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Characterization of the contractile response induced by 5-methoxytryptamine in rat stomach fundus strips

Norihito Amemiya *, Shinichi Hatta, Haruo Takemura, Hideyo Ohshika

Department of Pharmacology, School of Medicine, Sapporo Medical University, South-1, West-17, Chuo-ku, Sapporo 060, Japan

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Abstract

This study examined effects of 5-methoxytryptamine (5-MOT), an agonist at 5-HT_4 and 5-HT_{2B} receptors, on the contractile response and acetylcholine release in rat stomach fundus strips. 5-MOT (10^{-9}–10^{-5} M) produced a concentration-dependent increase in the contraction, while it evoked acetylcholine release in a 'bell-shaped' concentration-dependent manner. Atropine reduced 5-MOT (10^{-8}–10^{-6} M)-induced contractions, but it had little effect on the contractions evoked by higher concentrations. 5-MOT-induced contraction and acetylcholine release were inhibited 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 atropine, both SDZ 205-557 and yohimbine, a 5-HT_{2B} receptor antagonist, inhibited the contraction. In the presence of tetrodotoxin, the contraction was inhibited by yohimbine, but not by SDZ 205-557. These results suggest that the contractile action of 5-MOT in rat stomach fundus involves atropine-sensitive and atropine-resistant components. The sensitive contraction appears to be mediated through 5-HT_4 receptors located on cholinergic neurons, whereas the resistant contraction is mediated through 5-HT_4 receptors located on non-cholinergic neurons and through 5-HT_{2B} receptors.

Keywords: 5-HT receptor; Stomach fundus, rat; Contractile response; Acetylcholine release

1. Introduction

5-Hydroxytryptamine (5-HT) has variable effects on the gastrointestinal tract of a variety of species, including contraction or relaxation of smooth muscle and stimulation of the intramural nerve plexus. In the rat oesophagus (Reeves et al., 1991; Baxter et al., 1991) and ileum (Tuladhar et al., 1991), stimulation of 5-HT_4 receptors located on the smooth muscle cells results in relaxation. The guinea-pig stomach is also relaxed by the administration of 5-HT (Yamaguchi, 1972; Buchheit and Buhl, 1994).

In contrast, 5-HT results in contraction and an enhancement of electrically evoked contraction in the guinea-pig ileum (Eglen et al., 1990), colon (Elswood et al., 1991; Wardle and Sanger, 1993; Kojima and Shimo, 1995) and stomach (Yamaguchi, 1972; Buchheit and Buhl, 1994). The 5-HT-evoked contraction and potentiation appear to be mediated by stimulation of non-cholinergic and cholinergic excitatory neurons (Costa and Furness, 1979). 5-HT-produced enhancement of the atropine-resistant neurogenic contraction is suggested to be mediated by the stimulation of 5-HT_4 receptors located on tachykininergic neurons (Kojima and Shimo, 1995; Ramírez et al., 1994). Atropine-sensitive contractions induced by 5-HT are mediated by the stimulation of 5-HT_4 receptors (Elswood et al., 1991) and are involved in the release of acetylcholine (Clarke et al., 1989; Eglen et al., 1990; Fozard, 1990).

5-HT is also known to be a potent contractile agonist of the rat stomach fundus (Vane, 1957). However, in the rat stomach fundus, the involvement of acetylcholine release in the contractile response to 5-HT has been studied less extensively, because it was shown in an earlier study that the 5-HT-induced contraction was not affected by hyoscine (Vane, 1957). In addition, the type of 5-HT receptor, whose activation leads to fundic smooth muscle contraction, has not been easy to characterize pharmacologically due to the lack of selective pharmacological probes for subtypes of 5-HT receptors. Several recent studies have suggested that the 5-HT_{2B} receptor participates in the contractile effect of 5-HT in the rat stomach fundus (Foguet et al., 1992; Kursar et al., 1992; Humphrey et al., 1993; Cohen and Fludzinski, 1987; Baxter et al., 1994). It has also been proposed that multiple 5-HT receptor subtypes mediate the contractile response of 5-HT receptor agonists in rat stomach fundus (Foguet et al., 1992), which may explain the occurrence of shallow (Clineschmidt et al.,

* Corresponding author. Tel.: (81-11) 611-2111; Fax: (81-11) 612-5861.

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1985) or overtly biphasic (Buchheit et al., 1986) agonist concentration-effect curves.

The present study was, therefore, designed to provide further information regarding the 5-HT-induced contractile effect in the rat stomach fundus by simultaneously examining the effect of 5-methoxytryptamine (5-MOT), an agonist of 5-HT_4 and 5-HT_{2B} receptors (Hoyer et al., 1994; Saxena, 1995), on spontaneous contractile force and spontaneous acetylcholine release in fundus strips. In addition, we used electrically stimulated strips from rat stomach fundus, which offers the advantage that neuromodulatory effects of 5-MOT on intrinsic nerves can be investigated (Yamaguchi, 1972).

2. Materials and methods

2.1. Tissue preparation

Male Wistar rats (300–350 g) were killed, and the stomachs were removed 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 examination.

2.2. Measurement of ^3H outflow and contractile activity

The release of [^{3}H]acetylcholine and the contractile activity in the fundus strips were examined simultaneously using a superfusion system as described by Taniyama et al. (1991). 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, containing hemicholinium-3 (10 μM) to prevent the reuptake of choline. Experiments were started 60 min after the spontaneous ^{3}H outflow had approached a plateau. 5-MOT with the indicated concentrations was added to the superfusion fluid 5 min after the start of the experiment. Antagonists were left in contact with the tissue for 10 min before the addition of 5-MOT and, unless stated otherwise, these agents remained 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. For the present experimental conditions, the validity of assuming total tritium as a measure of [^{3}H]acetylcholine release has been documented (Wikberg, 1977; Kusunoki et al., 1985; Yau et al., 1991; Taniyama et al., 1991). Basal and 5-MOT-evoked ^{3}H outflows were obtained from the total ^{3}H outflow during the 3 min before and after the drug addition, respectively. The amount of 5-MOT-induced ^{3}H outflow was expressed as a percentage of the total basal ^{3}H outflow. 5-MOT-induced change in contractile activity was simultaneously measured in the same fundus preparation. Contractility was recorded isometrically as changes in grams of force on a Nihon Koden TNF force-displacement transducer with a resting level of 2 g.

In some experiments, the acetylcholine release and the contractile activity induced by electrical field stimulation were examined. Square-wave pulses were applied by means of two platinum electrodes which were positioned parallel to the tissue. The parameters of electrical field stimulation were a 5-ms pulse duration at 45 V, with a range of frequency of 2.5–15 Hz for 5 s every 5 min. Basal and electrically evoked ^{3}H outflows were obtained from the total ^{3}H outflow during the 3 min before and after electrical field stimulation, respectively. The electrically evoked ^{3}H outflow was expressed as a percentage of the total basal ^{3}H outflow.

Effects of 5-MOT and various antagonists on spontaneous contraction and electrically evoked contraction were further examined in rat fundus strips 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 experiments. Effects of drugs on electrically evoked contraction were measured using the following protocol: Electrical field stimulation (45 V, 5 Hz, 5-ms pulse duration, for 5 s, intervals of 30 s) was achieved using bipolar platinum ring electrodes (5 mm internal diameter, 10 mm apart). After the contractile responses to electrical field stimulation were stable, control electrically evoked contraction was recorded for 4 min. Without electrical field stimulation, the strip was pretreated with various antagonists or the vehicle for 10 min. The strip was incubated with 5-MOT for 10 min in the presence or absence of antagonists and the 5-MOT-induced contraction was recorded. Electrical field stimulation was again given to the strip for 4 min to examine the effects of the drugs on electrically evoked contraction. Alteration in the contraction evoked by electrical field stimulation compared to the contraction induced by 5-MOT was taken to indicate the effect of 5-MOT on electrically evoked contraction. Contractility was expressed as a percentage of the height of the control electrically evoked contraction.

Effects of drugs on spontaneous contractions were measured using the following protocol: The strip was pretreated with or without 1 μM atropine for 5 min. Prevention of the acetylcholine-dependent contraction with atropine was confirmed by abolishment of the contraction evoked by electrical field stimulation during a 5-min atropine pretreatment. Then the strip was incubated with

various antagonists or the vehicle for 10 min and with 5-MOT for 10 min in the presence or absence of antagonists to record spontaneous contraction. Contractility was expressed as changes in grams of force.

2.3. Data analysis

Data are shown as means ± S.E.M. The significance of differences between data was analyzed by using the two-tailed Student's *t*-test for unpaired observations. Values of $P < 0.05$ were taken to indicate significance.

2.4. Materials

The following drugs were purchased from the suppliers indicated: [*methyl*-^{3}H]choline chloride (86.6 Ci/mmol, New England Nuclear, Boston, MA, USA); 5-methoxytryptamine hydrochloride (Research Biochemicals, Natick, MA, USA); atropine sulfate monohydrate (Tokyo Kasei Kogyo, Tokyo, Japan); tetrodotoxin (Wako, Osaka, Japan); hemicholinium-3 bromide and yohimbine hydrochloride (Sigma, St. Louis, MO, USA). SDZ 205-557 hydrochloride (2-methoxy-4-amino-5-chloro-benzoic acid 2-[diethylamino] ethyl ester hydrochloride) was kindly provided by Sandoz Pharmaceuticals (Basel, Switzerland). All other reagents used were of analytical grade. The drugs were dissolved in Tyrode solution immediately before use, except for tetrodotoxin. Tetrodotoxin was made up in a stock 0.1 mM concentration in distilled water.

3. Results

3.1. Effects of 5-MOT on spontaneous contraction and ^{3}H outflow

Effects of 5-MOT on ^{3}H outflow and contractility were examined simultaneously in the stomach fundus strips using a superfusion system. 5-MOT produced an increase in the concurrently recorded spontaneous contraction in a concentration-dependent manner at concentrations from 10^{-9} to 10^{-5} M, with a maximum stimulation of ~ 3.13 ± 0.27 g at 10^{-5} M (Fig. 1A). 5-MOT (10^{-9}–10^{-7} M) produced concentration-dependent increases in ^{3}H outflow, used as an index of [^{3}H]acetylcholine release, from rat stomach fundus strips but with higher concentrations (10^{-6}–10^{-5} M) the increased ^{3}H outflow returned toward the basal level (Fig. 1B). Maximum stimulation of ^{3}H outflow was observed at 10^{-7} M 5-MOT (185.9 ± 12.1% of basal ^{3}H outflow).

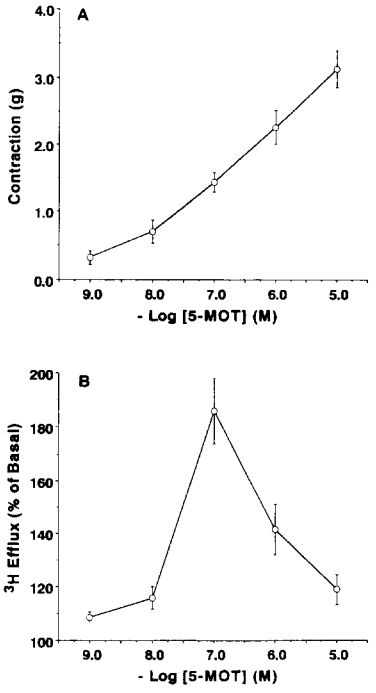

Fig. 1. Effects of 5-methoxytryptamine (5-MOT) on the spontaneous contractile response (A) and ^{3}H outflow (B) in rat stomach fundus strips. The contractile activity and ^{3}H outflow were determined simultaneously using a superfusion system. The ^{3}H outflow in the absence of 5-methoxytryptamine was taken as 100%. The values are means ± S.E.M. of 10 separate experiments.

3.2. Effects of antagonists on 5-MOT-induced contractions and ^{3}H outflow

Pretreatment of the fundus strips with atropine (1 μM) significantly prevented the contraction elicited by 5-MOT (Fig. 2A). In contrast, atropine was without effect on the 5-MOT (0.1 μM)-induced increase in ^{3}H outflow (Fig. 2B). Tetrodotoxin (1 μM) and SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-[diethylamino] ethyl ester) (1 μM), a selective 5-HT$_4$ receptor antagonist (Buchheit et al., 1991, 1992), significantly inhibited the stimulatory effects of 5-MOT both on contraction and ^{3}H outflow in the fundus strips (Fig. 2A and B).

3.3. Contraction and ^{3}H outflow evoked by electrical field stimulation

Electrical field stimulation produced an increase in contraction in the stomach fundus strips in a frequency-dependent manner in 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. Contractions evoked by electrical field stimulation were prevented by tetrodotoxin (1 μM) or the removal of Ca^{2+} from the superfusion medium, and atropine (1 μM) abolished the electrically evoked contraction (data not shown). The concurrently measured ^{3}H outflow evoked by electrical field stimulation showed a frequency-dependent (2.5–15 Hz) increase (2.5 Hz, 132.5 ± 2.5%; 5 Hz, 167.7 ± 11.4%; 10 Hz, 209.6 ± 16.5%; 15 Hz, 226.0 ± 12.8% of the basal level). The electrical field stimulation-induced increase in ^{3}H outflow was effectively prevented either by application

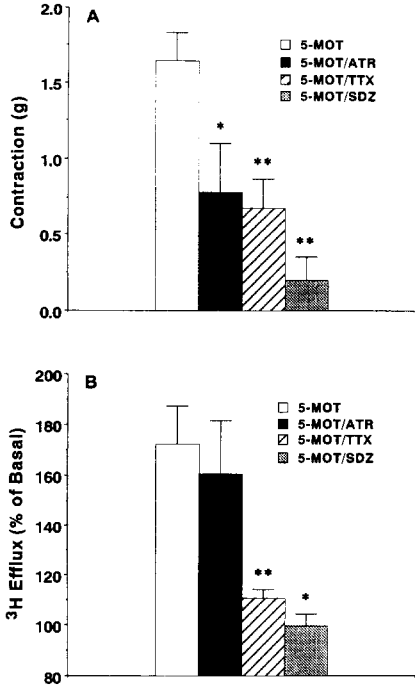

Fig. 2. Effects of atropine (ATR), tetrodotoxin (TTX), and SDZ 205-557 (SDZ) on the 5-MOT-induced increase in contraction (A) and ^{3}H outflow (B) in rat stomach fundus strips. The contractile activity and the ^{3}H outflow were determined simultaneously using a superfusion system. The concentrations of drugs used were as follows: 5-methoxytryptamine, 0.1 μM; atropine, 1 μM; tetrodotoxin, 1 μM; SDZ 205-557, 1 μM. The ^{3}H outflow in the absence of 5-methoxytryptamine was taken as 100%. The values are means ± S.E.M. of 5 separate experiments. * $P < 0.05$ and ** $P < 0.01$ indicate significant differences compared with the 5-methoxytryptamine-induced response.

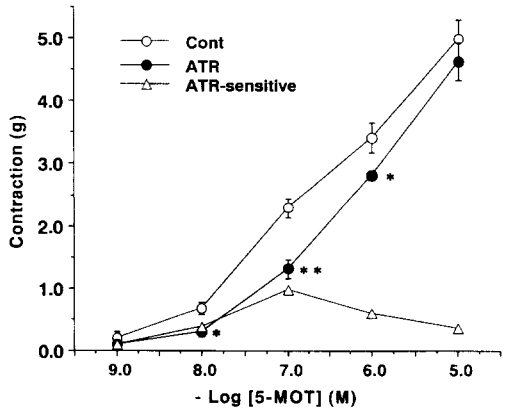

Fig. 3. Effects of 5-methoxytryptamine (5-MOT) on the contractile response in the presence or absence of atropine (ATR) in rat stomach fundus strips. The concentration of atropine used was 1 μM. The atropine-sensitive component (ATR-sensitive) of the 5-methoxytryptamine-induced contraction was calculated by subtracting the contractile force with atropine from that without atropine. The values are means ± S.E.M. of 4 separate experiments. * $P < 0.05$ and ** $P < 0.01$ indicate significant differences compared with the 5-methoxytryptamine-induced contraction in the absence of atropine.

of tetrodotoxin (1 μM) or the removal of Ca^{2+} from the superfusion medium (data not shown).

3.4. Effects of antagonists on 5-MOT-induced contractions

The contractile response of the rat stomach fundus strips to 5-MOT was further characterized using an organ bath system, because the contractile effect of 5-HT involves an atropine-resistant mechanism in the guinea-pig ileum (Buchheit et al., 1985; Ramírez et al., 1994) and proximal colon (Kojima and Shimo, 1995). To discriminate between atropine-sensitive and atropine-resistant contractions of the fundus, the contractile response to 5-MOT was examined in the presence of 1 μM atropine, which prevents the atropine-sensitive contraction through acetylcholine release elicited by activation of cholinergic neurons. As shown in Fig. 3, atropine significantly reduced the contractile response to 5-MOT at concentrations of 10^{-8} to 10^{-6} M and the antagonistic action of atropine appeared not to be simply competitive. The atropine-sensitive component of the 5-MOT-induced contraction, obtained by subtracting the contractile force with atropine from that without atropine, exhibited a 'bell-shaped' concentration-dependent response with a maximal contraction at 10^{-7} M 5-MOT (Fig. 3).

The contraction elicited by 5-MOT in the fundus strips was significantly inhibited by atropine (1 μM) and SDZ 205-557 (1 μM) in the organ bath system (Fig. 4). These results are consistent with those for the contractile experi-

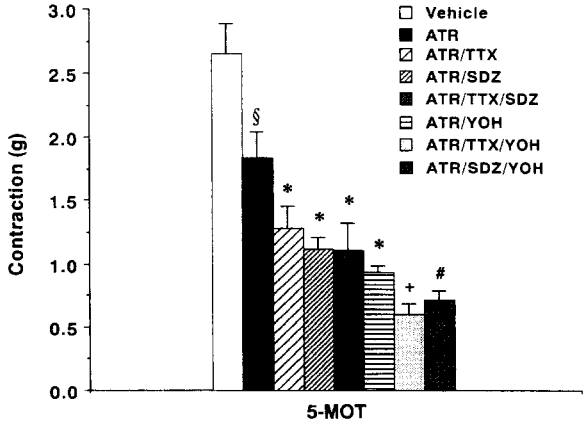

Fig. 4. Effects of atropine (ATR), tetrodotoxin (TTX), SDZ 205-557 (SDZ), and yohimbine (YOH) on 5-MOT-induced contractions in rat stomach fundus strips. The concentrations of drugs used were as follows: 5-methoxytryptamine, 0.1 μM; atropine, 1 μM; tetrodotoxin, 1 μM; SDZ 205-557, 1 μM; yohimbine, 1 μM. The values are means ± S.E.M. of 4–8 separate experiments. § $P < 0.05$ indicates a significant difference compared with the 5-methoxytryptamine-induced contraction. * $P < 0.05$ indicates a significant difference compared with the 5-methoxytryptamine-induced contraction in the presence of atropine alone. + $P < 0.05$ indicates a significant difference compared with the contraction in the presence of 5-methoxytryptamine, atropine and tetrodotoxin. # $P < 0.05$ indicates a significant difference compared with the contraction in the presence of 5-methoxytryptamine, atropine and yohimbine.

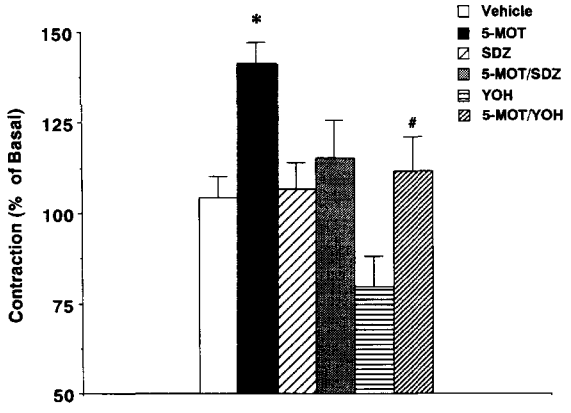

Fig. 5. Effects of 5-methoxytryptamine (5-MOT), SDZ 205-557 (SDZ), and yohimbine (YOH) on electrical field stimulation (45 V, 5 Hz, 5-ms pulse duration, for 5 s, intervals 30 s)-induced contractions in rat stomach fundus strips. The concentrations of drugs used were as follows: 5-methoxytryptamine, 0.1 μM; SDZ 205-557, 1 μM; yohimbine, 1 μM. The values are means ± S.E.M. of 5–7 separate experiments. * $P < 0.05$ indicates a significant difference compared with the contractile force in the absence of 5-methoxytryptamine. # $P < 0.05$ indicates a significant difference compared with the contraction in the presence of yohimbine alone.

ments with the superfusion system (Fig. 2A). Yohimbine, a 5-HT_{2B} receptor antagonist (Foguet et al., 1992; Wainscott et al., 1993; Baxter et al., 1994) (1 μM), effectively prevented 5-MOT-induced contractions (Fig. 4). Inhibitory effects of tetrodotoxin, SDZ 205-557, and yohimbine on the contractile response to 5-MOT were also observed in the presence of atropine. However, in the presence of atropine and tetrodotoxin, SDZ 205-557 did not cause a further inhibition to that observed with atropine and tetrodotoxin or with atropine and SDZ 205-557. In contrast, yohimbine inhibited the 5-MOT-induced contraction even in the presence of atropine and tetrodotoxin. The inhibitory effect of a combination of atropine, SDZ 205-557 and yohimbine was significantly greater than the inhibitory effect of atropine and SDZ 205-557 or atropine and yohimbine (Fig. 4).

3.5. Effect of 5-MOT on electrically (5 Hz) evoked contractions

5-MOT (0.1 μM) enhanced electrically (45 V, 5 Hz, 5-ms pulse duration, for 5 s, intervals 30 s) evoked contractions by approximately 40% of the control level in the organ bath system (Fig. 5). The stimulatory effect of 5-MOT on electrically evoked contractions was antagonized by SDZ 205-557, while the antagonist alone did not affect the contraction elicited by electrical field stimulation. Yohimbine reduced the 5-MOT-induced increase in electrically evoked contractions. It was observed, however, that yohimbine alone suppressed the control contraction elicited by electrical field stimulation. In fact, when the contraction evoked by electrical field stimulation in the presence of both 5-MOT and yohimbine was compared with that in the presence of yohimbine alone, the 5-MOT-induced increments in the electrically evoked contraction in both groups were essentially identical, being approximately 40% of the control.

4. Discussion

In the present study, we have shown that 5-MOT produced contraction and an increase in acetylcholine release in rat stomach fundus strips, suggesting that acetylcholine release is, at least partly, involved in the contractile effect of 5-MOT. However, 5-MOT caused a concentration-dependent muscle contraction, whereas it evoked acetylcholine release in a 'bell-shaped' concentration-dependent manner, having its maximal effect at 10^{-7} M 5-MOT (Fig. 1A and B). It is suggested, therefore, that the 5-MOT-induced contraction in the fundus strip is not due solely to acetylcholine released from cholinergic neurons.

Previous studies on the guinea-pig ileum (Buchheit et al., 1985; Ramírez et al., 1994) and the guinea-pig proximal colon (Kojima and Shimo, 1995) have demonstrated that the contractile effects of 5-HT involve non-cholinergic, atropine-resistant, excitatory mechanisms. To discriminate between atropine-sensitive and atropine-resistant components of the contractile response to 5-MOT in the stomach fundus strip, the 5-MOT-induced contraction was examined in the presence or absence of 1 μM atropine. With 1 μM atropine, the contractile response to 1 μM acetylcholine in the stomach fundus strips was abolished (data not shown) and the contraction evoked by electrical field stimulation (45 V, 10 Hz, 5-ms pulse duration, for 5 s), under which the extent of increase in acetylcholine release was nearly identical to that of the maximal effect of 5-MOT (0.1 μM) (Fig. 1B and Section 3), was blocked (data not shown). Atropine (1 μM) significantly suppressed the 5-MOT (10^{-8}–10^{-6} M) contraction of the fundus strips by 22–56% (Fig. 3). However, the contractile effect of 5-MOT (10^{-8}–10^{-6} M) remained even after treatment with atropine, and the contraction induced by 5-MOT at higher concentrations ($> 10^{-6}$ M) was not influenced by atropine. These results appear to indicate that the 5-MOT-induced contraction in the rat stomach fundus consists of both an atropine-sensitive component and an atropine-resistant component. At a lower concentration of 5-MOT ($< 10^{-7}$ M), the contractile effect of the agonist seemed to be mediated through both atropine-sensitive and atropine-resistant pathways, since the proportions of the sensitive and resistant components of the 5-MOT-induced contraction were 40–55% for both components, as evidenced from blockade by atropine (Fig. 3). However, the atropine-resistant component was more predominant at higher concentrations ($> 10^{-7}$ M), as shown by the only 8–22% inhibition of the 5-MOT-induced contraction by atropine. Such a concentration-dependent change in the contribution of the atropine-resistant compo-

nent to the contractile effect of 5-HT has also been demonstrated in the guinea-pig ileum (Buchheit et al., 1985).

The atropine-sensitive component of the 5-MOT-induced contraction was seen as a 'bell-shaped' concentration-dependent response (Fig. 3). This concentration-response pattern of the atropine-sensitive contractions resembled that for 5-MOT-elicited ^3H outflow (Fig. 1B). It was observed that the 5-MOT (0.1 μM)-induced increases in both acetylcholine release and contraction in the strips were sensitive to the sodium channel blocker tetrodotoxin (1 μM) and the 5-HT_4 receptor antagonist SDZ 205-557 (1 μM) (Fig. 2A and B). Furthermore, 5-MOT (0.1 μM) enhanced the electrical field stimulation-evoked contraction, which was considered to be mediated by acetylcholine release (Fig. 5). The 5-MOT-induced enhancement of the electrical field stimulation-evoked contraction was effectively antagonized by SDZ 205-557. Taken together, these data suggest that 5-MOT-evoked atropine-sensitive contractions are mediated through acetylcholine release by the stimulation of 5-HT_4 receptors located on cholinergic neurons in the rat stomach fundus, as has been proposed in previous studies on the guinea-pig ileum (Craig and Clarke, 1990; Kilbinger and Wolf, 1992; Kilbinger et al., 1995).

The possible mechanism(s) for the atropine-resistant contractile effect of 5-MOT was examined by using several antagonists. In the presence of atropine (1 μM), both tetrodotoxin (1 μM) and SDZ 205-557 (1 μM) significantly inhibited the contractile effect of 5-MOT (0.1 μM), whereas no further inhibition was observed with a combination of tetrodotoxin and SDZ 205-557 as compared with that seen with tetrodoxin or SDZ 205-557 alone (Fig. 4). It is suggested, therefore, that the resistant contraction might partly involve the release of a second neurotransmitter through the stimulation of 5-HT_4 receptors presumably located on non-cholinergic neurons. It has been reported for the guinea-pig proximal colon that the stimulation of 5-HT_4 receptors located on tachykininergic neurons leads to the release of tachykinins (Buchheit et al., 1985; Ramírez et al., 1994; Kojima and Shimo, 1995).

Neither tetrodotoxin nor SDZ 205-557 at the concentrations used completely abolished the atropine-resistant contraction elicited by 5-MOT (Fig. 4). Furthermore, yohimbine, a 5-HT_{2B} receptor antagonist, (1 μM) significantly inhibited the 5-MOT-induced contraction in the presence of atropine (Fig. 4). With a combination of yohimbine and SDZ 205-557, the inhibition of the atropine-resistant contraction was significantly greater than that seen with yohimbine or with SDZ 205-557 alone. Thus, these results seem to indicate the involvement of the 5-HT_{2B} receptor as well as the 5-HT_4 receptor in the atropine-resistant contractile effect of 5-MOT in rat stomach fundus. This is consistent with the proposed role of the 5-HT_{2B} receptor in mediating the contractile response to 5-HT in rat stomach fundus (Kursar et al., 1992; Foguet et al., 1992; Wainscott et al., 1993; Baxter et al., 1994). The 5-HT_{2B} receptor-mediated contractile response did not seem to be mediated through the release of acetylcholine or other neurotransmitters, since yohimbine did not affect the 5-MOT-induced increase in electrically evoked contractions (Fig. 5) and the antagonist further inhibited the atropine-resistant contraction in the presence of tetrodotoxin (Fig. 4).

In summary, the results of the present study suggest that, in rat stomach fundus, the contractile action of 5-MOT involves both an atropine-sensitive component and an atropine-resistant component, which are mediated by activation of at least two independent 5-HT receptors. The atropine-sensitive contraction appears to be mediated by the stimulation of 5-HT_4 receptors located on cholinergic neurons, leading to the release of acetylcholine, while the atropine-resistant contraction is mediated by the activation of 5-HT_4 receptors located on non-cholinergic neurons and by the activation of 5-HT_{2B} receptors (presumably located on the smooth muscle cells).

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