The Selective 5-Hydroxytryptamine 1A Antagonist, AZD7371 [3(R)-(N,N-Dicyclobutylamino)-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide (R,R)-tartrate Monohydrate] (Robalzotan Tartrate Monohydrate), Inhibits Visceral Pain-Related Visceromotor, but Not Autonomic Cardiovascular, Responses to Colorectal Distension in Rats

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ABSTRACT

5-Hydroxytryptamine 1A (5-HT1A) 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-HT1A antagonist AZD7371 [3(R)-(N,N-dicyclobutylamino)-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide (R,R)-tartrate monohydrate] showed no symptomatic improvement in IBS patients. We characterized the mechanisms mediating potential analgesic effects of AZD7371 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 mm Hg). Effects of AZD7371 (3–300 nmol/kg i.v.; 1–30 /H9262 mol/kg p.o.) and a reference 5-HT1A antagonist, WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexancarboxamide maleate salt; 3–300 nmol/kg i.v.), were assessed. Effects of intracerebroventricular AZD7371 were also evaluated. Intravenous AZD7371 or WAY-100635 and oral AZD7371 dose-dependently inhibited visceromotor responses to CRD (ED50, 203, 231, and 14 /H9262 mol/kg, respectively). In telemetrized rats, oral AZD7371 inhibited visceromotor responses to CRD without affecting the concomitant hypertensive and tachycardic responses. Intracerebroventricular AZD7371 did not affect visceromotor responses, whereas 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. Although agents effective on multiple pain-related readouts in the CRD model (e.g., pregabalin or clonidine) alleviate IBS symptoms, AZD7371, which is effective on only one pain-related pseudoaffective readout, does not. Data from preclinical CRD models of visceral pain need to be interpreted cautiously as it relates to their clinical translational value.

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). There is also evidence that serotonin-mediated signaling is altered in individuals with IBS (Mawe et al., 2006). Most of

ABBREVIATIONS: IBS, irritable bowel syndrome; 5-HT, 5-hydroxytryptamine; WAY-100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexancarboxamide maleate salt; CRD, colorectal distension; AZD7371, 3(R)-(N,N-dicyclobutylamino)-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide (R,R)-tartrate monohydrate; VMR, visceromotor response; AUC, area under the curve; bpm, beats per min; CNS, central nervous system.
the treatments for IBS to date have been designed to modulate serotonin signaling, such as the 5-HT$_3$ antagonist, alosetron, and the 5-HT$_4$ partial agonist (and 5-HT$_{1B}$ and 5-HT$_{2B}$ receptor antagonist), tegaserod (Spiller, 2008). Other serotonin receptors have been proposed as potential targets for the treatment of IBS, including 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{7}$ (Danzebrink and Gebhart, 1991; Coelho et al., 1998, 2001; Sivaraao et al., 2004; Tonini et al., 2005). For instance, it has been shown that the 5-HT$_{1A}$ antagonist, WAY-100635, inhibits pain-related responses to colorectal distension (CRD) in rats (Coelho et al., 1998), and 5-HT$_{7}$ 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-HT$_{1A}$ receptor antagonist AZD7371 tartrate monohydrate (robalzotan tartrate monohydrate, NAD299, here termed AZD7371) (Johansson et al., 1997) on visceral pain-related responses, a telemetric system was used. Rats with chronically implanted intracerebroventricular guide cannulae were stereotaxically implanted into the right lateral brain ventricle according to coordinates in the atlas of Paxinos and Watson (1998), i.e., from the bregma posterior, −0.8 mm; lateral, −1.5 mm; and dorsoventral, −3.5 mm, as described previously (Martínez 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.

**Materials and Methods**

**Animals**

Adult female Sprague-Dawley rats (250–300 g; Harlan, Horst, The Netherlands) 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, Kimstad, Sweden) and water under controlled conditions of temperature (21°C) and humidity (50%) on a 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 i.p.) of ketamin (88 mg/kg; Ketalar Vet; Pfizer AB) and xylazin (5 mg/kg; Rompun Vet; Bayer AG), and were surgically equipped with intraperitoneal radio transmitters (PhysioTel C50-PXT; Data Sciences International, St. Paul, MN). The catheter of the transmitter was inserted into the abdominal aorta and fixed with tissue adhesive (Vetbond; 3M, St. Paul, MN) for blood pressure measurements. The animals recovered from surgery in a quiet and dim postoperative room for 24 h and also received antibiotic (Bactrim; Roche Diagnostics, Basel, Switzerland) and analgesic (Fentanyl; Schering Plough, Kenilworth, NJ) treatment. Thereafter, a 7- to 10-day recovery period was allowed before starting any experimental procedures.

**Implantation of Intracerebroventricular Cannulae.** For intracerebroventricular administrations, rats with chronically implanted intracerebroventricular cannulae were used. Rats were anesthetized with a mixture (2 ml/kg i.p.) of ketamin (88 mg/kg; Ketalar Vet; Pfizer AB) and xylazin (5 mg/kg; Rompun Vet; Bayer AG), and the intracerebroventricular guide cannulae were stereotaxically implanted into the right lateral brain ventricle according to coordinates in the atlas of Paxinos and Watson (1998), i.e., from the bregma posterior, −0.8 mm; lateral, −1.5 mm; and dorsoventral, −3.5 mm, as described previously (Martínez 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.

**CRD**

Rats were habituated to Boumann cages (Plexiglas tubes; length, 18 cm; diameter, 6 cm; AstraZeneca, Mölndal, Sweden) 30 min/day for 3 consecutive days before experiments to reduce motion artifacts and confounding effects because of 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 Pharmaeuticals, Ballerup, Denmark). The balloons were connected to pressure transducers (P-602, CFM-k33, 100 mm Hg; Bronkhorst High-Tech, Ruurlo, 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) 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 mm Hg, 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; Martínez 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 high-pass 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 predesigned automatic analysis paradigms. Hence, manual analysis and potential bias by the investigator were avoided. Data represent the average rectified value of the high-pass-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 high-pass-filtered balloon pressure signals, the first and last seconds of each pulse were excluded because these reflect artifact signals produced by the barostat during inflation and deflation and do not originate from the animal.

**Drugs**

AZD7371 (AstraZeneca R&D) (Johansson et al., 1997) and WAY-100635 (Sigma-Aldrich) (Fletcher et al., 1993) were dissolved in 0.9% saline solution at the appropriate concentration. Saline solution was administered intravenously between distensions 3 and 4 of the CRD protocol.

**Experimental Protocols**

**Dose-Related Effects of Intravenous AZD7371 and WAY-100635 and Oral AZD7371 on Visceromotor Responses to Experimental Protocols**

Animal and Oral AZD7371 on Visceromotor Responses to Experimental Protocols

Saline solution was administered intravenously 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) was administered intravenously between distensions 3 and 4 of the CRD protocol.

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 crossover fashion in which vehicle and different doses of compounds were tested during the same experiment and repeated on 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 Intracerebroventricular AZD7371 on Visceromotor Responses to CRD and Micturition.** Rats with chronically implanted intracerebroventricular cannulae were used for these experiments. The intracerebroventricular injection was performed in lightly restrained animals using a 28-gauge injection cannula, 1 mm longer than the guide cannula, 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 injection 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) was injected slowly, over a 30-s period, between distensions 3 and 4 of the CRD paradigm. At the same time, a piece of preweighed 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 intracerebroventricular injections, vehicle and treatment, with an interval of 4 to 5 days. At the end of the experiments, at the time of euthanasia, the correct location of the cannula 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 intracerebroventricular 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 after dosing to determine the complete pharmacokinetic profile of AZD7371 after oral administration. Total plasma levels of AZD7371 were determined using standard high-performance liquid chromatography combined with mass spectroscopy procedures (limit of quantification, 1 nM).

**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 nonrepeated measures one-way analysis of variance, as appropriate, followed, when necessary, by a Student-Newman-Keuls multiple comparisons test. ED₅₀ 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 < 0.05.

**Results**

**Dose-Related Effects of AZD7371 and WAY-100635 on Visceromotor Responses to CRD.** CRD at 80 mm Hg induced a VMR manifested as a 4-fold increase in the activity of the abdominal musculature, compared with basal activity (basal, 0.05 ± 0.003; first distension at 80 mm Hg, 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 intravenously between distensions 3 and 4, inhibited in a dose-dependent manner the response to CRD with an ED₅₀ of 203 nmol/kg (95% confidence interval, 109–377 nmol/kg; r² = 0.3281; Fig. 1A). The overall response to CRD during the 4th to 12th distensions 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) = 3.95; P = 0.0086], which inhibited the response to CRD, with an ED₅₀ of 231 nmol/kg (95% confidence interval, 113–473 nmol/kg; r² = 0.2722; Fig. 1B).

Likewise, oral AZD7371 (1, 3, 10, or 30 µmol/kg) inhibited the response to CRD, with an ED₅₀ of 14 µmol/kg (95% confidence interval, 9–23 µmol/kg; r² = 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; first CRD, 0.17 ± 0.02; n = 30; P < 0.05), whereas 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, 32 ± 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 (30 min after dosing) and 0.75 ± 0.06 μM (90 min after 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 i.v. and 30 μmol/kg p.o. 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 of the 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). A 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 $P < 0.05$ versus the VMR before treatment; $F(3,7) = 8.75, P = 0.0033$; Fig. 4).

Effects of Intracerebroventricular AZD7371 on Visceromotor Responses to CRD and Micturition. Visceromotor responses to CRD were of similar magnitude after the intracerebroventricular 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. 5, top). Urine production during the 45 min after treatment, corresponding to the time interval of the CRD procedure, was reduced by 32% after intracerebroventricular AZD7371, compared with the responses observed after vehicle administration (Fig. 5, bottom). The inhibitory effects of AZD7371 on micturition were clear in six of the seven animals tested.
Nevertheless, because of 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 mm Hg) and heart rate (mean increase, 23 ± 7 beats per min (bpm)). Although 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 intravenously 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. 6, top). In the same animals, AZD7371 did not affect either the hypertensive (mean increase in blood pressure; vehicle, 18 ± 5 mm Hg; 100 nmol/kg, 18 ± 6 mm Hg; 300 nmol/kg, 19 ± 4 mm Hg; P > 0.05; Fig. 6, middle) or 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. 6, bottom). AZD7371, per se, did not affect resting (between distensions) blood pressure (vehicle, 126 ± 2 mm Hg; 100 nmol/kg, 129 ± 2 mm Hg; 300 nmol/kg, 129 ± 1 mm Hg) or resting (between distensions) heart rate (vehi-
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, probably through a peripheral or spinal mechanism. It is interesting that autonomic cardiovascular responses, generated concomitantly with the VMRs as part of the response to pain, were not affected by AZD7371.

Pseudoaffective 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 intravenous administration, AZD7371 and the 5-HT_{1A} antagonist WAY-1060635 displayed similar potency and efficacy. This, together with the previously reported analgesic effects of WAY-1060635 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 pseudoaffective cardiovascular autonomic responses associated to pain, at doses significantly inhibiting concomitant VMRs. Pseudoaffective 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), probably 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 (Loewy and McKellar, 1980; Martínez et al., 2006). 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 because VMRs were inhibited simultaneously. This finding was rather unexpected because 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. 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 and Goshgarian, 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-TH1A-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 because AZD7371 freely enters the brain upon peripheral administration, as previously demonstrated (Larsson et al., 1998; Johansson et al., 1997; Farde et al., 2000; Andrée et al., 2003; S. Hjorth, unpublished observations). In addition, in the present experiments, intracerebroventricular AZD7371 was effective at inhibiting micturition, a well characterized, 5-HT1A- and centrally mediated effect of AZD7371 (Pehrson et al., 2002; Yoshiyama et al., 2003), thus indicating that the lack of effects of intracerebroventricular AZD7371 on pseudoaffective VMRs is likely not due to an ineffective blockade of central 5-HT1A receptors. Moreover, the lack of effects of AZD7371 on CRD responses after intracerebroventricular administration reported here reinforces the view that inhibitory effects of the compound on VMRs are exerted, probably 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 improvement of symptoms 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 pseudoaffective 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-HT1A 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 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 cannot 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 cannot 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 to further understand the mechanism of action of AZD7371 and the negative outcome as it relates to improvement of symptoms in IBS patients.

In summary, the present results show that the selective 5-HT1A antagonist, AZD7371, inhibited pain-related visceralomotor, but not cardiovascular, autonomic, responses in a model of CRD-induced visceral pain in rats. This suggests that AZD7371 might not show a complete inhibitory profile on pseudoaffective 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 readouts should be monitored in preclinical models of visceral pain to increase the clinical translational value of preclinical data as it relates to the visceral analgesic effects of compounds.

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