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Effects of ondansetron on electrically evoked contraction in rat stomach fundus: Possible involvement of 5-HT_{2B} receptors

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Abstract

This study examined the effects of ondansetron, an antagonist of the 5-hydroxytryptamine (5-HT_3) receptor without 5-HT_4 receptor agonistic activity, on electrically evoked contractions and acetylcholine release in rat stomach fundus strips. Ondansetron (10^{-8}–10^{-4} M) produced a concentration-dependent increase in the magnitude of the electrically evoked contraction, while it inhibited the release of acetylcholine induced by electrical field stimulation. The stimulatory effect of ondansetron (10^{-6} M) on electrically evoked contractions was antagonized by yohimbine, a 5-HT_{2B} receptor antagonist, but not by SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-[diethylamino] ethyl ester), a 5-HT_4 receptor antagonist. In the presence of tetrodotoxin, ondansetron (10^{-7}–10^{-5} M) enhanced the contractions induced by acetylcholine. This stimulatory effect of ondansetron on acetylcholine-induced contractions was antagonized by yohimbine. These data suggest that ondansetron potentiates the contractile response to acetylcholine in the rat stomach fundus through the activation of 5-HT_{2B} receptors. © 1997 Elsevier Science B.V.

Keywords: Ondansetron; Stomach fundus; Contractile response; Acetylcholine release; (Rat)

1. Introduction

5-Hydroxytryptamine (5-HT) plays a physiological role in gastrointestinal motility (Ormsbee and Fondacaro, 1985; Costall and Naylor, 1990). A variety of different 5-HT receptor subtypes have been identified in the gastrointestinal tract (Saxena, 1995). Several compounds that possess 5-HT_3 receptor antagonistic activity, such as ondansetron, granisetron, cisapride, and metoclopramide, have been shown to accelerate gastric emptying in several species (for review see Briejer et al., 1995). Since the potencies of these compounds in stimulating this gastric emptying can be positively correlated to their antagonist potency at 5-HT_3 receptors, estimated by the inhibition of the von Bezold–Jarisch reflex (Schiavone et al., 1990; Haga et al., 1994; Briejer et al., 1995), it was hypothesized that antagonism of the 5-HT_3 receptor is the mechanism underlying the stimulatory effects of these compounds on gastric emptying. It has been demonstrated, however, that some selective 5-HT_3 receptor antagonists such as LY 277359 lack an accelerating effect of gastric emptying in rats (Cohen et al., 1990). These findings question the involvement of the 5-HT_3 receptor antagonistic mechanism in the stimulating effect of gastric emptying in rats.

Benzamide derivatives such as cisapride and metoclopramide possess 5-HT_4 receptor agonistic activity in addition to 5-HT_3 receptor antagonistic activity (Dumuis et al., 1989; Bockaert et al., 1990; Flynn et al., 1992; Rizzi et al., 1992). Several lines of evidence have suggested that the prokinetic properties of benzamide derivatives are mediated by facilitated cholinergic neurotransmission through stimulation of 5-HT_4 receptors (Schiavone et al., 1990; Linnik et al., 1991; Rizzi et al., 1994; Briejer et al., 1995; Hegde et al., 1995).

Ondansetron, a selective 5-HT_3 receptor antagonist, has been demonstrated to enhance gastric emptying in rats (Buchheit et al., 1989; Yoshida et al., 1992; Eglen et al., 1993; Miyata et al., 1995), mice (Haga et al., 1994) and guinea pigs (Costall et al., 1987), although the drug does not have gastroprokinetic activity in dogs (Rizzi et al., 1994) and humans (Nielsen et al., 1990; Talley et al., 1989). However, because ondansetron lacks 5-HT_4 receptor agonistic activity (Butler et al., 1988; Farthing, 1991), the mechanisms of its stimulatory effect on gastric emptying still remain elusive.

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The aim of the present study was to provide further information about the mechanisms of the accelerating effect of ondansetron, a selective 5-HT_3 receptor antagonist, on gastric emptying in rats. The proximal stomach is suggested to play a major role in the regulation of gastric emptying of liquids through contractions that raise the intraluminal pressure and move liquids into the duodenum (Wilbur and Kelly, 1973; Rizzi et al., 1994). In the present study, we examined the effects of ondansetron on electrically evoked contractions and acetylcholine release in rat stomach fundus strips, and compared the results with those obtained with cisapride, a benzamide derivative possessing 5-HT_4 receptor agonistic properties (Dumuis et al., 1989).

2. Materials and methods

2.1. Tissue preparation

All experiments were conducted in accordance with guidelines established by the Committee for the Care and Treatment of Laboratory Animals of the Institute of Animal Experimentation of Sapporo Medical University.

Whole stomachs were obtained from male Wistar rats (300–350 g) and placed in Tyrode solution of the following composition (mM): NaCl 136.9, KCl 2.7, NaH_2PO_4 0.4, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, glucose 5.6. The fundus portion was divided in half by making a midline incision along the greater curvature. The mucosa was carefully excised. A medial longitudinal strip (3×15 mm) was obtained from each half of the divided fundus by making cuts parallel to the midline incision. Two fundus strips were prepared from each animal for in vitro experiments.

2.2. Measurement of contractile activity

Contractile activity evoked by electrical field stimulation in rat stomach fundus strips was examined as described previously (Amemiya et al., 1996) with minor modifications. Briefly, rat stomach fundus strips were placed in a 10 ml organ bath containing Tyrode solution gassed with 95% O_2/5% CO_2 and maintained at 37°C. A tension of 2 g was applied to the tissues, which were allowed to equilibrate for 60 min before the start of the experiment. Square wave pulses (45 V, 5 Hz, 5 ms pulse duration, for 5 s at intervals of 30 s) were applied, using bipolar platinum ring electrodes (5 mm internal diameter, 10 mm apart). After the contractile responses to electrical field stimulation were stable, basal electrically evoked contractions were recorded for 4 min. Cumulative concentration–response curves for drugs were made by adding increasing concentrations of drugs at 4 min intervals with electrical field stimulation.

The effects of antagonists on ondansetron-induced and cisapride-induced changes in electrical field stimulation were examined as follows. After basal electrically evoked contractions were recorded for 4 min, the strips were pretreated with antagonists or the vehicle for 10 min without electrical field stimulation. The strip was incubated with drugs for another 10 min in the presence or absence of antagonists without electrical field stimulation. Electrical field stimulation was again given to the strip for 4 min to the examine effects of the drugs on electrically evoked contractions (see Fig. 1). Changes in the contraction elicited by electrical field stimulation compared to the contractile effects of each drug on basal tension were taken to indicate the effects of drugs on the electrically evoked contraction. Contractility was expressed as a percentage of the height of the basal electrically evoked contraction.

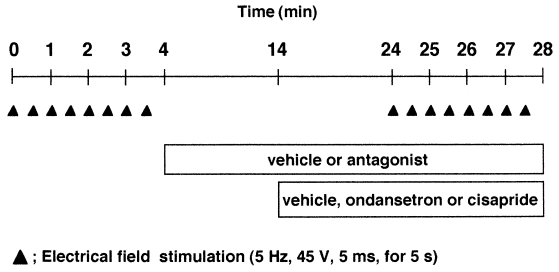

Fig. 1. Experimental protocol used to evaluate the effects of antagonists on ondansetron-induced and cisapride-induced changes in the response to electrical field stimulation in rat stomach fundus strips.

For estimation of the pA_2 value for yohimbine, two concentration–effect curves for ondansetron during electrical field stimulation were made in the absence or presence of yohimbine (incubated for 20 min) in each preparation. The response was expressed as a percentage of the maximum response determined from the first ondansetron concentration–effect curve (without yohimbine). The pA_2 value was calculated according to the method of Arunlakshana and Schild (1959). Concentration-ratios were obtained by dividing the EC_{50} for the 2nd ondansetron concentration–effect curve by that for the 1st concentration–effect curve made in the presence and absence of yohimbine, respectively.

In some experiments, the effects of ondansetron on acetylcholine-induced contractions and the contractile response to ondansetron without electrical field stimulation were examined as described previously (Amemiya et al., 1996) with minor modifications. The fundus strips were pretreated with tetrodotoxin for 10 min. After the pretreatment, the contractile response to acetylcholine (added exogenously to the organ bath) was obtained (basal contraction). After being washed with fresh medium for 30 min, the strip was pretreated with or without antagonists in the presence of tetrodotoxin for 10 min. Then it was incubated with or without ondansetron for 10 min, followed by measurement of the contractile response to acetylcholine. The contractile response to acetylcholine was obtained in the absence of ondansetron and antagonists (control contraction) or in the presence of ondansetron without or with

antagonists. Contractile activity was expressed as a percentage of the height of the basal acetylcholine-induced contraction.

The contractile response to ondansetron without electrical field stimulation was measured by incubating the strips with the drug to record contractile force. Contractility was expressed as changes in grams of force.

2.3. Measurement of ^3H outflow

The release of [^{3}H]-acetylcholine from rat fundus strips was examined using a superfusion system as described by Kusunoki et al. (1985). The fundus strips were incubated with [*methyl*-^{3}H]-choline chloride (3.2 μCi/ml) in Tyrode solution gassed with 95% O_2/5% CO_2 at 37°C for 60 min in a 5 ml organ bath. After being washed in fresh medium for 15 min, the strips were mounted in the apparatus and superfused at a flow rate of 1 ml/min with Tyrode solution gassed with 95% O_2/5% CO_2 at 37°C and containing hemicholinium-3 (10 μM) to prevent reuptake of choline. Experiments were started 60 min after the spontaneous ^{3}H outflow had approached a plateau. Square-wave pulses were applied by means of two platinum electrodes positioned parallel to the tissue. The parameters of electrical field stimulation were a 5 ms pulse duration at 45 V, with a frequency of 5 Hz for 5 s. The superfusates were collected in 1 min fractions and the radioactivity of the samples was determined by counting in a liquid scintillation spectrometer. The electrically evoked ^{3}H outflows (S1) were obtained from the total ^{3}H outflow during the 3 min after electrical field stimulation. Ondansetron at the indicated concentrations or vehicle was added to the superfusion fluid for 5 min. Then the electrically evoked ^{3}H outflow (S2) was obtained from the total ^{3}H outflow during 3 min with electrical field stimulation. The effects of ondansetron on the electrically evoked ^{3}H outflow were estimated by calculating the ratio S2/S1. The validity of assuming total tritium as a measure of [^{3}H]-acetylcholine release has been documented for the current experimental conditions (Wikberg, 1977; Kusunoki et al., 1985; Taniyama et al., 1991; Yau et al., 1991).

In some experiments, the effect of ondansetron on the spontaneous release of [^{3}H]-acetylcholine was examined. Ondansetron at the indicated concentrations was added to the superfusion fluid 5 min after the start of the experiment. The drug was kept in the superfusion fluid. The superfusates were collected in 1 min fractions and the radioactivity of the samples was determined by counting in a liquid scintillation spectrometer. Basal and ondansetron-evoked ^{3}H outflows were obtained from the total ^{3}H

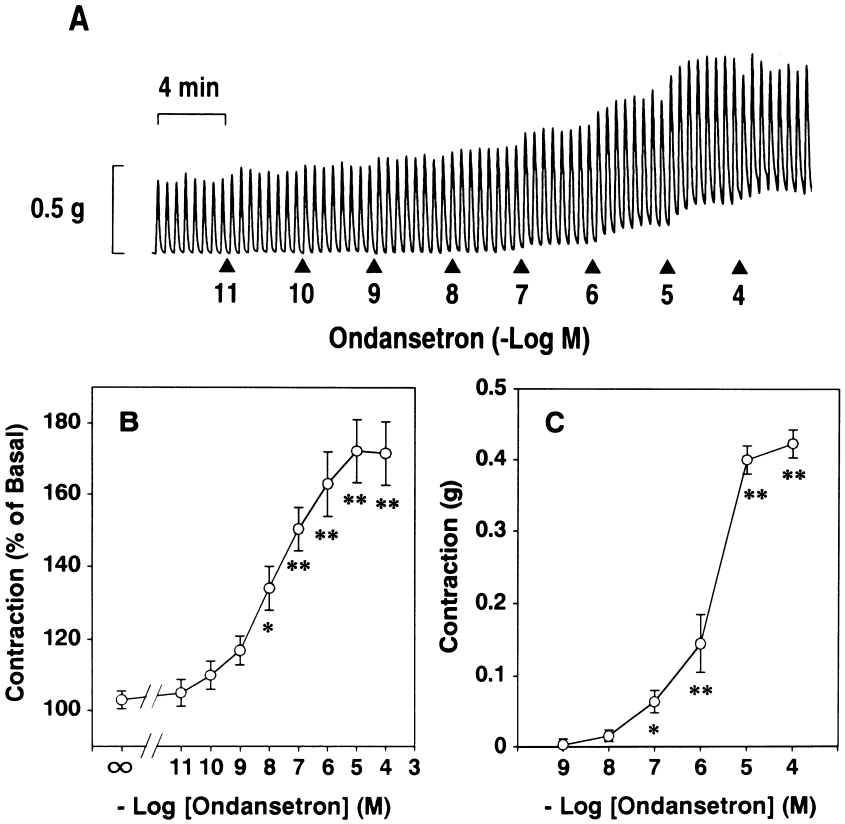

Fig. 2. Effects of ondansetron on electrical field stimulation-induced contractions (A and B) and the contractile response to ondansetron without electrical field stimulation (C) in rat stomach fundus strips. Typical tracing showing the effects of ondansetron on electrically evoked contractions (A). The values are means ± S.E.M. of 4–5 separate experiments (B and C). $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences compared with the contractile force in the absence of ondansetron, as determined by one-way analysis of variance with Dunnett's test.

outflow during the 3 min before and after drug addition, respectively. The amount of ondansetron-induced ^{3}H outflow was expressed as a percentage of the total basal ^{3}H outflow.

2.4. Data analysis

Data are shown as means ± S.E.M. The significance of differences among data was analyzed by using a one-way analysis of variance, followed by Dunnett's test and a two-way analysis of variance with Tukey's test as noted in the figure legends. Values of $P < 0.05$ were taken to indicate significance.

2.5. Materials

The following drugs were purchased from the suppliers indicated: [*methyl*-^{3}H] choline chloride (86.6 Ci/mmol, New England Nuclear, Tokyo); acetylcholine chloride (Daiichi Pharmaceutical, Tokyo); atropine sulfate monohydrate (Tokyo Kasei Kogyo, Tokyo); tetrodotoxin (Wako, Osaka); hemicholinium-3 bromide and yohimbine hydrochloride (Sigma Chemical, St. Louis). Ondansetron, cisapride, and SDZ 205-557 hydrochloride (2-methoxy-4-amino-5-chloro-benzoic acid 2-[diethylamino] ethyl ester hydrochloride) were kindly provided by Glaxo Group Research (Greenford), Yoshitomi Pharmaceutical Industries, (Osaka), and Sandoz Pharmaceuticals, (Basel), respectively. All other reagents used were of analytical grade.

3. Results

3.1. Alteration in stomach fundus contractions with or without electrical field stimulation

Electrical field stimulation produced an increase in contractions in the stomach fundus strips in a frequency-dependent manner over the range of frequencies tested (2.5–15 Hz) (2.5 Hz, 0.17 ± 0.04 g; 5 Hz, 0.44 ± 0.03 g; 10 Hz, 0.98 ± 0.02 g; 15 Hz, 1.29 ± 0.04 g) and the contractions were reproducible for several hours. These electrically evoked contractile responses could be blocked by tetrodotoxin (10^{-6} M) and by atropine (10^{-6} M) (data not shown). As shown in Fig. 2A and B, ondansetron (10^{-8}–10^{-4} M) produced concentration-dependent increases in electrically evoked contractions (45 V, 5 Hz, 5 ms pulse duration, for 5 s, intervals 30 s). Maximum stimulation was observed at 10^{-5} M ondansetron (172.1 ± 9.4% of the basal level). Similarly, ondansetron (10^{-7}–10^{-4} M) produced an increase in contractile force without electrical field stimulation in the strips in a concentration-dependent manner (Fig. 2C).

Ondansetron (10^{-6} M) and cisapride (10^{-7} M) significantly enhanced electrically evoked contractions to 143.1 ± 4.8% and 143.9 ± 7.4% of the basal level, respectively

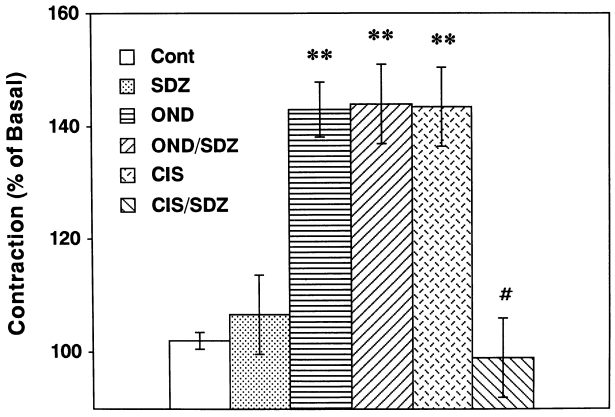

Fig. 3. Effects of ondansetron (OND), cisapride (CIS), and SDZ 205-557 (SDZ) on electrical field stimulation-induced contractions in rat stomach fundus strips. The concentrations of drugs used were as follows: ondansetron, 10^{-6} M; cisapride, 10^{-7} M; SDZ 205-557, 10^{-6} M. The values are means ± S.E.M. of 3–6 separate experiments. $^{**}P < 0.01$ indicates a significant difference compared with the contractile force in the absence of drugs (Cont) and $^{\#}P < 0.05$ indicates a significant difference compared with the contraction elicited by cisapride alone, as determined by two-way analysis of variance with Tukey's test.

(Figs. 3 and 5). Pretreatment of the fundus strips with SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-[diethylamino] ethyl ester) (10^{-6} M), a selective 5-HT_4 receptor antagonist (Buchheit et al., 1991, 1992), effectively antagonized the stimulatory effect of cisapride (Fig. 3). In

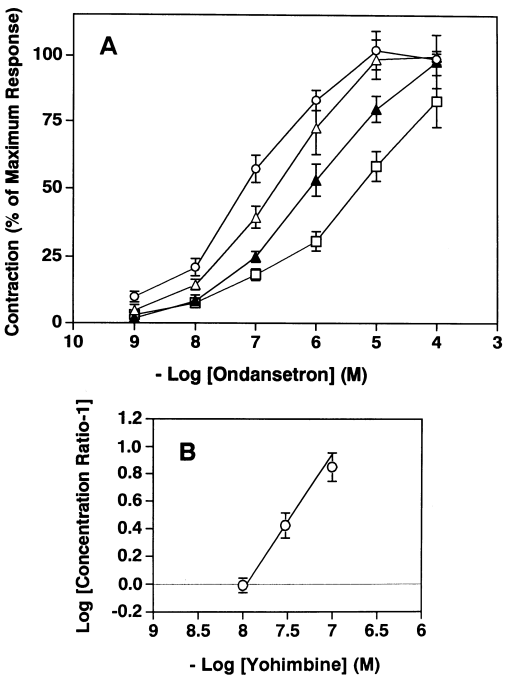

Fig. 4. Cumulative concentration–effect curve for ondansetron in the absence (○) and presence of 10^{-8} M (△), 3×10^{-8} M (▲) and 10^{-7} M (□) yohimbine on electrical field stimulation-induced contractions in rat stomach fundus strips (A) and Schild regression analysis derived from agonist concentration-ratios with 10^{-8} M, 3×10^{-8} M, and 10^{-7} M yohimbine (B).

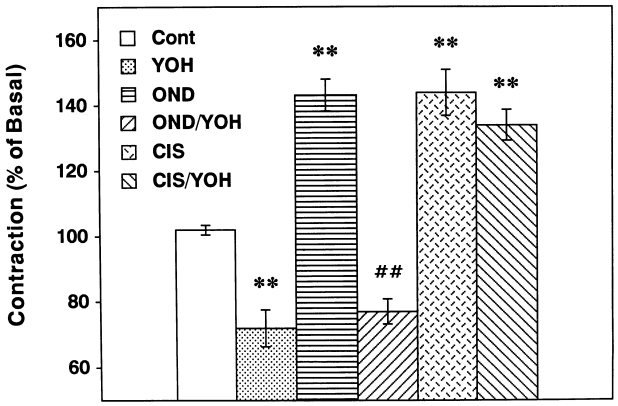

Fig. 5. Effects of ondansetron (OND), cisapride (CIS), and yohimbine (YOH) on electrical field stimulation-induced contractions in rat stomach fundus strips. The concentrations of drugs used were as follows: ondansetron, 10^{-6} M; cisapride, 10^{-7} M; yohimbine, 10^{-6} M. The values are means ± S.E.M. of 3–6 separate experiments. $^{**}P < 0.01$ indicates a significant difference compared with the contractile force in the absence of drugs (Cont) and $^{\#\#}P < 0.01$ indicates a significant difference compared with the contraction elicited by ondansetron alone, as determined by two-way analysis of variance with Tukey's test.

contrast, SDZ 205-557 did not inhibit the ondansetron-induced enhancement of electrically evoked contractions.

We used yohimbine to examine the possible contribution of 5-HT_{2B} receptors to the enhancement of electrically induced contractions by ondansetron. Yohimbine has high affinity for 5-HT_{2B} receptors and has been used as a 5-HT_{2B} receptor antagonist (Foguet et al., 1992; Wainscott et al., 1993; Baxter et al., 1994). Yohimbine caused concentration-dependent rightward displacements of the concentration–effect curves for ondansetron during electrical field stimulation (Fig. 4A). Schild regression analysis yielded an apparent pA_2 estimate of 7.95 with a slope of 0.96 (Fig. 4B). The inhibitory effect of yohimbine on this ondansetron-induced enhancement was more apparent at 10^{-6} M yohimbine ($P < 0.01$), although yohimbine alone suppressed the control contraction elicited by electrical field stimulation (Fig. 5). However, yohimbine at the same concentration did not block the potentiation of the electrically evoked contraction by cisapride. The stimulatory effect of ondansetron on electrically evoked contractions was not affected by the α-adrenoceptor antagonist phentolamine (10^{-6} M) (152.1 ± 4.5% of the basal contraction), whereas it was abolished by atropine (10^{-6} M) (data not shown).

3.2. Effects of ondansetron on electrically evoked and spontaneous ^{3}H outflows

The effects of ondansetron on ^{3}H outflow, used as an index of [^{3}H]-acetylcholine release, were examined in the rat stomach fundus strips by using a superfusion system. In the absence of ondansetron (control experiments), the ratio S2/S1 of ^{3}H outflow induced by electrical field stimulation was 0.97 ± 0.05 (Fig. 6A). Electrically evoked ^{3}H outflow was effectively prevented by either application of tetrodotoxin (10^{-6} M) or the removal of Ca^{2+} from the superfusion medium (data not shown). Ondansetron (> 10^{-7} M) caused a reduction rather than an enhancement of electrically evoked ^{3}H outflow (Fig. 6A). Furthermore, ondansetron (10^{-11}–10^{-5} M) had no stimulatory effect on spontaneous ^{3}H outflow from the fundus strips (Fig. 6B).

In contrast, cisapride (10^{-7} M) produced significant increases in electrically evoked ^{3}H outflow (control, 0.98 ± 0.06; cisapride, 1.30 ± 0.07 ($n = 7$, $P < 0.01$)) and in spontaneous ^{3}H outflow (control, 99.2 ± 2.3%; cisapride, 111.3 ± 2.1% ($n = 5$, $P < 0.05$)) in the fundus strips.

3.3. Effects of ondansetron on the contractile response to acetylcholine in stomach fundus strips

The effects of ondansetron on the contractile response to acetylcholine, added exogenously to the organ bath, were examined. Acetylcholine (10^{-7} M) produced an increase in contractile force (1.49 ± 0.21 g of force) and this increase was abolished by pretreatment with atropine (10^{-6} M) (data not shown). In the presence of tetrodotoxin (10^{-6} M), ondansetron (10^{-7}–10^{-5} M) enhanced acetylcholine-induced contractions in a concentration-dependent manner in rat stomach fundus strips (Fig. 7A). However, cisapride (10^{-7} M) did not affect acetylcholine-induced contractions in fundus strips (data not shown). The stimulatory effect of ondansetron (10^{-6} M) was effectively antagonized by

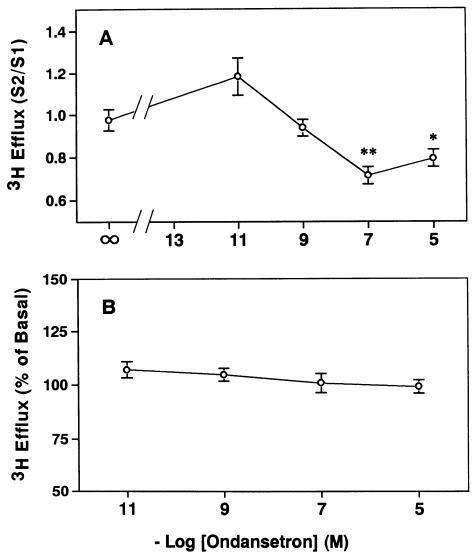

Fig. 6. Effects of ondansetron on electrically evoked (A) and spontaneous (B) ^{3}H outflow in rat stomach fundus strips. The electrically evoked ^{3}H outflow is expressed as the ratio S2/S1 as described in Section 2. The spontaneous ^{3}H outflow is expressed as a percentage of the basal ^{3}H outflow. The values are means ± S.E.M. of 4–5 separate experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences compared with the ^{3}H outflow in the absence of ondansetron, as determined by one-way analysis of variance with Dunnett's test.

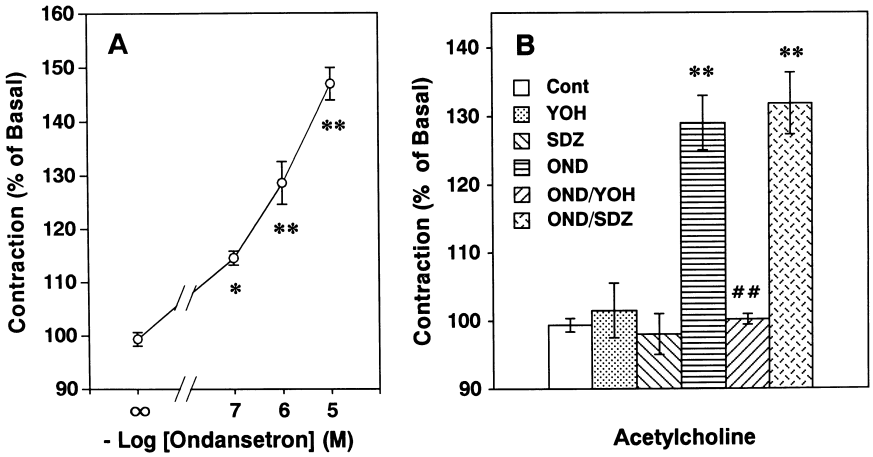

Fig. 7. Effects of ondansetron (OND), yohimbine (YOH) and SDZ 205-557 (SDZ) on acetylcholine-induced contractions in rat stomach fundus strips. The contractile response to acetylcholine (10^{-7} M) was determined in the presence of tetrodotoxin (10^{-6} M). Contractile activity is expressed as a percentage of the height of the basal acetylcholine-induced contraction. The concentrations of drugs used in B were: ondansetron, 10^{-6} M; yohimbine, 10^{-6} M; SDZ 205-557, 10^{-6} M. The values are means ± S.E.M. of 4–7 separate experiments. In (A), $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences compared with the contractile force in the presence of acetylcholine alone (Cont), as determined by one-way analysis of variance with Dunnett's test. In (B), $^{**}P < 0.01$ indicates significant differences compared with the contractile force in the presence of acetylcholine alone (Cont) and $^{\#\#}P < 0.01$ indicates a significant difference compared with the contraction elicited by ondansetron and acetylcholine, as determined by two-way analysis of variance with Tukey's test.

yohimbine (10^{-6} M), but not by SDZ 205-557 (10^{-6} M) (Fig. 7B). Ondansetron had little or no effect on the contractions induced by exogenous prostaglandin $F_{2\alpha}$ (3×10^{-8} M) and KCl (30 mM) in the presence of tetrodotoxin (prostaglandin $F_{2\alpha}$ and KCl produced 1.71 ± 0.16 g and 5.42 ± 0.35 g contractions and, with 10^{-6} M ondansetron, their contractions were $100.2 \pm 2.3\%$ and $103.3 \pm 3.3\%$ of the corresponding control contractions, respectively).

4. Discussion

Ondansetron enhanced electrically evoked, acetylcholine-mediated contractions and produced an increase in contractile force without electrical field stimulation in a dose-dependent manner in rat stomach fundus strips (Fig. 2A–C). The stimulatory effect of ondansetron (10^{-6} M) on electrically evoked contractions was not inhibited by the 5-HT_4 receptor antagonist SDZ 205-557 (10^{-6} M), whereas the enhancement of electrically evoked contractions by 10^{-7} M cisapride, which possesses 5-HT_3 receptor antagonistic and 5-HT_4 receptor agonistic properties, was effectively antagonized by SDZ 205-557 to the control levels (Fig. 3). Thus, ondansetron seemed to potentiate electrically evoked contractions by a mechanism unrelated to 5-HT_4 receptors. In contrast, the contractile action of cisapride appeared to depend on 5-HT_4 receptors presumably located on cholinergic neurons, leading to the release of acetylcholine (Section 3), as has been proposed in previous studies of the guinea pig ileum (Linnik et al., 1991; Rizzi et al., 1992).

Gastroprokinetic activity in vivo has sometimes been ascribed to the augmented release of acetylcholine by a mechanism similar to that operating in isolated gastrointestinal preparations (Buchheit et al., 1985; Schuurkes et al., 1985). The present results on acetylcholine release showed that ondansetron did not enhance, but rather decreased ($> 10^{-7}$ M), the electrically evoked release of acetylcholine, and it had little or no effect on the spontaneous release of acetylcholine (Fig. 6A and B). A previous report (Fox and Morton, 1990) indicated that activation of 5-HT_3 receptors in the guinea pig ileum causes acetylcholine release from cholinergic nerve terminals, leading to an increase in contractile activity. Thus, the inhibitory effect of ondansetron on electrically evoked acetylcholine release appears to be related to its antagonistic effect at 5-HT_3 receptors, that is, ondansetron blocks stimulation of 5-HT_3 receptors by endogenous 5-HT, which might be released by electrical field stimulation in the rat stomach fundus. However, the reduction in acetylcholine release elicited by ondansetron was relatively small (~25% of basal electrically evoked release of acetylcholine) and this effect was opposite to the stimulatory effect of ondansetron on the contractile activity with electrical field stimulation. It is unlikely, therefore, that the ondansetron-induced potentiation of the electrically evoked contraction in rat stomach fundus is related to its 5-HT_3 receptor antagonistic activity.

Recently, a novel receptor, the 5-HT_{2B} receptor, has been cloned from rat stomach fundus. The 5-HT_{2B} receptor is suggested to participate in the contractile effect of 5-HT in this tissue (Foguet et al., 1992; Kursar et al., 1992;

Humphrey et al., 1993; Baxter et al., 1994). To evaluate the possible contribution of 5-HT_{2B} receptors to the stimulatory effect of ondansetron on electrically induced contractions, we used yohimbine, which has high affinity for 5-HT_{2B} receptors (Foguet et al., 1992; Wainscott et al., 1993; Baxter et al., 1994), as a 5-HT_{2B} receptor antagonist.

Yohimbine (10^{-8}–10^{-7} M) caused concentration-dependent inhibition of the ondansetron-induced potentiation of electrically evoked contractions in rat stomach fundus strips (Fig. 4A). Schild regression analysis yielded an apparent pA_2 estimate of 7.95 with a slope of 0.96 (Fig. 4B), which is consistent with the estimated pA_2 value for yohimbine at rat stomach fundus 5-HT_{2B} receptors ($pA_2 = 7.9$; Baxter et al., 1994). Inhibition by yohimbine of the ondansetron (10^{-6} M) induced potentiation was ~70% at 10^{-7} M of the antagonist (Fig. 4A), and the inhibitory effect of yohimbine was more apparent at 10^{-6} M (Fig. 5). The α-adrenoceptor antagonist phentolamine (10^{-6} M) did not influence the effect of ondansetron (Section 3), suggesting that the blocking action of yohimbine did not result from its being an α-adrenoceptor antagonist. It might be argued that yohimbine at a concentration of 10^{-6} M could exert its antagonistic effect at other 5-HT receptors, e.g. 5-HT_{2C} receptors. However, yohimbine is reported to exhibit low affinity for cloned rat 5-HT_{2C} receptors ($pK_i = < 5.0$; Bonhaus et al., 1995). It seems likely, therefore, that ondansetron potentiates contractile activity during electrical field stimulation through a mechanism related to its agonistic activity at 5-HT_{2B} receptors in rat stomach fundus. The involvement of 5-HT_{2B} receptors in cisapride-induced stimulation seems unlikely, since the effect of cisapride was not antagonized by yohimbine (Fig. 5).

It was observed that yohimbine (10^{-6} M) alone suppressed the control contraction evoked by electrical field stimulation (Fig. 5). The reason for this is not clear. It might be due to antagonism by yohimbine of the stimulation of 5-HT_{2B} receptors by endogenous 5-HT, which might be released by electrical field stimulation in the rat stomach fundus, since the antagonist alone did not reduce the contractions occurring without electrical field stimulation in the presence of tetrodotoxin (Fig. 7B).

Since electrically evoked contractions were effectively inhibited by atropine (10^{-6} M), tetrodotoxin (10^{-6} M) or the removal of Ca^{2+} from the medium (data not shown), the contractile response to electrical field stimulation was mediated mainly through activation of muscarinic acetylcholine receptors by released acetylcholine. Thus, the effect of ondansetron on the electrically evoked contraction would result from either potentiation of the release of acetylcholine or an increase in the sensitivity of the smooth muscles to the transmitter. However, as mentioned above, ondansetron did not cause acetylcholine release in rat stomach fundus strips. It was assumed, therefore, that ondansetron might modulate the responsiveness of rat stomach fundus to acetylcholine, resulting in potentiation of the electrically evoked contraction. Indeed, the present results showed that ondansetron (10^{-7}–10^{-5} M) significantly potentiated acetylcholine-elicited contractions in the presence of tetrodotoxin (10^{-6} M) (Fig. 7A). Furthermore, the potentiation by ondansetron of the contractile response to acetylcholine was significantly blocked by yohimbine (10^{-6} M), but not by SDZ 205-557 (10^{-6} M) (Fig. 7B). In addition, ondansetron failed to enhance contractile responses to prostaglandin $F_{2\alpha}$ and KCl in the fundus strips (Section 3). These data appear to be consistent with the idea that ondansetron potentiates the responsiveness of stomach fundus to acetylcholine through stimulation of 5-HT_{2B} receptors.

The precise mechanisms by which ondansetron potentiates the contractile response to acetylcholine via activation of 5-HT_{2B} receptors are not clear at present. It has been demonstrated that the 5-HT_{2B} receptor is coupled to the stimulation of intracellular Ca^{2+} release and the activation of protein kinase C in rat stomach fundus (Cox and Cohen, 1995). It is feasible to assume that ondansetron modulates the function of acetylcholine receptors through alterations in the signal transduction mechanisms linked to the 5-HT_{2B} receptor, resulting in sensitization of the cholinergic contractile activity in rat stomach fundus.

Recently, 5-HT_5, 5-HT_6, and 5-HT_7 receptors have been cloned from rat cDNA and these receptors have been observed to be predominantly expressed in the central nervous system (Hoyer et al., 1994). There is no information with regard to the affinity of ondansetron and yohimbine for these types of receptors. Therefore, the possibility that these types of 5-HT receptors are involved in the effect of ondansetron on rat stomach fundus cannot be excluded, although their functional characterization remains to be established. Further investigations will be required to achieve a better understanding of the mechanism of action of ondansetron in the rat stomach.

In summary, the results of the present study suggest that, in rat stomach fundus, ondansetron potentiates the contractile response to electrical field stimulation, at least partly, via sensitization of smooth muscle cells to acetylcholine. This effect of ondansetron may be mediated mainly through a mechanism involving activation of 5-HT_{2B} receptors, presumably located on the smooth muscle cells. Although studies of the affinity of ondansetron for 5-HT_{2B} receptors have not yet been reported, this is the first demonstration that ondansetron may possess 5-HT_{2B} receptor agonistic activity, which could be related to its stimulatory effect on gastric emptying in the rat.

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