ELSEVIER

European Journal of Pharmacology 328 (1997) 201–206

ejp

Ondansetron facilitates neuromuscular transmission in the guinea-pig ileum

Asensio A. González, Margarita M. Puig *

Anesthesiology Research Unit, IMIM, Department of Anesthesiology, Hospital Universitario del Mar, Paseo Marítimo, 25, 08003 Barcelona, Spain

Received 3 February 1997; revised 27 March 1997; accepted 28 March 1997

Abstract

The effects of ondansetron on the neuromuscular function of the guinea-pig ileum were investigated in vitro. Ondansetron, but not tropisetron or MDL 72222 (1α-*H*-3α-5α-*H*-tropan-3-yl-3,5-dichlorobenzoate), enhanced submaximal electrically induced contractions ($EC_{50} = 1.3 \times 10^{-5}$ M). Desensitization with 5-hydroxytryptamine (1×10^{-5} M) or 2-methyl-5-HT (1×10^{-5} M) abolished this facilitatory response, which remained unaltered after desensitization with 5-methoxytryptamine (1×10^{-5} M) or addition of tropisetron, MDL 72222, *N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan, SB203186 (1-piperidinylethyl-1*H*-indole-3 carboxylate hydrochloride), pirenzepine or hexamethonium. At higher concentrations, ondansetron decreased the electrically induced contractions ($EC_{50} = 1 \times 10^{-4}$ M); the inhibitory response was unaffected by (−)-naloxone (1×10^{-6} M) or idazoxan (1×10^{-6} M). We conclude that, in the guinea-pig ileum, ondansetron elicits a biphasic response: facilitation of neuromuscular transmission mediated by a serotonergic receptor distinct from the 5-HT_3, 5-HT_4 or putative 5-HT_{1P} receptors, and an inhibitory response that does not involve opiate or α_2-adrenoceptors.

Keywords: Ondansetron; Ileum, guinea-pig; 5-HT receptor

1. Introduction

5-Hydroxytryptamine (5-HT) is a neurotransmitter in the enteric nervous system of mammals where it seems to participate in the physiologic control of gastrointestinal motility (Gershon et al., 1994; Dhasmana et al., 1993; Talley, 1992) although its precise role remains unknown. In vitro studies have shown that exogenous 5-HT acts in a complex and sometimes antagonistic manner on the contractility of the smooth muscle of the gut (Sanger, 1985). The variety of responses of the gastrointestinal muscle to 5-HT could be explained on the basis of activation of different types of serotonergic receptors, located at various levels in the intramural pathways that control neuromuscular function. On the basis of pharmacological data (Burks, 1994), 5-HT_{1A}, 5-HT_3, 5-HT_4 and the putative 5-HT_{1P} neuronal receptors, and 5-HT_2 smooth-muscle receptors have been identified in the small intestine.

Some benzamide compounds which stimulate gastric and intestinal motility in mammals including man, have concurrent 5-HT_3 receptor antagonist and 5-HT_4 receptor agonist properties, although their ability to stimulate gastrointestinal motility is mediated by the activation of peripheral 5-HT_4 receptors (Briejer et al., 1995). However, some observations suggest that 5-HT may also increase gastrointestinal motility by interacting with receptors other than the 5-HT_4 receptor. For example, in the canine antrum, 5-HT enhances contractility in vitro through a mechanism resistant to 5-HT_4 receptor blockade (De Ridder and Schurkes, 1993). Also ondansetron, a 5-HT_3 receptor antagonist without affinity for the 5-HT_4 receptor, stimulates gastric emptying in guinea pigs and rats in vivo (Costall et al., 1987; Gidda et al., 1988; Ohshika et al., 1990; Buchheit and Buhl, 1994) and in vitro (Buchheit and Buhl, 1994). Although central actions could explain the in vivo data, the in vitro effect has not been well characterized. Interestingly, in the guinea-pig isolated ileum, the relaxation induced by 5-HT (Carter et al., 1995) or 2-methyl-5-HT (Gunning and Humphrey, 1987) is antagonized by ondansetron.

In the present investigation we studied the functional responses of the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum to ondansetron, at concentrations ranging from 10^{-7} to 2×10^{-4} M. Our aim

* Corresponding author. Tel.: (34-3) 221-1010, ext. 2185; Fax: (34-3) 221-0541.

0014-2999/97/$17.00 Copyright © 1997 Elsevier Science B.V. All rights reserved.

was to define the possible site(s) and mechanism(s) of action of ondansetron by using selective antagonists and discriminating desensitization.

2. Materials and methods

Male Dunkin-Hartley guinea pigs weighing 250–400 g were killed by a blow to the occipital region. The small intestine was removed, rinsed with Krebs solution at 37°C and the terminal 20 cm discarded. Immediately afterwards, the longitudinal muscle-myenteric plexus was dissected and placed in 10-ml tissue baths containing Krebs solution at 37°C and gassed with 5% CO_2 in O_2. The strips were stretched to a positive tension of 0.3 g and then allowed to equilibrate for a period of 1 h; during this time the bath solution was replaced every 20 min. The preparations were studied in three different ways: (a) non-stimulated; (b) stimulated electrically at supramaximally effective voltage; (c) stimulated electrically at submaximally effective voltage.

For electrical field stimulation of the strips, bipolar rectangular pulses were passed between two platinum wire electrodes placed 6.8 cm apart at the top and bottom of the tissue bath. The pulses were generated by S88 Grass stimulators, amplified with a Crown DC 300 amplifier and monitored with a Tektronix R5103 N oscilloscope. Pulses were delivered at a frequency of 0.1 Hz, 1 ms duration and supramaximally or submaximally effective voltage according to the experiments being performed. In the present study, the supramaximally effective voltage was determined by increasing the voltage until maximal muscle contractions were obtained (40 V). The submaximally effective voltage was the voltage that evoked contractions of approximately 50–60% of the supramaximal amplitude and was variable for each preparation (14–27 V). Changes in tension were isometrically monitored through Grass FT03 transducers and recorded with a Grass 7D polygraph.

Drugs were added as single (non-cumulative) randomly sequenced concentrations to the tissue bath, in aliquots of 50–100 μl. The effects of drugs are expressed as percent inhibition or percent increase over basal amplitude (in mm) in the absence of treatment. Unless otherwise stated, the results are expressed as mean values with 95% confidence limits in parentheses. The pharmacological parameters were obtained by using the Pharmacological Calculation System (Tallarida and Murray, 1986). Student's *t*-test was used for comparisons between groups, and a $P < 0.05$ was considered statistically significant. Each group consisted of a given number of strips obtained from at least five different animals.

2.1. Drugs

Ondansetron hydrochloride dihydrate (Glaxo); 5-methoxytryptamine, 2-methyl-5-hydroxytryptamine and idazoxan (RBI); *N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP) (Research Foundation for Mental Hygiene, NY, USA); tropisetron and MDL 72222 (1α-*H*-3α-5α-*H*-tropan-3-yl-3,5-dichloro-benzoate), were given by Dr. M.I. Loza. Pirenzepine hydrochloride (ICI); SB 203186 (1-piperidinylethyl-1*H*-indole-3 carboxylate) hydrochloride (Tocris Cookson, UK). All other chemicals were purchased from Sigma.

3. Results

3.1. Antagonism of the effects of 2-methyl-5-HT by ondansetron

2-Methyl-5-HT induced a prominent, fast contraction of the non-stimulated longitudinal muscle-myenteric plexus preparation, followed by a rapid but incomplete return to baseline. Regardless of the residual tone, which persisted for at least 3 min, only the initial contraction was quantified for the purpose of the study. The response was concentration-dependent and produced an EC_{50} value of 6.2 $(5.1–7.3) \times 10^{-6}$ M. Identical responses were obtained during electrical stimulation at submaximally effective voltage and the resulting concentration–response lines were indistinguishable.

Concentration–response curves for 2-methyl-5-HT were progressively shifted to the right in the presence of increasing concentrations of ondansetron (10^{-7} M, 5×10^{-7} M and 3×10^{-6} M) and Schild analysis of the interaction gave a pA_2 value of 7.33 (6.99–7.67) with a slope of 0.94 (0.81–1.08), indicating competitive antagonism.

3.2. Excitatory and inhibitory effects of ondansetron

At concentrations between 3×10^{-6} M and 2×10^{-5} M ondansetron enhanced the electrically induced contractions in a concentration-dependent manner. Supramaximally stimulated preparations showed a slight increase in amplitude that was not quantified. Instead, in submaximally stimulated strips the response was clearly measurable and consisted of a concentration-related increase in the amplitude of the twitch contractions (Fig. 1, upper trace). The estimated EC_{50} for this effect was 1.32 $(1.27–1.38) \times 10^{-5}$ M. In non-stimulated strips no measurable effect was seen at these concentrations of ondansetron, although a slight elevation of the basal tone was observed in 7 of 21 strips at concentrations above 1×10^{-5} M (Fig. 1, lower trace). It is worth noting that concentrations of ondansetron higher than 2×10^{-5} M also increased the amplitude of electrically induced contractions, but the presence of a superimposed inhibitory effect (see below) interfered with quantification, so that 2×10^{-5} M was the highest stimulatory concentration evaluated, reaching a 59% (51–67) increase over basal contractions. Other 5-HT_3 antagonists such as tropisetron and MDL 72222, used at

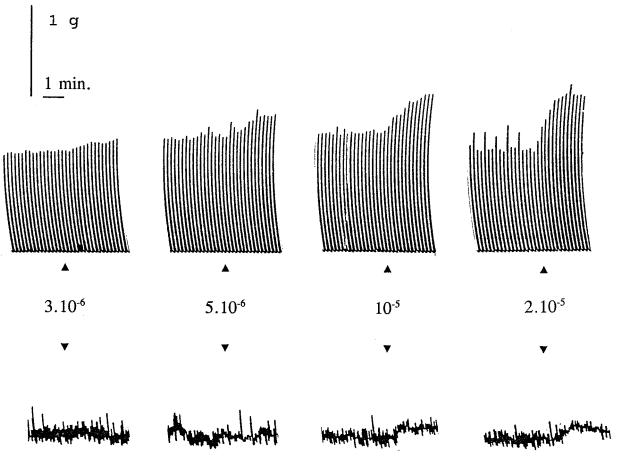

Fig. 1. Effect of ondansetron on an electrically stimulated (0.1 Hz; 1 ms; submaximally effective voltage) preparation of guinea-pig ileum (upper trace) and an unstimulated preparation (lower trace). Arrowheads indicate addition of ondansetron to the bath to obtain the concentrations (M) indicated.

concentrations between 10^{-7} M and 2×10^{-6} M, did not show the facilitatory effect induced by ondansetron (Fig. 2). Concentrations of ondansetron in the range of 2×10^{-5} to 2×10^{-4} M produced an inhibition of the twitch contractions in strips stimulated with either supra- or submaximally effective voltage. The concentration–response relationships generated a steep curve in both cases, reaching 100% inhibition at a concentration of ondansetron of 2×10^{-4} M. The EC_{50} for supramaximally stimulated strips was 1.03 (0.95–1.12) $\times 10^{-4}$ M with a slope of 155 (113–197). This inhibitory effect was readily and completely reversed by washing, but was unchanged in the presence of (−)-naloxone (1×10^{-6} M) or idazoxan (1×10^{-6} M). The inhibitory response was also observed with tropisetron and MDL 72222 at concentrations ranging between 2×10^{-6} M and 5×10^{-5} M (Fig. 2).

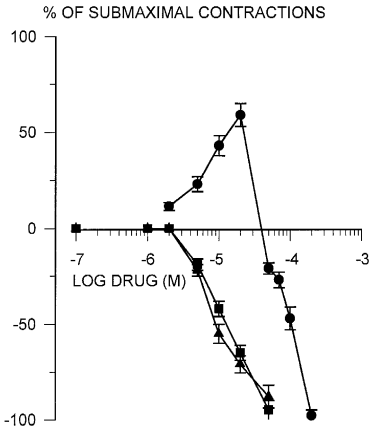

Fig. 2. Effects of ondansetron (●, $n = 38$), tropisetron (■, $n = 16$) and MDL 72222 (▲, $n = 15$) on the amplitude of electrically induced contractions of preparations stimulated at 0.1 Hz, 1 ms pulse duration and submaximally effective voltage. The points represent the mean values and vertical bars indicate the S.E.M.

3.3. Characterization of the excitatory effect of ondansetron

To test if smooth muscle becomes more sensitive to acetylcholine in the presence of ondansetron, we carried out a series of experiments in which concentration–response curves with acetylcholine were run in the absence (control) and presence of a fixed concentration of ondansetron (1×10^{-5} M). The results obtained from five experiments showed no significant differences between the curves in the absence and in the presence of ondansetron. The EC_{50} and slope of the concentration–response curves to acetylcholine were 1.03 (0.9–1.2) and 43 (36–51) in the absence, and 1.04 (0.7–1.4) and 44 (27–61) in the presence of ondansetron.

The effects of the ganglionic blocking agent, hexamethonium (1×10^{-5} M), and that of pirenzepine (1×10^{-7} M), a muscarinic M_1 receptor antagonist (Schuurkes et al., 1988), were also tested on our preparation. Both compounds failed to alter the excitatory response to ondansetron (Table 1).

In order to evaluate the involvement of the different types of 5-HT receptors in the excitatory response to ondansetron, we performed a series of desensitization studies. In these experiments, the excitatory effect of 1×10^{-5} M ondansetron was initially observed in preparations stimulated with submaximal voltage. Subsequently, the preparations were incubated for a period of 30 min with 5-HT or one of the receptor-selective agonists, and the effect of the same concentration of ondansetron was again tested. For each preparation the excitatory effect of ondansetron was measured and compared before and after the incubation with a given agonist.

The excitatory response to a single concentration of 1×10^{-5} M ondansetron in ten strips yielded a mean response with a 35.5% (31–40%) increase over basal contractions (Table 2). Adding 5-HT to the bath at a final concentration of 1×10^{-5} M initially increased the amplitude of the twitch contractions, which returned to their baseline level approximately 20 min afterwards. Thirty minutes after 5-HT addition, the excitatory response to

Table 1
Excitatory effect of 1×10^{-5} M ondansetron before (control) and 15 min after adding the various antagonists to the bath

Drug	Before	After	*P*
Hexamethonium 10^{-5} M ($n = 9$)	26.7 ± 2.7	30.3 ± 4.9	0.58
Pirenzepine 10^{-7} M ($n = 11$)	19.7 ± 2.3	21.7 ± 3.9	0.70
SB 203186 10^{-6} M ($n = 8$)	21.0 ± 1.9	24.8 ± 3.3	0.42
5-HTP-DP 10^{-5} M ($n = 12$)	25.7 ± 3.7	25.4 ± 2.5	0.95
MDL 72222 10^{-6} M ($n = 7$)	27.0 ± 6.6	32.6 ± 6.0	0.63
Tropisetron 5×10^{-7} M ($n = 10$)	26.3 ± 3.8	28.0 ± 4.2	0.79
Tropisetron 3×10^{-6} M ($n = 10$)	24.1 ± 3.3	26.0 ± 3.3	0.40
Vehicle ($n = 11$)	27.2 ± 3.9	34.1 ± 2.8	0.31

Results are means ± S.E.M.

Table 2
Excitatory response to 1×10^{-5} M ondansetron before (control), and after 30 min exposure to 5-HT (1×10^{-5} M), 2-methyl-5-HT (1×10^{-5} M) or 5-methoxytryptamine (5-MeOT) (1×10^{-5} M)

Agent	Before	After	P
5-HT ($n=10$)	35.5 ± 2.5	3.0 ± 0.9	<0.001
2-Methyl-5-HT ($n=10$)	35.7 ± 2.3	-2.6 ± 3.9	<0.001
5-MeOT ($n=10$)	33.6 ± 1.9	29.6 ± 2.8	0.23

Results expressed as percent increase over basal contractions for each set of experimental conditions (mean ± S.E.M.). P value is referred to control experiments.

ondansetron had decreased significantly ($P<0.001$). On the contrary, the excitatory response to ondansetron did not change significantly when vehicle (instead of 5-HT) was added to the bath. In another series of experiments, strips were exposed either to 2-methyl-5-HT (1×10^{-5} M) or to 5-methoxytryptamine (1×10^{-5} M) for 30 min, after which the effect of the same concentration of ondansetron was measured. The preparations exposed to 2-methyl-5-HT no longer responded to ondansetron, and even exhibited a slight inhibitory effect. In contrast, the excitatory response to ondansetron was unaltered in the strips incubated with 5-methoxytryptamine (Table 2).

The excitatory effect of ondansetron (1×10^{-5} M) was also evaluated in the presence of the following drugs: the 5-HT_3 receptor antagonists, MDL 72222 (1×10^{-6} M) and tropisetron (3×10^{-7}–1×10^{-6} M), the 5-HT_4 receptor antagonists, SB 203186 (1×10^{-6} M) and tropisetron (3×10^{-6} M, which at this concentration is a 5-HT_4 receptor antagonist), and the putative 5-HT_{1P} receptor antagonist, 5-HTP-DP (1×10^{-5} M). When added individually, none of these drugs had a significant effect on the submaximally effective twitch contractions; 5-HTP-DP at a concentration of 1×10^{-5} M induced a slight increase of less than 10% in two out of twelve preparations. Thus, our results show that none of the above mentioned antagonists blocked the excitatory effects of ondansetron (Table 1).

4. Discussion

The antiemetic properties of ondansetron are predominantly related to the blockade of 5-HT_3 receptors located on vagal afferent fibers projecting from the gastrointestinal tract. The longitudinal muscle-myenteric plexus of the guinea-pig ileum is a good in vitro preparation to study the functional effects of 5-HT_3 receptors in this species (Sanger et al., 1994). In this preparation, 5-HT or the more selective agonist 2-methyl-5-HT induces a neuronally mediated contraction which is antagonized by ondansetron and by other 5-HT_3 receptor antagonists such as tropisetron and MDL 72222.

The present investigation showed that, in addition to the above mentioned antagonism at 5-HT_3 receptors, higher concentrations of ondansetron also elicit an excitatory response in the longitudinal muscle-myenteric plexus preparation. Thus, at concentrations between 2×10^{-6} and 2×10^{-5} M, ondansetron increased the amplitude of electrically induced contractions in a concentration-dependent manner. The lack of a similar effect of other 5-HT_3 receptor antagonists suggests that the response is drug-specific for ondansetron and probably unrelated to the antagonism of 5-HT_3 receptors.

Other investigators have recently reported that ondansetron and, to a lesser extent, granisetron, enhance electrically induced contractions of guinea-pig gastric strips (Buchheit and Buhl, 1994), and that this effect is not observed with tropisetron or MDL 72222. Thus, our results support and extend the finding of an excitatory effect of ondansetron on the gastrointestinal tract of the guinea pig. In vivo, ondansetron can stimulate gastric emptying in guinea pigs and, in the rat, ondansetron and granisetron accelerate intestinal transit previously delayed by the ileal infusion of triglycerides (Brown et al., 1993). Although these effects have been postulated to be mediated by 5-HT_3 receptors in the central nervous system, it is also possible that the in vitro effects reported in our study could be related to an analogous excitatory mechanism.

The excitatory effect of high concentrations of ondansetron was readily replicated in all preparations; however, we were unable to fully characterize its possible mechanism(s). In our search, we excluded a possible increase in sensitivity of the smooth muscle to the activation of muscarinic receptors by completing concentration–response curves to exogenous acetylcholine in the presence of an excitatory concentration of ondansetron. A preganglionic mechanism was excluded by the lack of effect of hexamethonium. Moreover, muscarinic M_1 receptor blockade with pirenzepine did not alter the ondansetron-induced excitation, excluding the possibility that ondansetron antagonizes the muscarinic autoinhibition of acetylcholine release. Alternatively, we hypothesized that ondansetron could act on a neuronal 5-HT receptor located on the myenteric plexus that would facilitate or increase acetylcholine (or other excitatory neurotransmitter) release upon electrical stimulation.

In order to search for the type of 5-HT receptor involved, we performed desensitization experiments. The excitatory effects of 5-HT in the peripheral nervous system have been shown to be reduced or abolished after prolonged exposure to high concentrations of the endogenous agonist (desensitization). In our experiments, after exposure of the strips to 5-HT the excitatory effect of ondansetron was blocked (by approximately 90%), indicating that intact (not desensitized) serotonergic receptors are required for the excitatory response to ondansetron; therefore, we suggest that the response is mediated by 5-HT receptors.

Other investigators (Craig et al., 1990) have specifically desensitized 5-HT_3 and 5-HT_4 receptors in the guinea-pig

ileum after incubating the preparation with selective agonists. In order to investigate further the type of 5-HT receptor involved in the excitatory response to ondansetron, we attempted to apply the same pharmacological strategy. Under our experimental conditions, desensitization with 2-methyl-5-HT completely suppressed the facilitatory response, raising the possibility that ondansetron at these concentrations behaves as a 5-HT_3 receptor agonist. However, the excitatory response was unaltered in the presence of the 5-HT_3 receptor antagonists, tropisetron or MDL 72222. It is possible that 2-methyl-5-HT could desensitize other receptors besides the 'classic' 5-HT_3, since other investigators have suggested that the agonist activates 5-HT_{1P} putative receptors (Mawe et al., 1986). Putative 5-HT_{1P} receptors are present in the guinea-pig ileum and are readily desensitized, thus it is possible that the exposure to 2-methyl-5-HT induced tachyphylaxis to the effects mediated by these putative receptors. However, the selective antagonist, 5-HTP-DP, failed to antagonize the response, ruling out the involvement of putative 5-HT_{1P} receptors in the facilitatory response to ondansetron. On the other hand, the selective desensitization of 5-HT_4 receptors or the presence of 5-HT_4 receptor antagonists did not alter the excitatory response to ondansetron, indicating that these receptors are apparently not involved.

The inhibitory effect of ondansetron observed at concentrations above 2×10^{-5} M was also elicited by other 5-HT_3 receptor antagonists such as tropisetron and MDL 72222; this effect has been previously reported by other investigators, but its mechanism remains obscure. The steep slope of the concentration–response curve suggests a complex interaction (see Daniel et al., 1989). In cardiac myocytes, high concentrations of tropisetron (10^{-4} M) block sodium and potassium channels (Scholtysik et al., 1988), a possible mechanism to explain the inhibitory effect of ondansetron. In the present investigation, the reversal by (−)-naloxone, a non-specific opioid receptor antagonist, and idazoxan, an α_2-adrenoceptor antagonist, was also attempted. These experiments were carried out in order to rule out a non-specific interaction with other receptors/systems known to mediate inhibitory responses in this preparation (Puig et al., 1990). Since none of these drugs altered the inhibitory response to ondansetron, a local anesthetic-like effect seems to be the most likely explanation.

In conclusion, our results showed that ondansetron at concentrations slightly higher than those needed to antagonize 5-HT_3 receptors, can enhance neuromuscular transmission in the guinea-pig ileum. We showed that concentrations of ondansetron in the range of 2×10^{-6} to 2×10^{-5} M produce an excitatory effect in the electrically stimulated longitudinal muscle-myenteric plexus preparation of the guinea pig. The response seems to be mediated by serotonergic receptors, but we were unable to fully characterize the receptor/s involved. The continual advancement in the field of serotonergic neurotransmission in the gut will introduce new pharmacological tools that will permit elucidation of the mechanism(s) of the excitatory effect of ondansetron in the guinea-pig ileum.

Acknowledgements

Partially supported by Fondo de Investigaciones Sanitarias of Spain (Grant 94/1380). Part of the results shown in this paper have been presented at the XIX National Meeting of the Spanish Society of Pharmacology (Madrid, Spain, 1995).

References

Briejer, M.R., Akkermans, L.M.A., Schuurkes, J.A.J., 1995. Gastrointestinal prokinetic benzamides: the pharmacology underlying stimulation of motility. Pharmacol. Rev. 47, 631.

Brown, N.J., Horton, A., Rumsey, R.D., Read, N.W., 1993. Granisetron and ondansetron: effects on the ileal brake mechanism in the rat. J. Pharm. Pharmacol. 45, 521.

Buchheit, K.H., Buhl, T., 1994. Stimulant effects of 5-hydroxytryptamine on guinea pig stomach preparations in vitro. Eur. J. Pharmacol. 262, 91.

Burks, T.F., 1994. Neurotransmission and neurotransmitters. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract. Raven Press, New York, NY, p. 211.

Carter, D., Champney, M., Hwang, B., Eglen, R.M., 1995. Characterization of a postjunctional 5-HT receptor mediating relaxation of guinea-pig isolated ileum. Eur. J. Pharmacol. 280, 243.

Costall, B., Gunning, S.J., Naylor, R.J., Tyers, M.B., 1987. The effect of GR38032F, novel 5-HT_3 receptor antagonist, on gastric emptying in the guinea-pig. Br. J. Pharmacol. 91, 263.

Craig, D.A., Eglen, R.M., Walsh, L.K., Perkins, L.A., Whiting, R.L., Clarke, D.E., 1990. 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitization as a discriminative tool for the 5-HT_3 and putative 5-HT_4 receptors in guinea-pig ileum. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 9.

Daniel, E.E., Collins, S.M., Fox, J.E.T., Huizinga, J.D., 1989. Pharmacology of drugs acting on gastrointestinal motility. In: Schultz, S. (Ed.), Handbook of Physiology. The Gastrointestinal Tract: Motility and Circulation. Am. Physiological Society, Bethesda, p. 715.

De Ridder, W.J., Schuurkes, J.A., 1993. Cisapride and 5-hydroxytryptamine enhance motility in the canine antrum via separate pathways, not involving 5-hydroxytryptamine 1,2,3,4 receptors. J. Pharmacol. Exp. Ther. 264, 79.

Dhasmana, K.M., Zhu, Y.N., Cruz, S.L., Villalon, C.M., 1993. Gastrointestinal effects of 5-hydroxytryptamine and related drugs. Life Sci. 53, 1651.

Gershon, M.D., Kirchgessner, A.L., Wade, P.R., 1994. Functional anatomy of the enteric nervous system. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract. Raven Press, New York, NY, p. 381.

Gidda, J.S., Evans, D.C., Prime, P., Schenck, K., Cohen, M.L., 1988. Role of 5-HT_3 receptor antagonists in gastrointestinal motility. Gastroenterology 95, 867.

Gunning, S.J., Humphrey, P.P.A., 1987. Evidence for 5-HT_3-receptor mediated release of an inhibitory transmitter in guinea-pig isolated ileum. Br. J. Pharmacol. 91, 359P.

Mawe, G.M., Branchek, T.A., Gershon, M.D., 1986. Peripheral neural serotonin receptors: identification and characterization with specific antagonists and agonists. Proc. Natl. Acad. Sci. USA 83, 9799.

Ohshika, H., Kimura, H., Miyamoto, A., 1990. Stimulating effects of 5-HT_3 antagonist GR 38032 F on rat stomach. In: Proc. Second IUPHAR Satellite Meeting on Serotonin, Basel, 82P.

Puig, M.M., Turndorf, H., Warner, W., 1990. Effects of azepexole on opioid receptors and endogenous opioid release in the guinea pig ileum. Anesthesiology 72, 846.

Sanger, G.J., 1985. Three different ways in which 5-hydroxytryptamine can affect cholinergic activity in guinea-pig isolated ileum. J. Pharm. Pharmacol. 37, 584.

Sanger, G.J., Banner, S.E., Wardle, K.A., 1994. The role of the 5-HT_3 receptor in normal and disordered gastrointestinal function. In: King, F.D., Jones, B.J., Sanger, G.J. (Eds.), 5-Hydroxytryptamine-3 Receptor Antagonists. CRC Press, Boca Raton, FL.

Scholtysik, G., Imoto, Y., Yatami, Y., Brown, A.M., 1988. 5-HT antagonist ICS 205930 blocks potassium, sodium and calcium currents. J. Pharmacol. Exp. Ther. 245, 773.

Schuurkes, J.A.J., Van Bergen, P.J.E., Van Nueten, J.M., 1988. Prejunctional muscarinic (M_1)-receptor interactions on guinea-pig ileum: lack of effect of cisapride. Br. J. Pharmacol. 94, 228.

Tallarida, J., Murray, B., 1986. Pharmacologic Calculations with Computer Programs. Springer-Verlag, New York, NY.

Talley, N.J., 1992. 5-Hydroxytryptamine agonists and antagonists in the modulation of gastrointestinal motility and sensation: clinical implications. Aliment. Pharmacol. Ther. 6, 273.