The selective 5-HT$_{1A}$ antagonist, AZD7371 (robalzotan tartrate monohydrate), inhibits visceral pain-related visceromotor, but not autonomic cardiovascular, responses to colorectal distension in rats

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Running title: AZD7371 and visceral pain in rats: Clinical significance

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Abbreviations:
5-HT$_{1A}$: 5-hydroxytryptamine 1A
bpm: Beats per minute
CRD: Colorectal distension
ICV: Intracerebroventricular
IBS: Irritable Bowel Syndrome
VMR: Visceromotor response

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Abstract

5-hydroxytryptamine 1A (5-HT$_{1A}$) receptors have been suggested as a target for the treatment of irritable bowel syndrome (IBS). A recent clinical trial investigating the efficacy of the selective 5-HT$_{1A}$ antagonist AZD7371 showed no symptomatic improvement in IBS patients. We characterized the mechanisms mediating potential analgesic effects of AZD7371 [3(R)-(N,N-Dicyclobutylamino)-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide (R,R)-tartrate monohydrate] in a model of colorectal distension (CRD)-induced visceral pain in rats to understand its mechanism of action and the lack of clinical efficacy. Visceromotor and cardiovascular responses (telemetry) were assessed in conscious rats during noxious CRD (80 mmHg). Effects of AZD7371 (3-300 nmol/kg, iv; 1-30 µmol/kg, po) and a reference 5-HT$_{1A}$ antagonist, WAY-100635 (3-300 nmol/kg, iv), were assessed. Effects of intracerebroventricular (ICV) AZD7371 were also evaluated. Intravenous AZD7371 or WAY-100635 and oral AZD7371 dose-dependently inhibited visceromotor responses to CRD (ED$_{50}$s: 203 nmol/kg, 231 nmol/kg and 14 µmol/kg, respectively). In telemetrized rats, oral AZD7371 inhibited visceromotor responses to CRD without affecting the concomitant hypertensive and tachycardic responses. ICV AZD7371 did not affect visceromotor responses, while it inhibited micturition. None of the doses tested induced visible gross side-effects. AZD7371, likely acting at a spinal site, inhibited the visceromotor, but not the cardiovascular, responses to visceral pain in the CRD model in rats. While agents effective on multiple pain-related readouts in the CRD model (e.g. pregabalin or clonidine) alleviate IBS symptoms, AZD7371,
effective on only one pain-related pseudo-affective readout, does not. Data from preclinical CRD models of visceral pain need to be interpreted cautiously as it relates to their clinical translational value.
Introduction

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by symptoms of abdominal pain or discomfort associated with altered bowel habits (Azpiroz et al., 2007). Even if the etiology of IBS has been suggested to involve multiple factors, including, at least, abnormal central processing of gastrointestinal sensory signals, psychosocial disturbance, altered gastrointestinal motility, and visceral hypersensitivity (Drossman et al., 2002; Azpiroz et al., 2007) the pathophysiology of the disease remains largely unknown. The neurotransmitter and gut mucosal signaling molecule 5-hydroxytryptamine (5-HT, serotonin) plays a central role in normal gastrointestinal tract function through its modulatory effects on gut motility, intestinal secretion, and visceral sensitivity (Mawe et al., 2006, Gershon and Tack, 2007). Correspondingly, there is evidence that serotonin-mediated signaling is altered in individuals with IBS (Mawe et al., 2006). Indeed most of the treatments for IBS to date have been designed to modulate serotonin signaling, such are the 5-HT₃ antagonist alosetron and the 5-HT₄ partial agonist (and 5-HT₁B₁ and 5-HT₂B receptor antagonist) tegaserod (Spiller, 2008). Other serotonin receptors have been proposed as potential targets for the treatment of IBS, including 5-HT₁A, 5-HT₂ and 5-HT₇ (Danzebrink and Gebhart, 1991; Coelho et al., 1998, 2001; Sivarao et al., 2004; Tonini et al., 2005). For instance, it has been shown that the 5-HT₁A antagonist, WAY-100635, inhibits pain-related responses to colorectal distension (CRD) in rats (Coelho et al., 1998) and 5-HT₇ might affect sensitivity through the modulation of colonic compliance (Tonini et al., 2005).
The aims of the present study were to gain insight into the effects and mechanism of action of the selective, competitive, 5-HT₁A receptor antagonist AZD7371 tartrate monohydrate (robalzotan tartrate monohydrate, NAD299, here termed AZD7371) (Johansson et al., 1997) on visceral pain-related visceromotor and autonomic cardiovascular responses in a model of colorectal distension-induced visceral pain in conscious rats. AZD7371 was initially developed as a potential treatment for depression and anxiety disorders (Mucke, 2000). However, based on previous observations with the 5-HT₁A antagonist WAY-100635 (Coelho et al., 1998) and also in part of the preclinical observations presented in the present report, it was suggested that AZD7371 would be effective in reducing abdominal pain and discomfort in patients with IBS. In addition, because mental stress, anxiety, and depression have been implicated in the pathophysiology of IBS (Mayer et al., 2001), it was speculated that the potential anxiolytic properties of AZD7371 might provide further benefits in IBS patients. Nevertheless, the results obtained in a clinical study in IBS patients showed no symptomatic improvement over placebo, leading to discontinuation of development (Drossman et al., 2008). Therefore, we also aimed to understand the discrepancy between the positive preclinical findings and the negative clinical observations with AZD7371.
Methods

Animals

Adult female Sprague–Dawley rats (Harlan, The Netherlands; 250–300 g) were used. The rats were allowed to acclimatize to the animal facility for at least 1 week after arrival. Rats were housed in groups of five, unless otherwise stated, in an enriched environment with free access to food (Standard pellets, R3, Lactamin, Sweden) and water under controlled conditions of temperature (21 °C) and humidity (50%) on a 12:12 h light–dark cycle. The phase of the estrous cycle was not taken into consideration in the current study. All experiments were approved by the local animal ethics review committee in Göteborg, Sweden. All procedures were in accordance with current European laws concerning animal experimentation.

Surgical preparation

Implantation of radio transmitters

When assessing cardiovascular responses, a telemetric system was used. Rats were anesthetized with a mixture (2 ml kg/kg, i.p.) of ketamin (88 mg/kg; Ketalar® Vet; Pfizer AB, Täby, Sweden) and xylazin (5 mg/kg; Rompun® Vet; Bayer AG, Leverkusen, Germany) and were surgically equipped with intraperitoneal radio transmitters (PhysioTel® C50-PXT, DSI, St. Paul, MN, USA). The catheter of the transmitter was inserted into the abdominal aorta and fixed with tissue adhesive (Vetbond®, 3M, St. Paul, MN, USA) for blood pressure measurements. The animals recovered from surgery in a quiet and dim post-op room for 24 h and received also antibiotic (Bactrim®, Roche, Basel Switzerland) and analgesic
(Finadyne®, Shering-Plough, NJ, USA) treatment. Thereafter, a 7- to 10-day recovery period was allowed before starting any experimental procedures.

**Implantation of intracerebroventricular (ICV) canulae**

For ICV administrations rats with chronically implanted ICV canulae were used. Rats were anesthetized with a mixture (2 ml kg/kg, i.p.) of ketamin (88 mg/kg; Ketalar® Vet; Pfizer AB, Täby, Sweden) and xylazin (5 mg/kg; Rompun® Vet; Bayer AG, Leverkusen, Germany) and the ICV guide canulae were stereotaxically implanted into the right lateral brain ventricle according to coordinates in the Paxinos and Watson’s atlas (Paxinos and Watson, 1998), i.e. from the bregma posterior -0.8 mm; lateral -1.5 mm and dorsoventral -3.5 mm, as previously described (Martinez and Taché, 2001). After surgery animals were housed in individual cages with direct bedding and allowed a 7 day recovery period before starting any experiment.

**Colorectal distension (CRD)**

Rats were habituated to Bollmann cages (Plexi-glass tubes, length 18 cm, diameter 6 cm, AstraZeneca, Mölndal, Sweden) 30 min per day for three consecutive days prior to experiments, to reduce motion artifacts and confounding effects due to stress-related responses.

A 3 cm polyethylene balloon (made in-house) with connecting catheter (PE-50) was inserted in the distal colon, 2 cm from the base of the balloon to the anus, during light isoflurane anesthesia (Forene®, Abbott Scandinavia AB, Solna, Sweden). The catheter was fixed to the tail with tape. At the same time, if needed, an intravenous catheter (Neoflon®, Becton Dickinson AB, Helsingborg,
Sweden) was inserted in the tail vein for vehicle or compound administration. The intravenous catheter was flushed with 0.2 ml of heparin (50 IE/KY/ml, Leo Pharma, Ballerup, Denmark). The balloons were connected to pressure transducers (P-602, CFM-k33, 100 mmHg, Bronkhorst HI-TEC, Veenendal, The Netherlands). Rats were allowed to recover from sedation in the Bollmann cages for at least 15 min before the start of experiments.

A customized barostat (AstraZeneca, Mölndal, Sweden) was used to manage air inflation and balloon pressure control. A customized computer software (PharmLab on-line 5.0) running on a standard computer was used to control the barostat and to perform data collection. The distension paradigms generated by the barostat were achieved by generating pulse patterns on an analog output channel. For the assessment of visceral pain responses, the CRD paradigm consisted of repeated phasic distensions, 12 times at 80 mmHg, with a pulse duration of 30 s at 5 min intervals. This protocol has been used before to assess noxious responses to colorectal distension in rats (Käll et al., 2007; Martinez et al., 2007; Brusberg et al., 2008, 2009a; Lindström et al., 2008; Ravnefjord et al., 2008).

**Data collection and analysis**

The analog input channels were sampled with individual sampling rates, and digital filtering was performed on the signals. The balloon pressure signals were sampled at 50 samples/s. A highpass filter at 1 Hz was used to separate the contraction-induced pressure changes from the slow varying pressure generated by the barostat. A resistance in the airflow between the pressure generator and the pressure transducer further enhanced the pressure variations
induced by abdominal contractions of the animal. Data analysis was performed using pre-designed automatic analysis paradigms. Hence, manual analysis and potential bias by the investigator was avoided. Data represent the average rectified value of the highpass-filtered balloon pressure signals, calculated for 30 s before the pulse (i.e. baseline response) and for the duration of the pulse, and is given in arbitrary units. When calculating the magnitude of the highpass-filtered balloon pressure signals, the first and last seconds of each pulse were excluded since these reflect artifact signals produced by the barostat during inflation and deflation and do not originate from the animal.

**Drugs**

AZD7371 [3(R)-(N,N-Dicyclobutylamino)-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide (R,R)-tartrate monohydrate, robalzotan tartrate monohydrate, NAD299; AstraZeneca R&D] (Johansson et al., 1997) and WAY-100635 [N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]- N-(2-pyridyl)cyclohexanecarboxamide maleate salt; Sigma-Aldrich] (Fletcher et al., 1993) were dissolved in 0.9% saline solution at the appropriate concentration. Saline solution was used as vehicle control.

**Experimental protocols**

*Dose-related effects of intravenous AZD7371 and WAY-100635 and oral AZD7371 on visceromotor responses to CRD*

AZD7371 (3, 10, 30, 100 or 300 nmol/kg; equivalent approximately to 1.5, 5, 15, 50 and 150 µg/kg, respectively), WAY-100635 (3, 10, 30, 100 or 300 nmol/kg; equivalent to approximately to 1.3, 4.2, 13, 42 and 130 µg/kg, respectively) or vehicle (0.9%
saline solution, 1 ml/kg) was administered i.v. between distensions 3 and 4 of the CRD protocol.

In a separate experiment, the dose-related effect of oral AZD7371 was also characterized. In this case, AZD7371 (1, 3, 10 or 30 µmol/kg; equivalent approximately to 0.5, 1.5, 5 and 15 mg/kg) or vehicle (0.9% saline solution, 2 ml/kg) was administered orally (p.o.) 30 min before starting the CRD procedure.

In all experiments, each rat received both vehicle and a dose of compound on different occasions, with at least 4 days between experiments. Hence, each rat served as its own vehicle control. Experiments were performed in a counterbalanced cross-over fashion in which vehicle and different doses of compounds were tested during the same experiment, and repeated in several occasions.

**Effects of repetitive oral dosing with AZD7371 on visceromotor responses to CRD**

Separate groups of rats received a daily oral dose of AZD7371 (30 µmol/kg) or vehicle (2 ml/kg) for 9 consecutive days. Visceromotor responses (VMRs) to CRD were assessed before starting any treatment and on days 1, 5 and 9 of treatment (30 min after the administration of the corresponding treatment).

**Effects of AZD7371 on autonomic cardiovascular responses to CRD**

Telemetrized animals were used for this experiment. Animals were dosed i.v. with either AZD7371 (100 or 300 nmol/kg, n=4) or vehicle (1 ml/kg, n=4) between distensions 3 and 4 of the CRD procedure. In this case, cardiovascular parameters (blood pressure and heart rate) and VMRs to CRD were assessed
simultaneously. The same group of animals was used in these studies, with a 4 day interval between the two treatments.

Effects of ICV AZD7371 on visceromotor responses to CRD and micturition

Rats with chronically implanted ICV canulae were used for these experiments. The ICV injection was performed in lightly restrained animals using a 28 ga injection canula, 1 mm longer than the guide canula, connected to a 50 µl Hamilton syringe by a PE-50 catheter filled with distilled water. A small air bubble (1 µl) was drawn at the distal end of the PE-50 catheter to separate the injecting solution from the water and for visual inspection of the 10 µl injection (Martinez and Taché, 2001). Either AZD7371 (1 µg/rat) or vehicle (sterile saline, 10 µl/rat) were injected slowly, over a 30 s period, between distensions 3 and 4 of the CRD paradigm. At the same time, a piece of pre-weighed absorbent paper was placed under each Bollmann cage to collect any urine excreted during the remaining experimental time. Differences in weight of the absorbent paper before and after the procedure were taken as a measure of micturition. All animals received two ICV injections, vehicle and treatment, with an interval of 4-5 days. At the end of the experiments, at the time of euthanasia, the correct location of the canula into the lateral ventricle was verified by injecting 10 µl of dye (0.1% toluidine blue). Visualization of dye on the wall of the lateral ventricle indicates correctness of the ICV injections.

Plasma levels of AZD7371

Separate groups of animals were used for the determination of plasma levels of AZD7371. Animals were dosed orally with AZD7371 (30 µmol/kg, 2
ml/kg, n=3) and blood samples were obtained 15, 30, 60, 75, 90, 120, 180, 240
and 350 min post-dosing in order to determine the complete pharmacokinetic
profile of AZD7371 following oral administration. Total plasma levels of
AZD7371 were determined using standard HPLC combined with mass
spectroscopy procedures (limit of quantification: 1 nmol/l).

**Statistical analysis**

Data are expressed as mean±S.E.M. Differences between two groups
were assessed by paired or unpaired Student’s t-test, as appropriate.
Differences between multiple groups were determined by a repeated or non-
repeated measures one-way analysis of variance (ANOVA), as appropriate,
followed, when necessary, by a Student-Newman-Keuls multiple comparisons
test. ED$_{50}$ values were determined by nonlinear regression to a sigmoidal
equation with variable slope (Prism, version 4.01; GraphPad Software Inc., San
Diego, CA). Data were considered statistically significant when P was <0.05.
Results

**Dose-related effects of AZD7371 and WAY-100635 on visceromotor responses to CRD**

CRD at 80 mmHg induced a VMR manifested as a 4-fold increase in the activity of the abdominal musculature, compared to basal activity (basal: 0.05±0.003; first distension at 80 mmHg: 0.20±0.02; P<0.05; pooled data from 36 animals). In animals treated with vehicle the response to CRD increased by 164±20% from the first to the last distension indicating the development of acute mechanical hyperalgesia (Fig. 1). AZD7371 (3, 10, 30, 100 or 300 nmol/kg, n=8 for each), administered iv between distensions 3 and 4, inhibited in a dose-dependent manner the response to CRD with an ED$_{50}$ of 203 nmol/kg (95% confidence interval: 109 - 377 nmol/kg, $r^2=0.3281$; Fig. 1A). The overall response to CRD during distensions 4$^{th}$ to 12$^{th}$ was inhibited by 9±6 %, 4±10 %, 30±14 % (P<0.05), 41±10 % (P<0.05) and 48±10 % (P<0.05) at 3, 10, 30, 100 or 300 nmol/kg, respectively [F(5,70)=3.752; P=0.0046]. Similar effects were observed with WAY-100635 [3, 10, 30, 100 or 300 nmol/kg, n=4-8 for each; F(5,66)=3.395; P=0.0086], which inhibited the response to CRD with an ED$_{50}$ of 231 nmol/kg (95% confidence interval: 113 - 473 nmol/kg, $r^2=0.2722$; Fig. 1B).

Similarly, oral AZD7371 (1, 3, 10 or 30 µmol/kg) inhibited the response to CRD with an ED$_{50}$ of 14 µmol/kg (95% confidence interval: 9 - 23 nmol/kg, $r^2=0.4543$). Analgesic effects of AZD7371 were visible from the first distension. In vehicle-treated animals, the activity of the abdominal musculature increased 5-fold during the first CRD (basal: 0.03±0.002; 1$^{st}$ CRD: 0.17±0.02; n=30; P<0.05) while after AZD7371 the response was reduced to 0.13±0.02,
0.11±0.02 (P=0.099), 0.12±0.04 (P=0.077) and 0.07±0.006 (P<0.05) at 1, 3, 10 and 30 µmol/kg, respectively (n=7-8 for each dose). After oral AZD7371, the overall response to CRD (distensions 1 to 12) was inhibited by 25±8 %, 22±12 %, 41±7% (P<0.05) and 55±8% (P<0.05) at 1, 3, 10 and 30 µmol/kg, respectively (Fig. 2). At 30 µmol/kg, total plasma levels of AZD7371 at the time interval corresponding to the CRD procedure ranged between 2.21±0.35 µmol/l (30 min post-dosing) and 0.75±0.06 µmol/l (90 min post-dosing) as determined in a satellite group of animals (Fig. 3). The calculated T_{max} after oral dosing (0.33±0.08 h) corresponded to the start of the CRD procedure.

Although not systematically assessed, no gross side effects were observed at the doses tested after either intravenous or oral administration.

Based on these observations, the doses of 300 nmol/kg iv and 30 µmol/kg po were selected for further studies.

**Effects of repetitive oral dosing with AZD7371 on visceromotor responses to CRD**

Visceromotor responses to CRD were of similar magnitude in all animals before starting any treatment. Daily treatment with vehicle for 9 consecutive days (2 ml/kg/day, n=8) did not affect the response to CRD assessed on days 1, 5 and 9 [F(3,7)=0.07723, P=9716; Fig. 4]. In AZD7371-treated animals, after a single oral dose (30 µmol/kg, n=8) the response to CRD was reduced by 43.4±9 % compared with the response observed before starting the treatment procedure (P<0.05; Fig. 4). Similar degree of inhibition was observed after 5 or 9 days of repeated daily treatment [42.6±11.8 % and 44.7±10.0 %, respectively; both P<0.05 vs the VMR before treatment; F(3,7)=8.75, P=0.0033; Fig. 4].
**Effects of ICV AZD7371 on visceromotor responses to CRD and micturition**

Visceromotor responses to CRD were of similar magnitude after the ICV administration of vehicle or AZD7371 (AUC for distensions 4-12; vehicle: 2.42±0.39; AZD7371: 2.58±0.54; n=7 for each, Fig. 5A).

Urine production during the 45 min post-treatment, corresponding to the time-interval of the CRD procedure, was reduced by 32 % after ICV AZD7371, when compared with the responses observed after vehicle administration (Fig. 5B). The inhibitory effects of AZD7371 on micturition were clear in 6 out of the 7 animals tested. Nevertheless, due to the variability imposed by one of the animals, statistical significance was not reached.

**Effects of AZD7371 on visceromotor and autonomic cardiovascular responses to CRD in telemetrized rats**

In telemetrized rats, CRD induced a VMR, similar in magnitude to that described above, and a simultaneous increase in blood pressure (mean increase: 16±6 mmHg) and heart rate (mean increase: 23±7 beats per minute, bpm). While, as described above, the VMR to CRD increased over time with consecutive distensions (106±21 % increase from the first to the last distension; P<0.05), cardiovascular responses remained relatively stable along the CRD protocol (Fig. 6). AZD7371 (100 or 300 nmol/kg), administered iv between distensions 3 and 4, inhibited the VMR to CRD by 27 % and 30 %, respectively (AUC for distensions 4-12; vehicle: 4.69±0.77; 100 nmol/kg: 3.46±0.50; 300 nmol/kg: 3.32±0.35; n=4 for each; P<0.05; Fig. 6A). In the same animals,
AZD7371 did not affect neither the hypertensive (mean increase in blood pressure; vehicle: 18±5 mmHg; 100 nmol/kg: 18±6 mmHg; 300 nmol/kg: 19±4 mmHg; P>0.05; Fig. 6B) nor the tachycardic responses elicited by CRD (mean increase in heart rate; vehicle: 37±8 bpm; 100 nmol/kg: 40±10 bpm; 300 nmol/kg: 34±6 bpm; P>0.05; Fig. 6C). AZD7371, per se, did not affect neither resting (between distensions) blood pressure (vehicle: 126±2 mmHg; 100 nmol/kg: 129±2 mmHg; 300 nmol/kg: 129±1 mmHg) nor resting (between distensions) heart rate (vehicle: 397±3 bpm; 100 nmol/kg: 410±7 bpm; 300 nmol/kg: 402±5 bpm)
Discussion

This study shows that the selective 5-HT$_{1A}$ antagonist, AZD7371, inhibited pain-related VMRs in a model of mechanically (CRD)-induced visceral pain in rats, likely through a peripheral or spinal mechanism. Interestingly, autonomic cardiovascular responses, generated concomitantly with the VMRs as part of the response to pain, were not affected by AZD7371.

Pseudo-affective VMRs to CRD are considered a valid surrogate maker of visceral pain in rodents and are widely used in pharmacological studies assessing the potential analgesic effects of compounds on visceral pain (Ness and Gebhart, 1988; Käll et al., 2007; Brusberg et al., 2008, 2009a; Lindström et al., 2008; Ravnefjord et al., 2008). Results obtained here show that AZD7371 dose-dependently inhibited the VMRs to CRD after systemic administration. After iv administration, AZD7371 and the 5-HT$_{1A}$ antagonist WAY-100635 displayed similar potency and efficacy. This, together with the previously reported analgesic effects of WAY-100635 in several pain models, including CRD-evoked visceral pain (Coelho et al., 2001; Wei and Pertovaara, 2006), and the high selectivity of AZD7371 for 5-HT$_{1A}$ receptors (Johansson et al., 1997) indicate that the effects observed are likely to correspond to a blockade of 5-HT$_{1A}$ receptors.

On the other hand, AZD7371 failed to affect the pseudo-affective cardiovascular autonomic responses associated to pain, at doses significantly inhibiting concomitant VMRs. Pseudo-affective autonomic cardiovascular responses (changes in heart rate and blood pressure) have been characterized as a component of the pain response (Ness and Gebhart, 1988; Sivarao et al.,...
2007; Brusberg et al., 2008, 2009b; Lindström et al., 2008; Ravnefjord et al., 2008), likely generated at higher CNS centers, primarily in the brainstem, where the primary integration of ascending pain-related signals is likely to occur and autonomic cardiovascular centers are located (Martinez et al., 2006; Loewy and McKellar, 1980). Nevertheless, cardiovascular responses have been used to a lesser extent than the VMR as a surrogate marker for pain. The lack of effect of AZD7371 on pain-related cardiovascular changes is difficult to interpret, particularly since VMRs were inhibited simultaneously. This finding was rather unexpected since, in the same experimental conditions we have consistently observed that agents attenuating the VMR to CRD also inhibited autonomic cardiovascular responses to a similar degree (Brusberg et al., 2008, 2009b, Lindström et al., 2008; Ravnefjord et al., 2008). In the case of AZD7371, this might suggest that the effects on VMRs do not reveal a true analgesic effect of the compound but a motor-related effect, leading to the inhibition of the motor reflex implicated in the generation of contractions of the skeletal musculature of the abdominal wall during CRD. Such a motor inhibitory effect could take place at peripheral sites, directly in the muscle, or at a spinal level, modulating the activity of the efferent motor pathways. Indeed, several reports implicate spinal 5-HT$_{1A}$ receptors in the modulation of spinal motor reflexes; resulting in both the inhibition of withdrawal monosynaptic spinal reflexes and the enhancement of motor activity (Clarke and Ward, 2000; Hedo et al., 2002; Zimmer et al., 2006; Gajendiran, 2008). Therefore, it is possible that the inhibitory effects exerted by AZD7371, and by WAY-100635, on the VMRs to CRD are due to the 5-TH$_{1A}$-dependent modulation of motor reflexes at a spinal level, without interference with the afferent processing of pain signals. Nevertheless, no obvious motor-
related side-effects were noted in the animals at the doses tested. In addition, the fact that pain-related cardiovascular responses were not affected by AZD7371 suggests that the compound neither blocks pain pathways at supraspinal sites nor at peripheral afferent sites. The consequence of this will be that pain-related afferent information is still integrated at central levels and, therefore, might lead to the modulation of, at least, some pain-related efferent activity. Further studies using, for example, emerging animal imaging technologies might help to understand the effects of AZD7371 on brain activation and how the compound might affect the central integration of sensory signals elicited by visceral pain.

Alternatively, the lack of effects of AZD7371 on cardiovascular responses might be interpreted as an inadequate central exposure after peripheral systemic administration. However, this is unlikely to happen since AZD7371 freely enters the brain upon peripheral administration, as previously demonstrated (Larsson et al. 1998; Johansson et al., 1997; Farde et al., 2000; Andréé et al., 2003; Hjorth, unpublished observations). In addition, in the present experiments, ICV AZD7371 was effective at inhibiting micturition, a well-characterized, 5-HT$_{1A}$- and centrally-mediated effect of AZD7371 (Pehrson et al., 2002; Yoshiyama et al., 2003); thus indicating that the lack of effects of ICV AZD7371 on pseudo-affective VMRs is likely not due to an ineffective blockade of central 5-HT$_{1A}$ receptors. Moreover, the lack of effects of AZD7371 on CRD responses after ICV administration, reported here, reinforces the view that inhibitory effects of the compound on VMRs are exerted, likely, at a spinal location and are independent of any effect at supraspinal sites.
Overall, the present observations might help to understand the negative results obtained recently in a clinical trial with AZD7371 assessing symptoms improvement in IBS patients (Drossman et al., 2008). In that study, AZD7371 failed to show any symptomatic improvement over a 12-week treatment period. This, together with the undesirable profile observed (associated to the presence of CNS-related side-effects), lead to discontinuation of development (Drossman et al., 2008). It is worth pointing out that Drossman et al., (2008) did not assess the possible effects of AZD7371 on pain/discomfort thresholds during experimental CRD and assessed only symptomatic changes. Although the relationship between visceral hypersensitivity and IBS symptoms is not completely clear, the lack of effects on pain/discomfort symptoms in IBS patients partially agrees with the preclinical observations presented here and suggests that the analgesic-like effects observed when assessing pseudo-affective VMRs during CRD in rats are probably not sufficient to confidently demonstrate true analgesic effects of the compound. Alternatively, the CNS profile of side-effects reported in humans (Drossman et al., 2008) might mask a positive analgesic effect, leading to a negative outcome as it relates to the reporting of symptoms. Finally, species-related differences in 5-HT$_{1A}$ receptor expression and distribution between rat and humans and their role in modulating visceral sensitivity may offer an additional explanation to these discrepant results.

The implications of the current observations might be of importance in the selection of compounds intended for clinical development for the treatment of IBS, at least for compounds targeting primarily visceral pain/discomfort. This is particularly important in light of the failure of AZD7371 to prove clinical
efficacy in the clinical trial in IBS patients mentioned above (Drossman et al., 2008). For instance, other compounds, tested in the CRD model in the same experimental conditions described here, with proven clinical efficacy for the treatment of visceral pain, such as clonidine or pregabalin, not only reduced VMRs to CRD but simultaneously reduced the concomitant hypertensive and tachycardic responses associated to visceral pain (Viramontes et al., 2001; Houghton et al., 2007; Brusberg et al., 2008, 2009b; Ravnefjord et al., 2008). This suggests that animal data derived from the CRD model has to be interpreted carefully as it relates to its clinical translation to humans. This might imply that multiple pain readouts should be tested at preclinical level as to support, with enough confidence, the progression into man of new chemical entities targeting visceral pain/discomfort. One could also consider that VMRs mainly reflect the animal’s attempt to expel the balloon by increasing intraabdominal pressure and are not valid as a marker of pain. Therefore, alternative surrogate markers, such as cardiovascular parameters, should be used to assess the efficacy of new drugs targeting visceral pain. Nevertheless, we can not exclude the possibility that AZD7371, because of its characteristic pharmacological profile, represents a unique case in its clinical translation to humans. AZD7371, as mentioned above, was developed as a potential treatment for depression and anxiety disorders (Mucke, 2000). According to the side-effect profile described in the clinical trial carried out with AZD7371 in IBS patients (Drossman et al., 2008), it can not be excluded that central effects of the compound not directly implicated in pain processing might interfere with any beneficial effects on pain perception leading to a negative outcome as it relates to pain-related symptomatology. Unfortunately no other studies, such as
experimental colorectal balloon distension for the assessment of visceral sensitivity, have been performed in humans as to further understand the mechanism of action of AZD7371 and the negative outcome as it relates to symptoms improvement in IBS patients.

In summary, the present results show that the selective 5-HT₁A antagonist, AZD7371, inhibited pain-related visceromotor, but not cardiovascular autonomic, responses in a model CRD-induced visceral pain in rats. This suggests that AZD7371 might not show a complete inhibitory profile on pseudo-affective responses in rats, which might explain its failure to improve pain/discomfort in IBS patients in a recent clinical trial. From these observations, we suggest that multiple pain-related read-outs should be monitored in preclinical models of visceral pain as to increase the clinical translational value of preclinical data as it relates to the visceral analgesic effects of compounds.
References


Footnotes

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Legends for Figures

Fig. 1  Dose-related effects of AZD7371 and WAY-100635 on visceromotor responses to CRD in rats. Either AZD7371 (3 – 300 nmol/kg), WAY-100635 (3 – 300 nmol/kg) or vehicle (1 ml/kg) was administered iv between distensions 3 and 4 of the CRD protocol (see methods for details). Data correspond to the overall response to CRD for distensions 4 to 12 (AUC) (mean±SEM, n = 4 – 8 for each dose and compound). *: P<0.05 vs vehicle.

Fig. 2  Dose-related effects of oral AZD7371 on visceromotor responses to CRD in rats. Either AZD7371 (1 – 30 µmol/kg) or vehicle (2 ml/kg) were administered orally 30 min before starting the CRD protocol (see methods for details). A: Response to repetitive noxious CRD (12 distensions at 80 mmHg) in the different experimental groups. The broken line corresponds to the mean basal activity of the abdominal musculature between distensions. B: Overall response to CRD for distensions 1 to 12 (AUC) of the data included in panel A. Data are mean±SEM of n = 7 – 8 for each dose and compound. *: P<0.05 vs vehicle.

Fig. 3  Plasma levels of AZD7371 after oral administration in rats. Data are mean±SEM (n=3) and represents total plasma levels of AZD7371 after a single oral dose (30 µmol/kg) at time 0. Pharmacokinetic parameters calculated for AZD7371 are included. The shadowed area indicates the time interval after oral dosing at which CRD experiments were performed.
Fig. 4  Effects or repetitive oral dosing with AZD7371 on the visceromotor response to CRD in rats. Animals were treated orally with AZD7371 (30 µmol/kg/day) or vehicle (2 ml/kg/day) during 9 consecutive days. Responses to CRD were assessed before treatment, after a single dose, after a 5-day treatment and at the end of the treatment period (9 days). Data are mean±SEM of 8 animals per group. *: P<0.05 vs response before treatment (day 0).

Fig. 5  Effects of intracerebroventricular (ICV) AZD7371 on visceromotor responses to CRD and urinary output in rats. A: Responses to CRD. AZD7371 (1 µg/rat) or vehicle (10 µl/rat) was administered ICV between distension 3 and 4 of the CRD protocol (arrow). Broken lines with open symbols represent the basal activity of the abdominal musculature. Data are mean mean±SEM of 7 animals per group, treated with vehicle and AZD7371 in separate experiments. B: Urinary output in the same animals included in A during the time corresponding to distensions 4 to 12 of the CRD protocol. Each point represents an individual animal (open symbols with broken lines). The black symbols with errors represent the mean±SEM of each group. Notice how, compared with vehicle treatment, AZD7371 inhibited micturition in all animals except one.

Fig. 6  Effects of intravenous AZD7371 on visceromotor and autonomic cardiovascular responses to CRD in telemetrized rats. A: Visceromotor responses to CRD. The broken line corresponds to the mean basal activity of the abdominal musculature between distensions. B: Changes in mean arterial blood pressure (Δ mmHg) during CRD. C: Changes in heart rate (Δ beats per minute, bpm) during CRD. AZD7371 or vehicle was administered iv between
distension 3 and 4 of the CRD protocol (arrow). Data are mean ±SEM of 4 animals treated with vehicle and AZD7371 in separate experiments. *: P<0.05 vs corresponding response in the vehicle-treated group.
Figure 3

CRD

C_{max} = 3.16 \pm 0.24 \, \mu \text{mol/l}

t_{max} = 0.33 \pm 0.08 \, h

t_{1/2} = 1.80 \pm 0.30 \, h
Figure 4

Vehicle
(5 ml/kg/day, po)

AZD7371
(30 μmol/kg/day, po)

Response to CRD
(AUC Distensions 1-12)

Days of treatment

0  1  5  9

0  1  5  9

* * *
Figure 5

- Vehicle (10 μl/rat, ICV)
- AZD7371 (1 μg/rat, ICV)

Response to CRD

Distension number (80 mmHg)

Urine weight (mg)

Vehicle
(10 μl/rat, ICV)

AZD7371
(1 μg/rat, ICV)
Figure 6

![Graph showing response to CRD with different treatments: Vehicle, AZD7371 (100 nmol/kg, iv), and AZD7371 (300 nmol/kg, iv).]

- Vehicle (1 ml/kg, iv)
- AZD7371 (100 nmol/kg, iv)
- AZD7371 (300 nmol/kg, iv)

Response to CRD

Distension number (80 mmHg)

Δ Blood pressure (mmHg)

Δ Heart rate (bpm)

Distension number (80 mmHg)