Effects of alosetron on spontaneous migrating motor complexes in murine small and large bowel in vitro

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Received 8 February 2001; accepted in final form 21 May 2001

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Several lines of evidence support the use of serotonin subtype 3 (5-HT₃) receptor antagonists for the treatment of IBS. Originally developed for suppressing chemotherapy-induced nausea, 5-HT₃ receptor antagonists attenuate 5-HT-induced signaling in visceral afferents (23). Intestinal distension in animals leads to activation of reflex behavior that is used to model visceral pain. 5-HT₃ antagonists potently suppress these reflexes (34). These compounds also suppress gut motility in IBS patients, particularly in the large bowel (26, 38, 46). Additional therapeutic effects of 5-HT₃ receptor antagonists may be a consequence of their antianxiolytic properties (14, 28).

Numerous studies have investigated the effects of 5-HT₃ receptor antagonists on human bowel function in health and disease. Rectal desensitization by granisetron in IBS patients occurred in the absence of effect on rectal tone (38). In addition, postprandial motility was inhibited but distension-induced motility was unaffected. On the other hand, ondansetron significantly slowed whole gut transit in healthy men (22) but did not influence any index of colonic function in five patients with diarrhea-predominant IBS (25). A single intravenous dose of ondansetron failed to affect rectal or gastric sensitivity in IBS, although compliance of the colon was increased (51). A gender selectivity of action for ondansetron has not been reported.

Recent studies demonstrated that alosetron (Lotronex), a novel and highly potent 5-HT₃ antagonist, gives relief of symptoms selectively in female patients with IBS, although in both studies the number of male patients included was low (1, 10). A larger study, conducted entirely in women, has demonstrated 1 mg b.d. as the most effective dose for improving abdominal pain and discomfort, urgency, and stool frequency and consistency (11). Alosetron did not change the perception of colonic distension, yet it significantly increased the compliance of the colonic wall to distension in IBS patients (15). In carcinoid diarrhea, alosetron did not affect gastric emptying or small bowel transit but significantly slowed proximal colon emptying, the most abnormal physiological parameter of this disease (43, 28, 32). Alosetron appears to exert its effect predominantly on the large bowel in vivo (28). Alosetron does not slow gastric emptying, diarrhea, or nausea but may decrease overall visceral sensation (15). In addition, alosetron has antianxiolytic properties (14, 28). Alosetron also affects the cardiovascular system as an alpha₂ agonist, and analgesic activity as a COX-2 inhibitor (15).

Alosetron is a potent and selective 5-HT₃ receptor antagonist that is effective in treating diarrhea-predominant IBS. Alosetron is efficacious in female patients with IBS but not in males. Alosetron may act centrally, involve the enteric nervous system (ENS), or affect gastric emptying or small bowel transit but does not significantly change rectal or gastric sensitivity in IBS.

Irritable Bowel Syndrome (IBS) is a functional gut disorder characterized by abdominal pain, discomfort, and altered bowel function (41). The syndrome affects ≥15% of the population (9, 17) and is characterized by a female predominance of ≥70% (35, 47). Although the pathophysiology remains unclear, it is most likely multifactorial. Dysmotility of both the large and small bowel of IBS patients has been described (12, 30, 31, 45). Inappropriate secretory activity is also a feature of IBS (2). Increasingly, however, visceral hypersensitivity has been implicated in IBS (16, 36, 49). Unfortunately, current therapies for IBS, including dietary fiber, opioids for diarrhea, smooth muscle relaxants, and psychotropic agents are not selective and do not give consistent relief of symptoms (8).

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In both healthy volunteers and IBS patients, alosetron delayed colonic transit in the absence of an effect on small bowel transit (26).

To investigate whether alosetron has a direct action on the gut and to determine whether this is a site of gender selectivity, we examined the effects of alosetron on spontaneous migrating motor complexes (MMCs) in the terminal ileum and colon of the C57BL/6 mouse in vitro. These events recorded from the murine bowel in vitro share pharmacological characteristics with phase III activity of the human MMC in vivo (20). Our earlier work (5) revealed no gender differences in frequency, amplitude, or duration of MMCs for either tissue. We therefore recorded the effects of alosetron and ondansetron on spontaneous MMCs in tissue from both males and females.

METHODS

Measurement of contractile activity. Nonfasted C57BL/6N mice (Simonsen Labs, Gilroy, CA) of either gender, 7–12 wk of age, were killed by cervical dislocation. The phase of the ovarian cycle in female mice was not determined. The entire colon and, separately, the terminal ileum of a comparable length (6–7 cm), were removed into modified Krebs solution. The luminal contents were flushed gently, a stainless steel rod (1.0-mm diameter) was inserted into the lumen of the bowel, and the tissue was secured to mounting posts fixed securely to the bottom of the bath using rubber O rings. The bath contained prewarmed Krebs solution at 37.0 ± 0.5°C and was gassed (3% CO₂-97% O₂ vol/vol) throughout the experiment. The Krebs solution was replaced approximately every hour until activity became coordinated.

Two stainless steel clips (micro-serrefines; Fine Science Tools, Foster City, CA) were attached to the tissue within 1.5–2 cm of the oral and anal ends. Suture silk was used to connect each clip to a force transducer (model TST125C; Biopac Systems, Santa Barbara, CA). Initial tension was routinely set to <5 mN to minimize local reflex stimulation of the bowel. Tension was monitored continuously using an MP100 interface and recorded on a PC running Acqknowledge software 3.2.6 (Biopac Systems).

Solutions and drugs. The composition of the Krebs solution was (in mM) 120.35 NaCl, 5.9 KCl, 15.5 NaHCO₃, 1.2 NaH₂PO₄, 1.2 MgSO₄, 2.5 CaCl₂, and 11.5 glucose. The solution was gassed continuously with a mixture of 3% CO₂-97% O₂ (vol/vol) to give a final pH of 7.3–7.4.

Stock solutions of 1 mM alosetron and ondansetron were made up in distilled water, aliquoted, and stored frozen. Dilution series were prepared daily and added sequentially to the bath.

Analysis of data and statistical methods. Contractile activity was analyzed from computer traces. Frequency and duration of coordinated peaks were assessed from the anal trace using established experimental parameters (5, 19). The anal trace was selected because peaks were more uniform and were more stable with increasing time ex vivo compared with the oral trace (see Figs. 2–4). The duration of each contraction was determined as the time between the half-maximal amplitude points on the rising and falling phases. The interval between contractions was determined by the time between the half-maximal amplitude points on the rising phase of consecutive contractions (Fig. 1). Amplitude, duration, and frequency were expressed as percentage of control values for statistical analysis. Control values were obtained from a minimum of five peaks immediately preceding the application of antagonist to the bath. Data are presented as means ± SE. Log IC₅₀ and Hill coefficient values were obtained using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). Statistical significance was determined using Student’s t-test (paired or unpaired as appropriate) or Mann-Whitney rank correlations.

RESULTS

Stability of MMC activity in large and small bowel. To demonstrate that stability of MMC activity exceeded the duration of the antagonist experiments, preparations were monitored continuously in the ab-
sence of any manipulation (Figs. 2A and 3A). Although
duration and frequency remained constant, there was
a tendency for the amplitude to wane. This effect was
significant in oral traces but did not reach significance
in anal traces (data not shown). All measurements of
effects of antagonists were measured using recording
of activity in anal traces.

**Effects of alosetron on spontaneous MMC activity in large and small bowel.** Alosetron dose-dependently de-
creased the frequency of MMC activity in both the
large and small bowel (Figs. 2B and 3B). The effect on
frequency occurred before effects on either amplitude
or duration. Although there was no effect of gender on
colic frequency, the small intestine from females
showed a significantly greater sensitivity than tissue
from males (Fig. 4). There was a 100-fold difference
in threshold concentrations required to slow MMC ac-
tivity in females and males (20 nM and 2 µM, respec-
tively). MMC activity in both tissues was abolished by
10 µM alosetron. In both intestinal regions, there was
a tendency for reduced amplitude in the presence of 2
µM alosetron, but this was only significant in the small
bowel (Fig. 4). With increasing doses of alosetron, the
duration of each MMC appeared to decrease in the
male colon. In contrast, there was a significant in-
crease in the duration of MMCs in female tissue. No
effect of gender on duration of MMC in the small
intestine was observed.

**Effects of ondansetron on spontaneous MMC activity in large and small bowel.** The effects of ondansetron on
spontaneous MMC activity in both the small and large
bowel of the mouse were qualitatively indistinguish-
able from the effects of alosetron (Fig. 5). As was
observed for alosetron, the threshold for effect of on-
dansetron on MMC frequency occurred before an effect
on either amplitude or duration. No effect of gender

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**Fig. 2.** Effect of alosetron on MMC ac-
tivity in male colon. Once established,
MMC activity was sustained in colonic
preparations for >3 h (A). Sequential
addition of alosetron caused a pro-
nounced slowing of MMC frequency
(B).
was observed on the decrease in MMC frequency in the small intestine. At low concentrations of ondansetron (<5 nM), we observed a small but significant increase in MMC frequency selectively in male small intestine preparations (Fig. 6). In the colon, however, we observed a large gender difference in the response to ondansetron. The threshold for inhibition of MMC frequency was 100-fold lower in the male colon compared with the female colon (Fig. 6; 20 nM and 2 μM, respectively).

The effects of ondansetron on amplitude were not affected by gender in either tissue. In contrast, and similar to the trend observed with alosetron, ondansetron appeared to reduce the duration of MMC activity in the colon selectively in male tissue. This effect was not observed in the small intestine.

Gender selectivity of effect of 5-HT₃ antagonists on MMC activity in large and small bowel. To determine IC₅₀ values for each drug, the inhibition of MMC frequency was plotted against drug concentration (Fig. 7). The values ranged from 0.1 to 1.5 μM (Table 1). No gender differences were apparent between any drugs or tissues, with the exception that ondansetron was significantly more potent in the male compared with the female colon. Although no differences in IC₅₀ values for alosetron were observed (Table 1), the inhibitory effect in the female small intestine was significantly greater than that in male tissue (Fig. 7). This was reflected in the large difference between the Hill coefficients for these tissues (Table 2).

DISCUSSION

Recent clinical trials have demonstrated that the 5-HT₃ receptor antagonist alosetron (Lotronex) relieves symptoms of IBS and that this occurs selectively in female patients (1, 10, 11). However, the sites of action and mechanisms that underlie both the action and gender selectivity of alosetron remain uncertain.
In this study, we found that alosetron and ondansetron had a direct, selective inhibitory effect on the frequency of spontaneous MMC in isolated small and large intestine from C57BL/6 mice. In addition, with the exception of human studies, this is the first study to demonstrate a gender difference in bowel response to 5-HT3 antagonism.

**Gender selectivity of action.** Our previous work (5) demonstrated that gender had no effect on the parameters of MMC activity. We therefore made comparisons between drugs, genders, and bowel regions. A striking and reciprocal effect of gender was observed for the two drugs. The threshold for effects of alosetron in the female small intestine (20 nM) was 100-fold lower than for male small intestine. There was no effect of gender in the colon. Ondansetron, on the other hand, showed no gender effect in the small intestine, but the threshold in the male colon (20 nM) was 100-fold lower than in the female colon. The threshold for effect and IC50 values, ranging from 0.1 to 1.5 μM, strongly suggest that 5-HT3 receptor activation regulates the frequency of MMCs in the murine bowel.

The therapeutic effect of alosetron in female IBS patients can be observed at 1 (11) and 2 (1) mg b.d. Pharmacokinetic studies have demonstrated that the peak plasma concentration after oral administration of 2 mg is between 10 and 20 ng/ml (approximating 30–60 nM) (24). However, because the alosetron shows a large first-pass effect and the gastrointestinal tract may be the site of this effect, the local concentration within the gastrointestinal tract may be much higher. The pharmacokinetic profiles of ondansetron and alosetron are similar. Systemic exposure after oral or intravenous dosing with ondansetron was significantly higher in

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**Fig. 4.** Effect of alosetron on MMC parameters. Alosetron showed a dose-dependent decrease in frequency of MMC activity in both the small and large bowel. No effect of gender was observed in the colon. However, the threshold for slowing of MMCs occurred at a 100-fold lower dose in female compared with male small intestine (20 nM and 2 μM, respectively). All MMC activity was abolished by 10⁻⁵ M alosetron. *P < 0.05, **P < 0.01 compared with control activity. SI, small intestine.
women than in men (39). A higher systemic exposure in women is also observed for alosetron (24). Three mechanisms may be responsible. First, women cleared the drug more slowly than men. Second, women showed higher bioavailability, presumably because of reduced first-pass uptake of drug. Third, the weight-adjusted volume of distribution was smaller in women. However, increasing the dose of alosetron in male IBS patients was ineffective in alleviating symptoms, suggesting that factors other than bioavailability are responsible for the gender selectivity of alosetron efficacy (10).

It is therefore currently unclear why alosetron shows efficacy selectively in female IBS patients. This is the first study to demonstrate a gender difference in the effects of 5-HT3 antagonists in an animal model of gut physiology. The principal gender difference in response to alosetron was a 100-fold lower threshold for decreasing frequency of ileal MMC activity in female mice. Because the distal small intestine has been implicated as the origin of symptoms in some patients with IBS (30, 31), it is tempting to speculate that the gender selectivity of alosetron is based on a direct effect on the female small intestine. Alosetron did not affect ileal transit in clinical studies, suggesting that effects on ileal motility are not the mechanism of its therapeutic efficacy (26). Our data also show that there are gender differences between individual 5-HT3 antagonists in this assay. One explanation for this effect is a differential distribution of 5-HT3 receptor subtypes. Recently the pharmacological properties of a shorter splice variant of the murine 5-HT3 receptor have been...
reported (4). Although most drugs exhibited almost identical properties at the native and variant receptor, ondansetron was more potent at the shorter splice variant. Whether gender differences in the expression patterns of the full-length 5-HT$_3$ receptor and the splice variant exist in either mice or humans is currently unknown.

Extensive preclinical pharmacology has demonstrated the potency and selectivity of alosetron. The pK$_i$ values for the rat (9.8) and human (9.4) 5-HT$_3$ receptors are very similar, with no other receptor demonstrating binding with a pK$_i$ >6 (13). The pK$_B$ for alosetron on rat 5-HT$_3$ receptors was 9.8 (13), whereas in the same assay ondansetron had a pK$_B$ of 8.6 (7). Unfortunately, interspecies comparisons must be interpreted with caution because large species difference in 5-HT$_3$ receptor pharmacology have been reported. In particular, binding of antagonists at the guinea pig 5-HT$_3$ receptor appears to require several orders of magnitude greater concentrations of drug compared with binding at the rat receptor (6). Although the binding affinity for the murine 5-HT$_3$ receptor has not been assessed directly, in vivo and tissue culture studies have shown that its pharmacological properties are similar to those of the rat receptor (4, 32, 50). In vivo studies have shown that ondansetron (3.2 mg/kg) does not disturb normal colonic transit in the rat (29). However, lower doses (0.1–1 mg/kg) dose dependently increased whole gut transit time in male mice (37). Similarly, the concentrations required to demonstrate the antianxiolytic effects of ondansetron are similar in rats and mice (28). Together, these results strongly suggest that the mouse 5-HT$_3$ receptor more closely resembles that of the rat than that of the guinea pig.

Fig. 6. Effect of ondansetron on MMC parameters. Ondansetron showed a dose-dependent decrease in frequency of MMC activity in both the small and large bowel. No effect of gender was observed in the small intestine. In the colon, and the reverse of the selectivity of alosetron, the threshold at which MMC frequency was inhibited in the male occurred at a 100-fold lower dose then in the female (20 nM and 2 μM, respectively). All MMC activity was abolished by 10$^{-5}$ M ondansetron. *P < 0.05, **P < 0.01 compared with control activity.
Possible enteric sites and modes of drug action. One of the original criteria for diagnosing IBS is abdominal pain relieved by defecation (33). It has been shown that pain associates temporally with eating but not defecation in IBS (40). A striking correlation between prolonged propagating contractions (PPCs) and abdominal pain was observed in IBS patients (30). Interestingly, this study also reported that PPCs were also associated with postprandial pain, similar to that regularly experienced in 44% of IBS patients. Together, these studies suggest that in some patients the ileum may be the site of origin of symptoms of IBS.

Although 5-HT3 receptors have central, peripheral, and enteric locations, our data suggest that alosetron and ondansetron have direct actions on both the small and large intestine. Recent studies also showed that 5-HT3 receptor antagonists can act directly on the large intestine, because LY-278584 slows the propulsion of pellets in the isolated guinea pig distal colon (27) and ondansetron reduces MMC activity in the isolated colon of nonaffected littermates of the piebald lethal mouse (18).

Table 1. Effect of 5-HT3 antagonists on MMC frequency

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<thead>
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<th>IC50 (95% CI), μM</th>
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<tbody>
<tr>
<td></td>
<td>Colon</td>
</tr>
<tr>
<td>Female</td>
<td>0.9 (0.3–2.9)</td>
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<tr>
<td>Male</td>
<td>0.6 (0.4–1.0)</td>
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5-HT3, serotonin subtype 3 receptor; MMC, migrating motor complex; CI, confidence interval; SI, small intestine. *P < 0.05 compared with equivalent gender group.

Table 2. Hill coefficient values for inhibition of MMC frequency by 5-HT3 receptor antagonists

<table>
<thead>
<tr>
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<th>Hill Coefficient</th>
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<tr>
<td></td>
<td>Alosetron</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>Male</td>
<td>1.6 ± 0.9</td>
</tr>
<tr>
<td>SI</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2.6 ± 1.0</td>
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<tr>
<td>Male</td>
<td>6.4 ± 2.9</td>
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5-HT3 receptor antagonists could affect neuro-neurotransmission down the bowel, because some descending interneurons in the mouse are serotonergic and ligand-gated 5-HT3 receptors have been demonstrated on different functional classes of enteric neurons, at least in the guinea pig (42, 44). However, both alosetron and ondansetron reduced MMC frequency more effectively than complex amplitude, suggesting that 5-HT3 receptors were more likely to be involved in the pacemaker responsible for the generation of the MMC than in the conduction of MMCs down the bowel. This latter phenomenon is critically dependent on cholinergic neurotransmission (5, 19). Alosetron and ondansetron could be blocking 5-HT3 receptors on the mucosal processes of intrinsic sensory neurons, which are readily activated by mucosally applied 5-HT (3). The generation of the MMC may therefore be dependent on spontaneous release of serotonin from enterochromaffin (EC) cells in the mucosa, which contain over 90% of the body’s serotonin, that excites intrinsic sensory neurons.
sensory neurons (44). EC cells themselves express 5-HT_{3} receptors that appear to enhance 5-HT release (21). 5-HT_{3} antagonism at this site would therefore reduce the frequency of 5-HT release and may in turn reduce MMC frequency.

In conclusion, our results show clear gender differences in the action of both alosetron and ondansetron on spontaneous migrating motor activity in the murine bowel. Compared with the male small intestine, alosenon had a significantly lower threshold for effects in the female small intestine. The ileum has been identified as a site of origin of symptoms in IBS in humans. Gender differences in the distribution of the 5-HT_{3} receptor or its splice variants have the potential to contribute to the gender selectivity of action of alose- 
tron in IBS.

Support for this project was provided by the National Institute of Diabetes and Digestive and Kidney Diseases (DK-10793 to T. K. Smith, PO1-DK-41315 to K. M. Sanders and T. K. Smith) and by Glaxo Pharmaceuticals (K. M. Sanders and T. K. Smith).

Preliminary results of this study were presented at the annual meeting of the American Gastroenterological Society, San Diego, CA, 2000, and at the International Society for Autonomic Neuroscience, London, UK, 2000.

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