens for help in statistical analyses.

Conflicts of interest

The authors state no conflicts of interest.

References

Alexander SPH, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC), 3rd edn. *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Bassil AK, Dass NB, Murray CD, Muir A, Sanger GJ (2005) Prokineticin-2, motilin, ghrelin and metoclopramide: prokinetic utility in mouse stomach and colon. *Eur J Pharmacol* **524**: 138–144.

Baxter GS, Murphy OE, Blackburn TP (1994). Further characterisation of 5-hydroxytrptamine receptors (putative 5-HT$_{2B}$) in rat stomach fundus longitundinal muscle. *Br J Pharmacol* **112**: 323–331.

Beattie DT, Smith JAM (2008). Serotonin pharmacology in the gastrointestinal tract: a review. *Naunyn-Schmiedeberg's Arch Pharmacol* **377**: 181–203.

Beattie DT, Smith JA, Marquess D, Vickery RG, Armstrong SR, Pulido-Rios T *et al.* (2004). The 5-HT$_4$ receptor agonist tegaserod, is a potent

5-HT$_{2B}$ receptor antagonist *in vitro* and *in vivo*. *Br J Pharmacol* **143**: 549–560.

Bockaert J, Claeysen S, Compan V, Dumuis A (2004). 5-HT$_4$ receptors. *Curr Drug Targets CNS Neurol Disord* **3**: 39–51.

Bonhaus DW, Flippin LA, Greenhouse RJ, Jaime S, Rocha C, Dawson M *et al*. (1999). RS-127445: a selective, high affinity, orally bioavailable 5-HT$_{2B}$ receptor antagonist. *Br J Pharmacol* **127**: 1075–1082.

Borman RA, Burleigh DE (1997). 5-HT$_{1D}$ and 5-HT$_{2B}$ receptors mediate contraction of smooth muscle in human small intestine. *Annals NY Acad Sci* **812**: 222–223.

Borman RA, Tilford NS, Harmer DW, Day N, Ellis ES, Sheldrick RLG *et al*. (2002). 5-HT$_{2B}$ receptors play a key role in mediating the excitatory effects of 5-HT in human colon in vitro. *Br J Pharmacol* **135**: 1144–1151.

Cheng Y, Prusoff W (1973). Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 percent inhibition (IC$_{50}$) of an enzymatic reaction. *Biochem Pharmacol* **22**: 3099–3108.

Cunningham B, Li P, Schulz S, Lin B, Baird C, Gerstenmaier J *et al*. (2004). Label-Free Assays on the BIND System. J Biomolecular. *Screening* **9**: 481–490.

De Maeyer JH, Lebebvre RA, Schuurkes JAJ (2008). 5-HT$_4$ receptor agonists: similar but not the same. *Neurogastroenterol Motil* **20**: 99–112.

Fang Y, Frutos A, Verklereen R (2008). Label-Free Cell-Based Assays for GPCR Screening. *Comb Chem High Throughput Screen* **11**: 357–369.

Fiorica-Howells E, Maroteaux L, Gershon MD (2000). Serotonin and the 5-HT$_{2B}$ receptor in the development of enteric neurons. *J Neurosci* **20**: 294–305.

Gore S, Gilmore IT, Haigh CG, Brownless SM. Stockdale H, Morris AL (1990). Colonic transit in man is slowed by ondansetron (GR38032F), a selective 5-hydroxytryptamine receptor (type 3) antagonist. *Aliment Pharmacol Ther* **4**: 139–144.

Kalvass JC, Maurer TS (2002). Influence of non-specific brain and plasma binding on CNS exposure: implications for rational drug discovery. *Biopharm Drug Dispos* **23**: 327–338.

McCullough JL, Armstrong SR, Hegde SS, Beattie DT (2006). The 5-HT$_{2B}$ antagonist and 5-HT$_4$ agonist activities of tegaserod in the anaesthetized rat. *Pharmacological Research* **53**: 353–358.

Summerfield S, Stevens A, Cutler L, Osuna MC, Hammond B, Tang SP *et al*. (2006). Improving the in vitro prediction of in vivo CNS penetration: integrating permeability, P-gp efflux and free fractions in blood and brain. *J Pharmaco Exp Ther* **316**: 1282–1290.

Talley NJ, Phillips SF, Haddad A *et al*. (1990). GR38032F (ondansetron), a selective 5-HT$_3$ receptor antagonist, slows colonic transit in healthy man. *Dig Dis Sci* **5**: 477–480.

Wardle KA, Bingham S, Ellis ES *et al*. (1996). Selective and functional 5-hydroxytryptamine$_4$ receptor antagonism by SB 207266. *Br J Pharmacol* **118**: 665–670.