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New benzimidazole derivatives: selective and orally active 5-HT_3 receptor antagonists

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Abstract

The synthesis of new 5-HT_3 receptor antagonists is an interesting field of research because of their wide therapeutic use. The aim of this work is to functionally characterise a new series of benzimidazole derivatives previously described. These compounds bind to 5-HT_3 receptors and have been evaluated using in vitro (rat *tunica muscularis mucosae*) and in vivo tests (Bezold–Jarisch reflex in rat and gastrointestinal motility and spontaneous motility in mice). Ondansetron and 1-[4-amino-5-chloro-2-(3,5-dimethoxyphenil)methyloxy]-3-[1-[2-methylsulfonylamino]piperidin-4-yl]propan-1-one hydrochloride (RS 39604) were used as well known 5-HT_3 and 5-HT_4 receptor antagonists. These benzimidazole derivatives have proved to be 5-HT_3 receptor antagonists. Interestingly, they are as active as ondansetron when they are intraperitoneally (i.p.) or orally (p.o.) administered and, in mice, they seem to induce fewer behavioural changes at similar effective doses than does ondansetron. The present results confirm the usefulness of the previously proposed pharmacophore and justify the interest in these new benzimidazole derivatives.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT_3 receptor antagonist; Gastrointestinal motility; Benzimidazole derivative

1. Introduction

Serotonin (5-HT) is a neurotransmitter and neuromodulator involved in multiple central and peripheral events. At least 14 different subtypes of 5-HT receptors have been described; nevertheless, their physiological roles are not yet well established. The 5-HT_3 receptors are ligand-gated cation channels and agonist drugs cause membrane depolarisation of single neurones (Derkach et al., 1989) and also nerve-mediated contractions of nerve–muscle preparations in vitro. This subtype has been widely studied because of its involvement in several physiological and pathological processes. The antagonists are the main drugs, acting on 5-HT_3 receptors, with therapeutic uses. Their prevalent clinical indication is treatment of the emesis associated with cancer chemotherapy and their effectiveness on irritable bowel syndrome is under evaluation (Balfour et al., 2000). However, several reports suggest that 5-HT_3 antagonists could be useful in the treatment of, for example, bulimia nervosa (Faris et al., 1998; Hartman et al., 1997), drug withdrawal, anxiety, schizophrenia, drug abuse and age-associated memory impairment (Buhot et al., 2000). There are also findings supporting the role of 5-HT_3 receptors in peripheral and central analgesia (Pelissier et al., 1996; Doak and Sawynok, 1997; Espejo and Gil, 1998; Farber et al., 2001).

Often, 5-HT_3 receptor antagonists also bind to 5-HT_4 receptors because of the structural similarity of these receptors. The development of selective antagonists of the 5-HT_3 receptor subtype is attracting considerable attention; recently, we reported the synthesis of a new series of benzimidazole derivatives (López-Rodríguez et al., 1999). Some of these compounds exhibited very high affinity for 5-HT_3 receptors

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with no significant affinity for the 5-HT_4 receptors. The biological activity of the three most interesting compounds: (*S*)-(−)-*N*-(1-azabicicle(2.2.2)oct-3-yl)benzimidazole-4-carboxamide (UCM-61196), (*S*)-(−)-*N*-(1-azabicicle (2.2.2) oct-3-yl)-6-chlorobenzimidazole-4-carboxamide (UCM-17197), (*S*)-(−)-*N*-(1-azabicicle (2.2.2)oct-3-yl)-6-chloro-7-nitrobenzimidazole-4-carboxamide (UCM-10197), respectively, was determined, analysing their activity in the isolated guinea pig ileum, and the results obtained suggested that they could act as selective 5-HT_3 receptor antagonists (López-Rodríguez et al., 1999).

Our aim was to study the effect of these three new compounds using different tests in order to: better characterise and confirm their functional activity as 5-HT_3 serotonergic receptor antagonists (Bezold–Jarisch reflex); rule out 5-HT_4 biological activity (rat *tunica muscularis mucosae*); determine their in vivo effect on gastrointestinal motility, diarrhoea and rate of gastric emptying induced by stimulation of the enteric serotonergic system by administration of 5-hydroxytryptophan (5-HTP), an endogenous precursor of 5-HT after intraperitoneal (i.p.) or oral (p.o.) administration; compare these new compounds with well-known 5-HT_3 and 5-HT_4 receptor antagonists such as ondansetron or 1-[4-amino-5-chloro-2-(3,5-dimethoxyphenil)methyloxy]-3-[1-[2-methylsulfonyl-amino]piperidin-4-yl]propan-1-one hydrochloride (RS 39604) (Derkach et al., 1989); and detect behavioural modifications, observing and recording spontaneous behaviour and motility.

2. Materials and methods

2.1. Bezold–Jarisch reflex

The Bezold–Jarisch reflex is one of the most selective and frequently used in vivo tests for study of 5-HT receptor agonists and antagonists (Jarisch and Zotterman, 1948).

Experiments were performed on anaesthetised spontaneously breathing male Wistar rats weighing 200–250 g. Anaesthesia was induced with Equithesin (3 ml/kg i.p.) (chloral hydrate 2.1 g, sodium pentobarbital 0.46 g, $MgSO_4$ 1.06 g, propylene glycol 21.4 ml, ethanol (90%) 5.7 ml, H_2O 23 ml). Blood pressure and heart rate were continuously recorded, after carotid cannulation, using a pressure transducer connected to a personal computer. The jugular vein was cannulated for intravenous (i.v.) drug administration. When the experiment was finished, the rats were killed by an anaesthetic overdose.

The Bezold–Jarisch reflex (Fozard, 1984) was evoked by i.v. administration of a dose of 5-HT (30 μg/kg) that reduced the heart rate by approximately 50%.

The rapid and transient drop in heart rate due to the administration of 5-HT was quantified 5 min before and 5 min after a single administration (0.1 ml/rat) of one i.v. dose of one the three new compounds (0.06, 0.1, 0.13 and 0.2 μg/kg) of the 5-HT_3 reference antagonist, ondansetron (Fortuno et al., 1999) (0.1, 1, 5, 15 or 30 μg/kg), or of the 5-HT_4 reference antagonist, RS 39604 (0.1–30 μg/kg). To evaluate the effect of the 5-HT receptor antagonists or of the new compounds, the decrease in heart rate induced by 5-HT under control conditions was evaluated; this value was taken as 100. Then, the effect of 5-HT was quantified 5 min after the administration of the 5-HT receptor antagonists or of the new compounds and the difference from the previous value was quantified as % of inhibition.

Each group of experiments was carried out in separate groups of at least five animals each.

2.2. Rat oesophagus tunica muscularis mucosae

The rat oesophagus *tunica muscularis mucosae* preparation was included because 5-HT induces a dose-dependent relaxation in carbachol-contracted preparations. This effect is taken to be specifically mediated through 5-HT_4 receptors (Hegde et al., 1995; Nagakura et al., 1999; Takeda et al., 1999). To better characterise functionally these compounds and to eliminate effects mediated through 5-HT_4 receptors, their ability to block the effect of serotonin on the rat oesophagus *tunica muscularis mucosae* was tested (Craig and Clarke, 1991; Taniyama et al., 1991; Barocelli et al., 1999).

The thoracic oesophagus was isolated from male Wistar rats weighing 150–200 g and placed in Tyrode solution (composition [mM]: NaCl 139.0, KCl 2.7, $MgCl_2 \cdot 6H_2O$ 1.1, NaH_2PO_4 0.4, glucose 5.6, $NaHCO_3$ 11.8 and $CaCl_2 \cdot 5H_2O$ 1.8).

The outer striated muscle coat was cut longitudinally and gently peeled away, to expose the inner *muscularis mucosae* as previously described (Baxter et al., 1991). The tissues were suspended under a resting tension of 0.25 g in 5-ml organ baths, containing Tyrode solution, maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2. The resting tension was readjusted during an equilibration period of 1 h. Methysergide (10^{-6} M) was included in the Tyrode solution in order to block 5-HT_1 and 5-HT_2 receptors (Hegde et al., 1995). Cocaine (3×10^{-5} M) and corticosterone (3×10^{-5} M) were also added to inhibit amine reuptake (Baxter et al., 1991).

After the equilibration period, carbachol (3×10^{-6} M) was added to induce a sustained contraction of the preparation. Upon the establishment of a stable contraction, a concentration–response curve was made by cumulative addition of 5-HT (10^{-9} to 5×10^{-6} M). Each dose was added to the organ bath when the maximum effect was reached. When the maximal concentration was added, the tissues were washed and re-equilibrated for a 60-min period. A second 5-HT concentration–response curve was made in presence of one of the new compounds or of reference compounds, ondansetron and RS 39604. The concentration used was 10^{-6} M. The EC_{50} of 5-HT was then calculated in the presence and absence of the drugs tested.

2.3. Gastrointestinal motility in mice

Adult male CD-1 mice weighing 25–30 g (Harlan, Barcelona, Spain) were used for this set of experiments. The animals were housed under standard laboratory conditions: controlled temperature: 23 ± 1 °C, 12/12-h light/dark cycle and free access to food and water until 24 h before the experiments. On the day of the experiments, the mice were screened to exclude animals with pre-existing diarrhoea or behaviour alterations. The mice were randomly divided into two separate groups and two tests were carried out to evaluate gastrointestinal motility.

2.3.1. 5-Hydroxytryptophan-induced diarrhoea

The mice, randomly divided into the appropriate groups, were treated: with i.p. saline (NaCl 0.9%) solution or one dose of UCM-61196, UCM-10197, UCM-17197, ondansetron or RS 39604 (1, 2 and 5 mg/kg), with p.o. (intragastric cannula) SS or one dose of one of the new compounds (5, 15, 25 and 50 mg/kg), ondansetron (5, 15 and 25 mg/kg) or RS 39604 (5, 15, 25 and 50 mg/kg).

Higher doses of ondansetron were not tested because of their toxicity (see Section 2.4) (Hendrie, 1990).

Five minutes after i.p. injection or 30 after p.o. administration, 50% ($n = 10–12$) of the animals of each group were treated with saline and 50% ($n = 10–12$) with 5-HTP (10 mg/kg i.p.) and immediately placed in a 1-l container.

The severity of diarrhoea was then scored using an arbitrary scale (0 = normal stools, 1 = wet but formed stools, 2 = swollen and unformed stools and 3 = watery diarrhoea) (Hegde et al., 1995) over a 30-min continuous observation period, by an observer who was unaware of the treatments.

2.3.2. Gastric emptying

To evaluate the gastric emptying rate, gastrointestinal transit (GIT) in mice was measured according to procedures previously described (Pol et al., 1994). Food was withheld from the animals 24 h before the experiment, but free access to water was allowed. Mice then received 0.3 ml of a suspension of charcoal consisting of 10% vegetable charcoal in 5% gum acacia, administered with an intragastric cannula. Gastrointestinal transit was evaluated 20 min after the administration of the marker. At this time, the animals were killed and the small intestine was removed. The length of the intestine from pyloric sphincter to the ileo-cecal junction and the distance travelled by the charcoal front were both measured and recorded. Then, gastrointestinal transit was calculated for each animal as a percentage of the distance travelled by the charcoal, relative to the total length of the small intestine (percentage gastrointestinal transit).

Separate groups ($n = 20–24$ per group) were treated with saline solution or one dose selected from our previous data (approximately 50% score diarrhoea inhibition) of each new compound: UCM-61196, UCM-10197 and UCM-17197 (5 mg/kg i.p.), ondansetron (3 mg/kg i.p.) or RS 39604 (5 mg/kg i.p.). Ten minutes after this treatment, 50% of the animals of each group were given saline ($n = 10–12$) and 50% 5-HTP (10 mg/kg i.p., $n = 10–12$). Then, 20 min later, the mice received 0.3 ml of a suspension of charcoal. Twenty minutes after the administration of the marker, the percentage of gastrointestinal transit was evaluated as described above.

To discard non-specific effects, new compounds were also tested after an inflammatory reaction had been induced in the mouse small intestine with croton oil. It is well known that croton oil increases gastric emptying rate (Pol et al., 1994). Four groups of mice ($n = 12$) were given croton oil (0.05 ml CO, 50% olive oil) p.o. and one saline. Three of the croton oil-treated groups of animals were treated with UCM-61196, UCM-10197 and UCM-17197 (5 mg/kg i.p.) and one with saline 6 h later. Saline was also administered to the control group that had previously been treated with saline. Then, 5 min later, all mice received 0.3 ml of a suspension of charcoal and were killed after 20 min. Gastrointestinal transit was then quantified as described above.

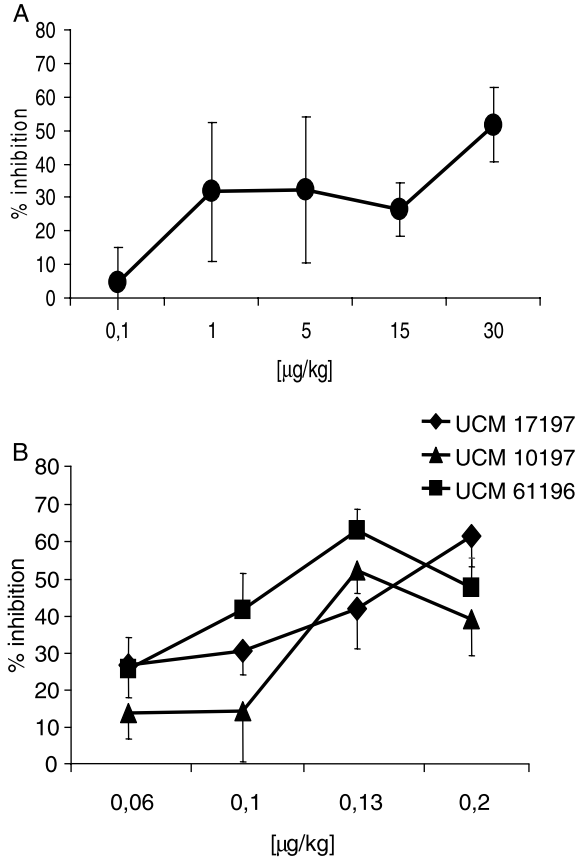

Fig. 1. Bezold–Jarisch reflex. The lines represent the mean ± S.E.M. of the percentage inhibition by ondansetron (A) and the new compounds (B), at different doses (µg/kg i.v.), of the bradycardia induced by 5-HT (30 µg/kg i.v.) (Bezold–Jarisch reflex) in anaesthetised rats ($n = 5$ per group).

2.4. Behavioural modifications

To evaluate behavioural alterations after the different treatments, spontaneous behaviour was always observed in the cage before the performance of the different tests. Any modification in activity or in ambulation was recorded. An observer unaware of the treatments recorded changes in spontaneous behaviour.

Spontaneous locomotor activity was evaluated using individual photocell activity chambers. The mice were i.p. injected with 3 mg/kg of ondansetron, 5 mg/kg of RS 39604 or of one of the new compounds (5 mg/kg). The mice were then placed in the chambers and, starting 5 min later, the number of interruptions of photocell beams was recorded over a 30-min period. The mean number of crossing was compared with that from a control group of mice that had received saline.

Drugs: ondansetron hydrochloride dihydrate (kindly supplied by Glaxo-Smith-Kline, Madrid, Spain), RS 39604 (Tocris, Madrid, Spain), 5-HTP and gum acacia were obtained from Sigma-Aldrich (Madrid, Spain). New compounds (UCM-61196, UCM-10197 and UCM-17197) were synthesised in our laboratory. All drugs were dissolved in physiological SS.

Statistical analysis: The data are expressed as means ± standard error of the mean (S.E.M.) Statistical analyses for differences between groups were performed with Student's *t*-test. Statistical analyses, for differences between multiple groups, were performed with an analysis of variance followed by Newman–Keuls test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Bezold–Jarisch reflex

As expected, the i.v. administration of 5-HT (30 μg/kg) induced short-lasting modifications of arterial pressure and a drop in heart rate. The mean percentage reduction of heart rate was 46.2 ± 5.4 ($n = 22$) in control animals.

The i.v. administration of the new compounds: UCM-61196, UCM-10197 and UCM-17197, as well as of ondansetron, produced a dose-dependent inhibition of the 5-HT-induced bradycardia in anaesthetised rats (Fig. 1). It is interesting that the doses needed to inhibit the Bezold–Jarisch reflex were smaller when the new compounds were used.

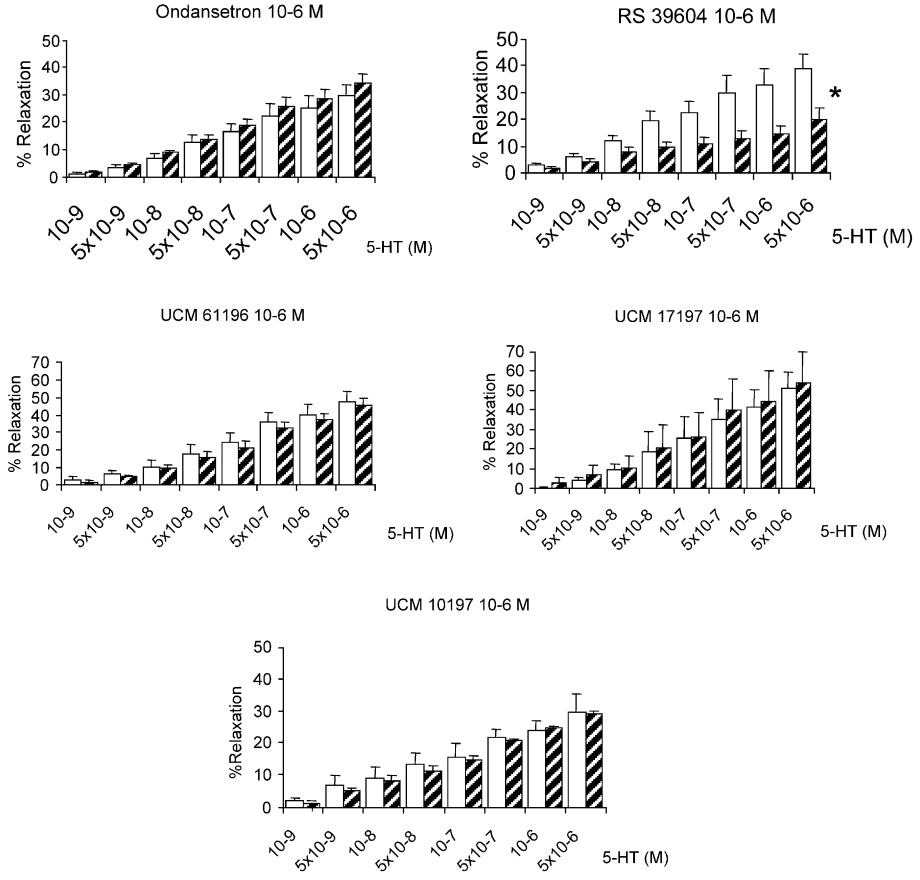

Fig. 2. Rat oesophagus. Bars represent the mean of the percentage relaxation ± S.E.M. induced by 5-HT on the carbachol pre-contracted rat oesophagus in absence (open bars) or presence of ondansetron, RS 39604, UCM-61196, UCM-17197 and UCM-10197 (closed bars), *$p < 0.05$ vs. control; $n \geq 6$.

The compounds tested themselves did not affect baseline heart rate or arterial pressure at any of the doses used (data not shown).

RS 39604, at the doses tested (0.1–30 μg/kg), did not modify the baseline heart rate or arterial pressure. The mean percentage reduction of the cardiac frequency for 30 μg/ kg was -11.68 ± 10.3; $n = 5$. RS 39604 did not affect the 5-HT effect.

3.2. Rat oesophagus tunica muscularis mucosae

The addition of carbachol (3×10^{-6} M) induced a lasting contraction of the rat oesophagus *tunica muscularis mucosae* preparation.

UCM-61196, UCM-10197, UCM-17197, ondansetron and RS 39604 had no effect when administered alone on basal tone or on carbachol-induced contraction (data not shown).

5-HT (5×10^{-9} to 5×10^{-6} M) produced concentration-dependent relaxation of the carbachol pre-contracted rat oesophagus. RS 39604 (10^{-6} M) induced a significant inhibition of the 5-HT effect, whereas the new compounds and ondansetron did not induce significant modifications of the 5-HT inhibitory effects (Fig. 2).

The 5-HT EC_{50} and confidence limits ( ) for each group before and after drug incubation were: control: 5.6×10^{-8} M (4.1×10^{-8} to 7.6×10^{-8}) after RS 39604: 4.8×10^{-6} M (2.0×10^{-6} to 1.1×10^{-5}); control: 1.4×10^{-7} M (1.1×10^{-7} to 1.7×10^{-7}) after ondansetron: 7.7×10^{-8} M (5.7×10^{-8} to 1.1×10^{-7}); control: 6.2×10^{-8} M (4.1×10^{-8} to 9.3×10^{-8}) after UCM-10197: 8.7×10^{-8} M (5.9×10^{-8} to1.3×10^{-7}); control: 1.4×10^{-7} M (1.1×10^{-7} to 1.9×10^{-7}), UCM-17197: 1.0×10^{-7} M (7.8×10^{-8} to 1.3×10^{-7}); control: 8.8×10^{-8} M (6.9×10^{-8} to 1.1×10^{-7}), UCM-61196: 1.3×10^{-7} M (1.1×10^{-7} to1.6×10^{-7}). Significant differences were only

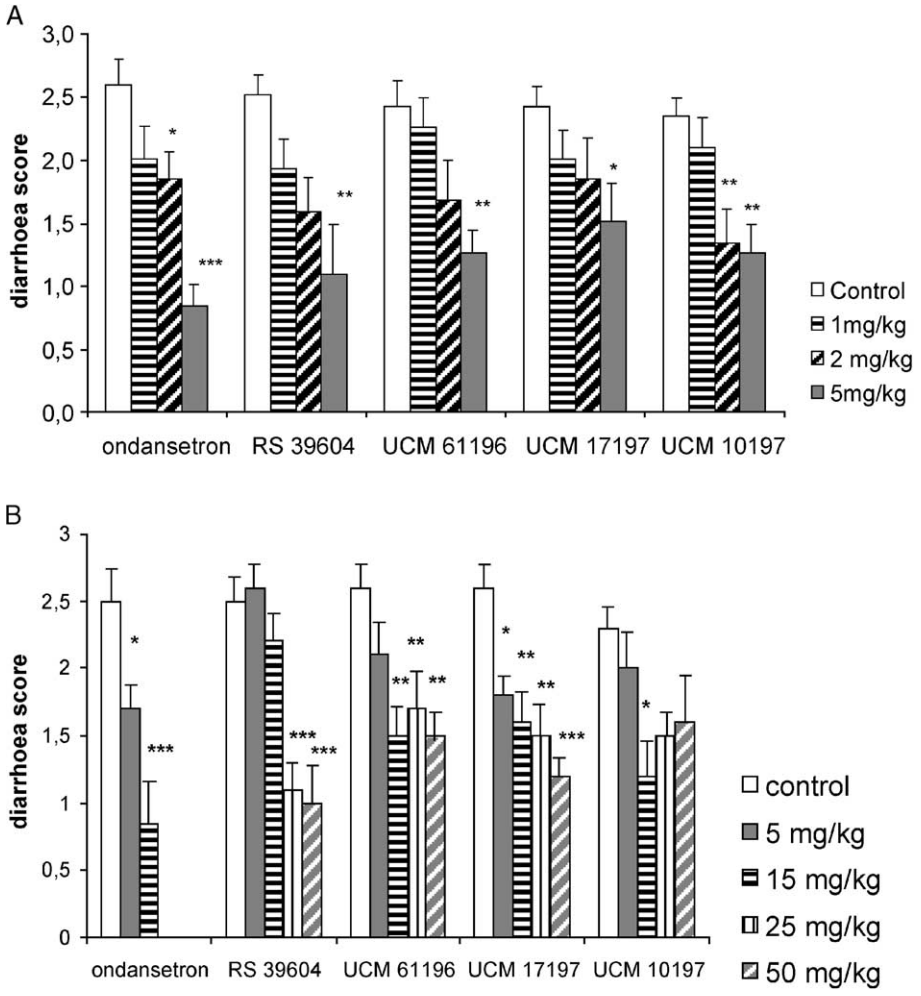

Fig. 3. 5-HTP-induced diarrhoea. The effect of intraperitoneal (A) and oral (B) administration of the different compounds at different doses on 5-HTP-induced diarrhoea in mice. (Controls were treated with vehicle + 5-HTP.) The severity of diarrhoea was assessed using an arbitrary scoring scale ranging from 0 to 3: 0 = normal stools, 1 = wet but formed stools, 2 = swollen and unformed stools and 3 severe watery diarrhea. Each bar represents the mean ± S.E.M. $n = 12$ per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control.

found when the 5-HT EC_{50} obtained after RS 39604 was compared with its respective control value ($p < 0.0001$); $n \geq 6$.

3.3. Gastrointestinal motility in mice

3.3.1. 5-Hydroxytryptophan-induced diarrhoea

The i.p. or p.o. administration of the tested compounds (UCM-61196, UCM-10197, UCM-17197, ondansetron or RS 39604) did not induce any significant modification in the number or appearance of the stools in control animals.

The administration of 5-HTP (10 mg/kg i.p.) induced a significant increase in the degree of diarrhoea scored. This effect was prevented by treatment with the reference compounds or with the new compounds. In all cases, the treatment was equally effective whether drugs were i.p. or p.o. administered (Fig. 3).

3.3.2. Gastric emptying

The i.p. treatment with UCM-61196, UCM-10197 and UCM-17197 (5 mg/kg i.p.) or with the 5-HT antagonists, ondansetron (3 mg/kg i.p.) and RS 39604 (5 mg/kg i.p.) did not significantly modify the gastric emptying rate in mice treated with saline.

Treatment with 5-HTP (10 mg/kg i.p.) induced a significant increase in the percentage of gastrointestinal transit, this effect was prevented by treatment with the new compounds or with the reference antagonists (Fig. 4).

Treatment with croton oil significantly increased the percentage of gastrointestinal transit, which the new compounds were not able to prevent. Twenty minutes after the administration of the marker, % GIT was 61.3 ± 6.4% in the control group, whereas it was 77.1 ± 3.1% in CO-treated animals. The effect of the new compounds (5 mg/kg), ondansetron (3 mg/kg) and RS 39604 (5 mg/kg) on gastrointestinal transit was UCM-61196: 85.41 ± 15.29%, UCM-17197: 81.50 ± 11.86%, UCM-10197: 71.15 ± 13.82%, ondansetron 86.55 ± 7.32% and RS 39604: 78.98 ± 8%. The differences were not significant.

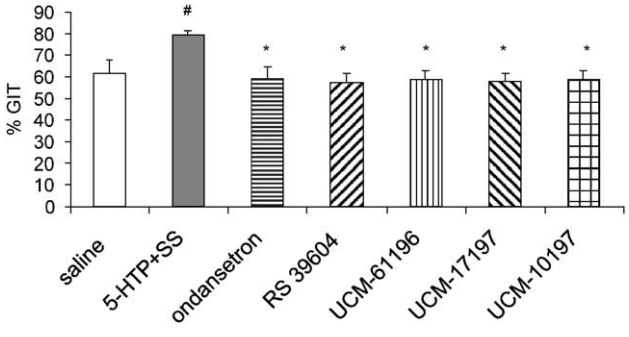

Fig. 4. Gastrointestinal transit in 5-HTP-induced diarrhoea. Bars represent the mean ± S.E.M. of the percentage gastrointestinal transit in 5-HTP-induced diarrhoea. *$p < 0.05$ vs. 5-HTP + saline group, #$p < 0.05$ vs. saline group; $n \geq 10$.

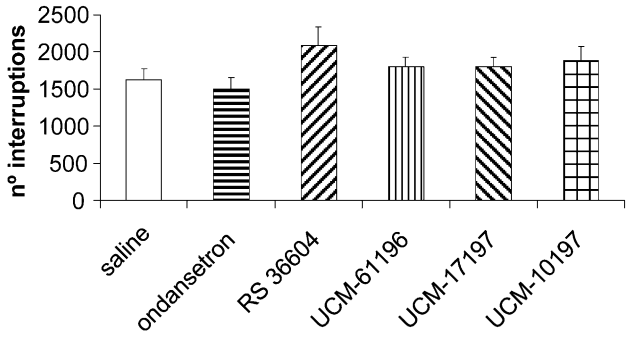

Fig. 5. Locomotor activity in mice. Bars represent the number of interruptions of photocell beams recorded over a 30-minute period. The doses used were 3 mg/kg of ondansetron, 5 mg/kg of RS 39604 or 5 mg/kg of one of the new compounds. The mean number of crossing ± S.E.M. was compared with that obtained from a control group of mice that had received saline solution; $n \geq 10$.

3.4. Behavioural modifications

The new compounds and RS 39604, at the doses tested, did not induce modifications in the characteristics of the spontaneous activity or ambulation of the mice. On the contrary, when ondansetron was i.p. injected at the highest dose (5 mg/kg) or when it was orally administered (≥ 25 mg/kg), the mice showed ambulatory impairments, convulsions and mortality that reached 20% of the those of treated animals after the administration of 15 mg/kg and 80% after treatment with 25 mg/kg.

Spontaneous locomotor activity evaluated in control animals or after administration of the highest i.p. doses used was not significantly different from the control values (Fig. 5). The effect of ondansetron was recorded after the administration of 3 mg/kg because of the side-effects seen.

4. Discussion

Previous results had shown that UCM-61196, UCM-10197 and UCM-17197 selectively bind to 5-HT_3 receptors in the cerebral cortex of rat. Preliminary pharmacological characterisation, using guinea pig ileum, had suggested that they could act as 5-HT_3 receptor antagonists (López-Rodríguez et al., 1999).

We now studied the in vivo and in vitro functional activity of these new compounds. The tests used were selected because they are generally accepted for characterising serotonergic ligands (Hegde et al., 1995; Malinowska et al., 1996; Eglen et al., 1994; Pires et al., 1998). The effects of these new antagonists were also studied on the main systems where 5-HT shows activity.

Serotonergic effects on the cardiovascular system involve a wide number of 5-HT receptors subtypes that are currently not clearly defined and that are dependent on the test carried out and on the animal used. In agreement with previous data (Bogle et al., 1990), the i.v. administration of 5-HT induced

changes in arterial pressure and in heart rate (Bezold–Jarisch reflex) that are known to be peripherally and centrally mediated and mainly due to 5-HT_3 receptor activation.

The i.v. administration of UCM-61196, UCM-10197 and UCM-17197 did not induce any significant change in arterial pressure or heart rate in anaesthetised rats. These results permit agonistic activity on 5-HT_1 or 5-HT_2 receptors to be eliminated since the stimulation of these receptors induces hypertension in rat (O'Connor et al., 2001). Moreover, the compounds are not 5-HT_3 receptor agonists because the selective stimulation of these receptors induces transient hypotension and bradycardia (Pires et al., 1998).

As expected, the i.v. administration of 5-HT induced a decrease in arterial pressure and heart rate that was blocked by ondansetron, a selective 5-HT_3 receptor antagonist, whereas RS 39604, the 5-HT_4 reference compound, did not modify the 5-HT effect. All the new compounds induced a significant reduction of the bradycardia (von Bezold–Jarisch reflex).

These results are consistent with our previous results showing that the new compounds displayed high affinity for the 5-HT_3 receptor. Concerning functional activity, the present data confirm their antagonistic activity previously suggested from their effects on guinea pig ileum (López-Rodríguez et al., 1999).

When the effect of new compounds was tested on the rat oesophagus tunica mucosae, none elicited relaxation in carbachol pre-contracted tissues, allowing 5-HT_4 agonistic effects to be eliminated (Hegde et al., 1995; Nagakura et al., 1999; Takeda et al., 1999). Moreover, neither ondansetron nor the new derivatives were able to reduce the relaxation induced by 5-HT, whereas RS 39604 induced a concentration–response inhibition of the 5-HT effect. These data agree with previously published binding data and permit functional antagonistic effects on 5-HT_4 receptors to be disregarded also.

The effect of the new compounds was assayed using two more tests, in order to test their in vivo efficacy and because the best-established uses and effects of 5-HT_3 and 5-HT_4 receptor antagonists are related to gastrointestinal motility control (Talley, 2001; Reeves et al., 2002; Grimsehl et al., 2002; Jin et al., 1999; Sevcik et al., 1998; Grider et al., 1998; Kiso et al., 2001).

Firstly, the effect of UCM-61196, UCM-10197, UCM-17197 and ondansetron on the diarrhoea induced by the 5-HT precursor, 5-HTP, was tested in mice (Woolley, 1958; Hegde et al., 1994). This is a useful model to study transit impairments, the induced diarrhoea being due to serotonergic stimulation and it is accepted that 5-HTP-induced diarrhoea may be used as a carcinoid syndrome model (Bourin et al., 1996).

The new compounds were able to prevent the diarrhoea both after p.o. and after i.p. administration and their effectiveness was similar to that of ondansetron.

When their effect on gastric emptying was tested, there were no modifications in either control animals or those with gastric emptying unselectively stimulated with croton oil. On the contrary, when 5-HTP was administered, and therefore gastrointestinal transit increased by serotonergic mechanisms, the oral or parenteral administration of UCM-61196, UCM-10197, UCM-17197 or ondansetron induced a significant reduction of the gastrointestinal transit, confirming the antagonistic activity of the new compounds.

The effectiveness of the new compounds when diarrhoea or gastric emptying were stimulated by serotonergic mechanisms without modifications in control animals suggests that they would be promising selective agents. Moreover, they were as effective as the reference compound when administered orally.

5-HT is also involved in the regulation of complex sensory, motor, affective and cognitive functions. These effects are mediated by stimulation of several subtypes of receptors (Varga et al., 2002; Nunes de Souza et al., 2002; Sakaue et al., 2001) including 5-HT_3 (Kankaanpaa et al., 2002; Fung and Ferrill, 2001). As a first approach to determine behavioural modifications in control animals and tolerability of these new compounds, spontaneous behaviour and spontaneous locomotor activity were recorded.

Although ondansetron is accepted as being a safe drug, it has been previously reported high doses may cause side-effects (Hendrie, 1990). In accordance with these data, it was found that the administration of high doses of ondansetron induces behavioural changes (ambulatory impairment and convulsions) and even mortality that limits the possibility of increasing the dosage. On the contrary, none of the new compounds elicited significant changes in spontaneous behaviour or motility.

Concerning the potency of the new compounds, it is interesting that as expected from their previously determined affinity (mean K_i [nM]±S.E.M. were: UCM-61196 2.6±0.3,UCM-17197 0.13±0.2 and UCM 10197 1.7 ± 0.2) (Lopez-Rodriguez et al., 1999), there were no significant differences. Otherwise, the present data do not explain the difference in the doses required to inhibit the Bezold–Jarisch reflex and those needed to prevent the diarrhoea. In any case, these are very different tests, involving different pathways and mechanisms mediating the effects induced by activation of 5-HT_4 receptors. Furthermore, bioavailability was not studied. Effectiveness, when they were orally administered, suggests a good absorption but differences in distribution may be the cause of these differences.

5. Summary

Our present results focussed on the serotonergic system, demonstrating that UCM-61196, UCM-10197 and UCM-17197 display 5-HT_3 antagonistic activity, lack effects mediated through 5-HT_4 receptors, are effective when administered in vivo (i.p. or p.o.) and seem to induce fewer

behavioural changes at similar effective doses than does ondansetron, the reference compound.

These results suggest that the compounds are promising and support our earlier hypothesis (López-Rodríguez et al., 1999) about the interest of azabicyclic benzimidazole-4-carboxamides derivatives as 5-HT_3 receptor-selective antagonists.

References

Balfour, J.A., Goa, K.L., Perry, C.M., 2000. Alosetron. Drugs 59, 511–518.

Barocelli, E., Ballabeni, V., Bertoni, S., Silva, C., Impicciatore, M., 1999. Interactions of new and conventional H_3-antagonists with non-histaminergic receptors involved in neurogenic and myogenic contractile responses of guinea-pig ileum. J. Auton. Pharmacol. 19, 7–17.

Baxter, G.S., Craig, D.A., Clarke, D.E., 1991. 5-Hydroxytryptamine$_4$ receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae. Naunyn-Schmiedeberg's Arch. Pharmacol. 343, 439–446.

Bogle, R.G., Pires, J.G.P., Ramage, A.G., 1990. Evidence that central 5-HT_{1A}-receptors play a role in the von Bezold–Jarisch reflex in the rat. Br. J. Pharmacol. 100, 757–760.

Bourin, M., Hascoet, M., Deguiral, P., 1996. 5-HTP induced diarrhea as a carcinoid syndrome model in mice? Fundam. Clin. Pharmacol. 10, 450–457.

Buhot, M.C., Martin, S., Segu, L., 2000. Role of serotonin in memory impairment. Ann. Med. 32, 210–221.

Craig, D.A., Clarke, D.E., 1991. Peristalsis evoked by 5-HT and renzapride: evidence for putative 5-HT_4 receptor activation. Br. J. Pharmacol. 102, 563–564.

Derkach, V., Surprenant, A., North, R.A., 1989. 5-HT_3 receptors are membrane ion channels. Nature 339, 706–709.

Doak, G.J., Sawynok, J., 1997. Formalin-induced nociceptive behaviour and edema: involvement of multiple peripheral 5-hydroxytryptamine receptor subtypes. Neuroscience 80, 939–949.

Eglen, R.M., Bonhaus, D.W., Clark, R.D., Johnson, L.G., Lee, C.H., Leung, E., Smith, W.L., Wong, E.H., Whiting, R.L., 1994. (*R*) and (*S*) RS 56532: mixed 5-HT_3 and 5-HT_4 receptor ligands with opposing enantiomeric selectivity. Neuropharmacology 33, 515–526.

Espejo, E., Gil, E., 1998. Antagonism of peripheral 5-HT_4 receptors reduces visceral and cutaneous pain in mice, and induces visceral analgesia after simultaneous inactivation of 5-HT_3 receptors. Brain Res. 788, 20–24.

Farber, L., Stratz, T.H., Bruckle, W., Spath, M., Pongratz, D., Lautenschlager, J., Kotter, I., Zoller, B., Peter, H.H., Neeck, G., Welzel, D., Muller, W., 2001. Short-term treatment of primary fibromyalgia with the 5-HT_3-receptor antagonist tropisetron. Results of a randomized, double-blind, placebo-controlled multicenter trial in 418 patients. Int. J. Clin. Pharmacol. Res. 21, 1–13.

Faris, P.L., Kim, S.W., Meller, W.H., Goodale, R.L., Hofbauer, R.D., Oakman, S.A., Howard, L.A., Stevens, E.R., Eckert, E.D., Hartman, B.K., 1998. Effect of ondansetron, a 5-HT_3 receptor antagonist, on the dynamic association between bulimic behaviors and pain thresholds. Pain 77, 297–303.

Fortuno, A., Ballaz, S., Del Rio, J., Barber, A., 1999. CCK-mediated response in the activation of 5-HT receptor types in the guinea-pig ileum. J. Physiol. Biochem. 55, 85–92.

Fozard, J.R., 1984. MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. Naunyn Schmiedeberg's Arch. Pharmacol. 326, 36–44.

Fung, S.M., Ferrill, M.J., 2001. Treatment of bulimia nervosa with ondansetron. Ann. Pharmacother. 35, 1270–1273.

Grider, J.R., Foxx-Orenstein, A.E., Jin, J.G., 1998. 5-Hydroxytryptamine$_4$ receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. Gastroenterology 115, 370–380.

Grimsehl, K., Whiteside, J.B., Mackenzie, N., 2002. Comparison of cyclizine and ondansetron for the prevention of postoperative nausea and vomiting in laparoscopic day-case gynaecological surgery. Anaesthesia 57, 61–65.

Hartman, B.K., Faris, P.L., Kim, S.W., Raymond, N.C., Goodale, R.L., Eckert, E.D., 1997. Ondansetron in bulimia nervosa. Arch. Gen. Psychiatry 54, 969–970.

Hegde, S.S., Moy, T.M., Perry, M.R., Loeb, M., Eglen, R.M., 1994. Evidence for the involvement of 5-hydroxytryptamine$_4$ receptors in 5-hydroxytryptophan-induced diarrhea in mice. J. Pharmacol. Exp. Ther. 271, 741–747.

Hegde, S.S., Bonhaus, D.W., Johnson, L.G., Leung, E., Clark, R.D., Eglen, R.M., 1995. RS 39604: a potent, selective and orally active 5-HT_4 receptor antagonist. Br. J. Pharmacol. 115, 1087–1095.

Hendrie, C.A., 1990. The 5-HT_3 antagonist GR 38032F is highly toxic in DBA/2 mice. Psychopharmacology (Berlin) 101, 429–430.

Jarisch, A., Zotterman, Y., 1948. Depressor reflexes from the heart. Acta Physiol. Scand. 16, 31–51.

Jin, J.G., Foxx-Orenstein, A.E., Grider, J.R., 1999. Propulsion in guinea pig colon induced by 5-hydroxytryptamine (HT) via 5-HT_4 and 5-HT_3 receptors. J. Pharmacol. Exp. Ther. 288, 93–97.

Kankaanpaa, A., Meririnne, E., Seppala, T., 2002. 5-HT_3 receptor antagonist MDL 72222 attenuates cocaine- and mazindol-, but not methylphenidate-induced neurochemical and behavioral effects in the rat. Psychopharmacology 159, 341–350.

Kiso, T., Ito, H., Miyata, K., Kamato, T., Naitoh, Y., Iwaoka, K., Yamaguchi, T., 2001. A novel 5-HT_3 receptor agonist, YM-31636, increases gastrointestinal motility without increasing abdominal pain. Eur. J. Pharmacol. 431, 35–41.

López-Rodríguez, M.L., Benhamú, B., Morcillo, M.J., Tejada, I., Orensanz, L., Alfaro, M.J., Martín, M.I., 1999. Benzimidazole derivatives: 2. Synthesis and structure-activity relationship of new azabicyclic benzimidazole-4-carboxilic acid derivatives with affinity for serotoninergic 5-HT_3 receptors. J. Med. Chem. 42, 5020–5028.

Malinowska, B., Godlewski, G., Buczko, W., Göthert, M., 1996. Facilitation by substance P and inhibition by (+)-tubocurarine of the 5-HT_3 receptor-mediated Bezold–Jarisch reflex in rats. Eur. J. Pharmacol. 315, 159–164.

Nagakura, Y., Akuzawa, S., Miyata, K., Kamato, T., Suzuki, T., Ito, H., Yamaguchi, T., 1999. Pharmacological properties of a novel gastrointestinal prokinetic benzamide selective for human 5-HT_4 receptor versus human 5-HT_3 receptor. Pharmacol. Res. 39, 375–382.

Nunes de Souza, R.L., Canto de Souza, A., Rodgers, R.J., 2002. Effects of intra-hippocampal infusion of WAY-100635 on plus-maze behavior in mice. Influence of site of injection and prior test experience. Brain Res. 927, 87–96.

O'Connor, S.E., Grosset, A., La Rochelle, C.D., Gautier, E., Bidouard, J.P., Robineau, P., Caille, D., Janiak, P., 2001. Cardiovascular effects of SL65.0472, a 5-HT receptor antagonist. Eur. J. Pharmacol. 414, 259–269.

Pelissier, T., Alloui, A., Caussade, F., Dubray, C., Cloarec, A., Lavarenne, J., Eschalier, A., 1996. Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-hydroxytryptamine$_3$ receptors: in vivo and in vitro evidence. J. Pharmacol. Exp. Ther. 278, 8–14.

Pires, J.G.P., Silva, S.R., Ramage, A.G., Futuro-Neto, H., 1998. Evidence that 5-HT_3 receptors in the nucleus tractus solitarius and other brainstem areas modulate the vagal bradycardia evoked by activation of the von Bezold–Jarisch reflex in the anesthetized rat. Brain Res. 791, 229–234.

Pol, O., Ferrer, I., Puig, M., 1994. Diarrhea associated with intestinal inflammation increase the potency of Mu and Delta opioids on the inhibition of gastrointestinal transit in mice. J. Pharmacol. Exp. Ther. 270, 386–391.

Reeves, J.J., Shannon, M.W., Fleisher, G.R., 2002. Ondansetron decreases vomiting associated with acute gastroenteritis: a randomized, controlled trial. Pediatrics 109, e62.

Sakaue, M., Ago, Y., Murakami, C., Sowa, C., Sakamoto, Y., Koyama, Y., Baba, A., Matsuda, T., 2001. Involvement of benzodiazepine binding sites in an antiaggressive effect by 5-HT(1A) receptor activation in isolated mice. Eur. J. Pharmacol. 432, 163–166.

Sevcik, J., Ruzicka, V., Slansky, J., Smejkal, J., Masek, K., 1998. Is nitric oxide involved in 5-HT_3 receptor-mediated neurogenic relaxation of guinea pig proximal colon? Jpn. J. Pharmacol. 77, 265–270.

Takeda, M., Tsukamoto, K., Mizutani, Y., Suzuki, T., Taniyama, K., 1999. Identification of SK-951, a novel benzofuran derivative, as an agonist to 5-HT_4 receptors. Jpn. J. Pharmacol. 79, 203–212.

Talley, N.J., 2001. Serotoninergic neuroenteric modulators. Lancet 358, 2061–2068.

Taniyama, K., Nakayama, S., Takeda, T., Matsuyama, S., Shirakawa, J., Sano, I., Tanaka, C., 1991. Cisapride stimulates motility of the intestine via the 5-hydroxytryptamine receptors. J. Pharmacol. Exp. Ther. 258, 1099–1104.

Varga, V., Sik, A., Freund, T.F., Kocsis, B., 2002. GABA(B) receptors in the median raphe nucleus: distribution and role in the serotonergic control of hippocampal activity. Neuroscience 109, 119–132.

Woolley, D.W., 1958. Convenient method for assay in vivo of antimetabolites of serotonin. Proc. Soc. Exp. Biol. Med. 98, 367–370.