Effects of 5-hydroxytryptamine on electrical responses of circular smooth muscle isolated from the guinea-pig gastric antrum

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Abstract

The effects of 5-hydroxytryptamine (5-HT) on electrical responses of the membrane were investigated in circular smooth muscle isolated from the guinea-pig stomach antrum. Small segment of circular muscle tissue produced a periodical generation of slow potentials at frequency of 0.1–2 cycles min⁻¹, during random generation of unitary potentials. Application of 5-HT (10⁻⁷–10⁻⁵ M) hyperpolarized the membrane and either increased or decreased the frequency of slow potentials, both with associated increase in amplitude of slow potential. These effects of 5-HT were abolished by methysergide. Nω-nitro-L-arginine (L-NA) increased the frequency of spontaneously generated slow potentials and also increased the frequency of slow potentials generated during stimulation with 5-HT, suggesting an involvement of the increased production of nitric oxide (NO) by 5-HT. Atropine did not alter spontaneous and 5-HT-induced electrical responses. The hyperpolarization produced by 5-HT was associated with a decrease in input resistance and time constant of the membrane. The amplitude of the 5-HT-induced hyperpolarization was increased in low [K⁺]₀ solution and decreased in high [K⁺]₀ solution or in the presence of glybenclamide, suggesting that the hyperpolarization was produced by activation of ATP-sensitive K⁺channels. The increase in amplitude of slow potentials by 5-HT may be secondary due to hyperpolarization of the membrane. The inhibition by 5-HT of the frequency of slow potentials may be partly due to the increased release of NO, however the mechanism by which dual effects of 5-HT on the frequency of slow potentials remains unsolved.

Key words: gastric muscle, 5-hydroxytryptamine, slow potential, hyperpolarization, ATP-sensitive K⁺channel
Introduction

Gastrointestinal smooth muscles are spontaneously active with periodic generation of slow waves and/or spike potentials (Tomita, 1981). Investigation of the pathophysiological alteration of gastrointestinal smooth muscle tissue in c-kit mutant mice revealed that a lack of interstitial cells of Cajal distributed in the myenteric region (ICC-MY) was causally related to the absence of slow waves in intestinal smooth muscle, thereby suggesting that ICC-MY might be the pacemaker of gastrointestinal activity (Ward et al. 1994; Huizinga et al. 1995; Sanders, 1996; Huizinga et al. 1997; Sanders et al. 1999). In isolated smooth muscle tissues of the guinea-pig gastric antrum, three types of electrical responses are recorded; slow waves recorded from circular smooth muscle cells, driving potentials recorded from ICC-MY and follower potentials recorded from longitudinal smooth muscle cells (Dickens et al. 1999). Analysis of the electrical connection between these cells revealed that the driving potential generated in ICC-MY propagates to both circular and longitudinal smooth muscle cells in an electrotonic manner (Dickens et al. 1999; Cousins et al. 2003; Hirst and Ward, 2003).

However, isolated circular smooth muscle bundles of the guinea-pig stomach antrum, which do not contain longitudinal muscle and ICC-MY, are also spontaneously active with periodic generation of regenerative potentials (slow potentials) (Suzuki and Hirst, 1999; Suzuki, 2000). The circular muscle segment produces ongoing small transient depolarizing potentials called unitary potentials, which are propagated electrical responses from interstitial cells of Cajal distributed within muscle bundles (ICC-IM) (Dickens et al., 2001). The frequency spectrum analysis of unitary potentials indicates that slow potentials are formed by a summation of unitary potentials generated in intramuscular interstitial cells of Cajal (ICC-IM) (Edwards et al., 1999). Slow potentials can also be evoked by the membrane depolarization in an all or none fashion, with a refractory of 3–5 s (Suzuki and Hirst, 1999; Kito et al., 2002). In intact tissues with attached longitudinal muscle layer and ICC-MY, slow potentials generated in circular muscle layer form the 2nd component of slow waves (Dickens et al., 2001; Hirst et al., 2002; Hirst and Ward, 2003).

Heterogeneous distribution of 5-hydroxytryptamine (5-HT) receptors in the gastrointestinal tissues suggests that 5-HT may be taking an important role for the digestive activity (Gershon, 2004). Many subtypes of 5-HT receptor distribute heterogeneously in gastrointestinal tissues, in prejunctional and postjunctional membranes and also in ICC, and each subtype of receptors has different actions (Liu et al., 2005). Gastrointestinal smooth muscle has a distribution of 5-HT₁ receptors, and their activation produces a muscle relaxation in the rat oesophagus (Baxter et al., 1991), human intestine (Grider et al., 1998; Grider, 2003), human colon and rat ileum (Tuladhar et al., 1994). Activation of presynaptic 5-HT receptors enhances the release of acetylcholine (ACh) in the myenteric neurons (Kilbinger and Wolf, 1992; Pan and Galligan, 1994; Galligan et al., 2003), and facilitates peristaltic reflexes (Grider et al., 1998; Foxx-Orenstein et al., 1998; Grider, 2003). Two types of 5-HT receptor (5-HT₁ and 5-HT₄) distribute in the prejunctional membrane of myenteric cholinergic nerves, and activation of 5-HT₁ receptors increases and that of 5-HT₄ inhibits the release of ACh from nerves (Taniyama et al., 1991).

Experiments were carried out to investigate the effects of 5-HT on smooth muscle segment isolated from the guinea-pig stomach antrum. This muscle tissue contains smooth muscle,
myenteric nerve endings and ICC-IM, and slow potentials are generated periodically by the activity of ICC-IM (Suzuki and Hirst, 1999; Edwards et al., 1999), while excitation of myenteric nerve endings releases mainly ACh and nitric oxide (NO) (Teramoto et al., 2003; Lee et al., 2004). This tissue has an advantage that the electrical events appeared in any cells coupled within the tissue could be detected easily by recording membrane responses from smooth muscle cell, because the size of tissue is significantly small compared to the length constant of the tissue (equal to 2.5 mm; Tomita, 1981).

Methods

Preparations

Male albino guinea-pigs, weighting 200–300 g, were anesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (sevoflurane, Maruishi Pharm., Osaka, Japan), and exsanguinated by decapitation. All animals were treated ethically according to the guiding principles for the care and use of animals in the field of physiological sciences, approved by The Physiological Society of Japan. The stomach was excised, and opened by cutting along the small curvature in Krebs solution. The mucosal layers were removed by cutting with fine scissors, and smooth muscle tissues were isolated from the antrum region.

After removal of longitudinal muscle layer and attached myenteric layer, single circular muscle bundles, with about 100 μm wide and 200–300 μm long, were prepared. The bundle segment was pinned out on a silicone rubber plate fixed at the bottom of an organ bath (8 mm wide, 8 mm deep, 20 mm long), with the mucosal side uppermost, and was superfused with warmed (36°C) Krebs solution, at a flow rate of about 2 ml min⁻¹. The ionic composition of the Krebs solution was as follows (mM): Na⁺ 137.4, K⁺ 5.9, Ca²⁺ 2.5, Mg²⁺ 1.2, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134, and glucose 11.5. Solutions containing different concentrations of potassium ions ([K⁺]o) (1.2, 3.5, 13.8 mM) were prepared by changing the ratio of KCl with NaCl. These solutions were aerated with O₂ containing 5% CO₂, and the pH of the solutions was maintained at 7.2–7.3.

Membrane potentials were recorded from circular smooth muscle cells using glass capillary microelectrode (capillary outer diameter, 1.2 mm, inner diameter 0.6 mm; Hilgenberg, Germany) filled with 0.5 M KCl (the tip resistance ranged between 80 and 150 MΩ). Electrical responses recorded with a high input impedance amplifier (Axoclamp-2B, Axon Instruments, Inc., Foster City, California, U.S.A.) were displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Osaka, Japan) and were also stored on a personal computer for later analysis.

Chemicals used were 5-hydroxytryptamine (5-HT), atropine sulphate, glybenclamide, N⁶-nitro-L-arginine (LNA) (Sigma Chem., MO, U.S.A.) and methysergide (Sandoz Pharm., Swiss). These chemicals were dissolved with distilled water or dimethyl sulfoxide (DMSO) as a stock solution, and were further diluted with Krebs solution, with the ratio less than 1 : 1000. The dilution of chemicals did not alter the pH of the solution.

Experimental values were expressed by the mean value ± standard deviation (SD). Statistical significance was tested using Student’s t-test, and probabilities of less than 5% (P<0.05) were considered significant.
Results

Effects of 5-HT on slow potentials

In circular smooth muscle bundles isolated from the guinea-pig stomach antrum, the resting membrane potential varied between preparations, from -65 mV to -75 mV (mean, -66.8 ± 2.5 mV, n=16). Slow potentials were observed in all preparations tested, with the frequency ranging between 0.21 and 3.46 cycles min⁻¹ (mean, 1.75 ± 0.71 cycles min⁻¹, n=35) and the amplitude ranging between 18.6 and 42.2 mV (mean, 32.3 ± 6.6 mV, n=35). Unitary potentials ranging up to 5 mV were also generated in a random fashion, between the interval of slow potentials. These properties were similar to those reported previously (Suzuki and Hirst, 1999; Kito et al., 2002).

Increasing concentrations of 5-HT (10⁻⁸–10⁻⁶ M) were applied to the superfusate for 3–5 min, while electrical responses had been recorded from single cells in the isolated circular smooth muscle segment. In response to stimulation with 10⁻⁸ M 5-HT, no detectable change in electrical responses was produced in all preparations tested (n=6). During stimulation of muscle with 5-HT in concentrations above 10⁻⁷ M, the frequency of slow potentials was increased in 15 preparations (Fig. 1A) and decreased in 20 preparations (Fig. 1B). The concentration-dependent change in the frequency of slow potentials in response to 10⁻⁷–10⁻⁵ M 5-HT was summarized by expressing the effects as relative to the background frequency of slow potentials (Fig. 1C). The peak response of the frequency-increased bundle appeared at 10⁻⁶ M, while the inhibition of the frequency developed in a concentration-dependent manner. In both the frequency-increased and frequency-decreased bundles, 5-HT equally increased the amplitude of slow potentials (Fig. 1D) and hyperpolarized the membrane (Fig. 1E), in a concentration-dependent manner.

The effects of methysergide, known as a non-selective inhibitor of 5-HT receptors (Bradley et al., 1986), on responses produced by 5-HT were investigated. In bundles which showed the frequency-inhibited response to 5-HT, methysergide (10⁻⁶ M) did not alter the resting membrane potential (control, -67.5 ± 2.5 mV; in methysergide, -68.2 ± 2.8 mV; n=10; P>0.05) and amplitude and frequency of slow potentials (Fig. 2, A and B). In the presence of 10⁻⁶ M methysergide, 5-HT (up to 10⁻⁵ M) did not produce any significant hyperpolarization of the membrane (Fig. 2C), nor did change the amplitude (Fig. 2D) and frequency (Fig. 2E) of slow potentials. Similar effects of methysergide were also observed in bundles which showed the frequency-increased response to 5-HT (n=2, data not shown). These results indicate that the hyperpolarization and modulation of the frequency and amplitude of slow potentials were mediated through activation of 5-HT receptors.

Alteration of the responses to 5-HT was investigated after incubation of tissues with solution containing L-NA or atropine, or both, to examine possible involvement of nitric oxide (NO) or acetylcholine (ACh) in the 5-HT-induced responses. Figure 3 shows a typical example of the 5-HT responses produced in bundles which showed frequency-increased response. The increase in frequency of slow potentials by 10⁻⁶ M 5-HT (Fig. 3A) was enhanced after the tissue had been exposed to the solution containing 10⁻⁵ M L-NA (Fig. 3B), but co-application of 10⁻⁶ M atropine with L-NA did not further alter the response (Fig. 3C). The summarized data indicated that the 5-HT induced hyperpolarization (Fig. 3D) and amplitude of slow potentials (Fig. 3F) were not
5-HT actions on gastric electrical activity

Fig. 1. Two types of electrical response produced by 5-HT. Excitatory (A) and inhibitory responses (B) of slow potentials produced by 5-HT (10^{-9} M) in circular smooth muscle bundle isolated from the guinea-pig stomach antrum. A and B were recorded from different bundles. Frequency (C) and Amplitude (D) of slow potentials and peak amplitude of hyperpolarization (E) produced by different concentrations of 5-HT (10^{-7} M-10^{-5} M) are summarized in graphs (●, excitatory response; ○, inhibitory response; mean ± S.D., n=5-8).

altered by L-NA and atropine. L-NA increased the frequency of slow potentials, and 5-HT further increased it, either in the absence or presence of atropine (Fig. 3E).

The effects of L-NA or atropine on responses to 5-HT were also observed in bundles which showed the frequency-inhibited response (Fig. 4). The inhibition of slow potentials by 5-HT (Fig. 4A) was not altered by atropine (Fig. 4B), and was slightly diminished in the co-application of L-NA (Fig. 4C). Summarized data indicated that the hyperpolarization (Fig. 4D) and the increase in amplitude of slow potentials by 5-HT (Fig. 4E) were not altered by atropine or L-NA, while the inhibition by 5-HT of the frequency of slow potentials was diminished by L-NA (Fig. 4F). Thus, the results shown in Figs. 3 and 4 suggested that the inhibitory response produced by 5-HT was partly due to the increased production of NO. No significant contribution of cholinergic effects was observed in the responses produced by 5-HT.

Properties of hyperpolarization produced by 5-HT

The effects of 5-HT on biophysical properties of the membrane were investigated in circular smooth muscle bundles isolated from the stomach antrum, by recording responses from two
Fig. 2. Effects of methysergide on the actions of 5-HT. Electrical responses produced by 5-HT \( (10^{-6} \text{ M}) \) were recorded in the absence (A) and presence of \( 10^{-6} \text{ M} \) methysergide (B). Effects of methysergide on the amplitude of hyperpolarization (C), and on the amplitude (D) and frequency (E) of slow potentials produced by \( 10^{-6} \text{ M} \) 5-HT are summarized (mean ± S.D., \( n=10 \) from 3 tissues). \* \( P<0.05 \).

different cells simultaneously. As reported previously (Suzuki and Hirst, 1999), electrical responses recorded simultaneously from two different cells showed a synchronization, indicating that these two cells were electrically coupled. In 5 bundles tested, any couple of cells showed a synchronization of responses. Stimulation of muscle with current applied to one electrode produced an electrotonic potential which was recorded from the second electrode. The amplitudes of electrotonic potential were linearly increased in response to increasing intensities of current (1–3 nA) (Fig. 5, A and C). The time constant of the onset of electrotonic potential was about 200 ms (Fig. 5D), and the calculated input resistance was about 5 MΩ (Fig. 5E). Application of 5-HT (\( 10^{-5} \text{ M} \)) hyperpolarized the membrane by 4.1 ± 0.9 mV \((n=5)\), and decreased the amplitude of electrotonic potentials produced by any given intensity of currents by about 26% (Fig. 5, B and C). In the presence of 5-HT, both the time constant and input resistance of the membrane were significantly decreased (Fig. 5, D and E).

The effects of 5-HT were observed in solutions containing different concentrations of potassium ion ([K⁺]). The amplitude of hyperpolarization produced by \( 10^{-5} \text{ M} \) 5-HT was about 5 mV in standard solution with 5.9 mM [K⁺] (Fig. 6A). Elevation of [K⁺] to 13.8 mM depolarized the membrane by about 7 mV, with associated increase in frequency and decrease in amplitude of slow potentials, and reduced the amplitude of hyperpolarization produced by 5-
HT to about 1 mV (Fig. 6B). Reduction of [K+]o to 3.5 mM hyperpolarized the membrane by about 2 mV, with associated decrease in the frequency and increase in the amplitude of slow potentials, and increased the 5-HT-induced hyperpolarization to about 8 mV (Fig. 6C). The relationship between the amplitude of hyperpolarization produced by 5-HT and concentration of [K+]o plotted on a logarithmic scale indicated that the amplitude was linearly related with the concentration of [K+]o (Fig. 6D).

The effects of glybenclamide, an inhibitor of ATP-sensitive K\(^{-}\)-channels, on 5-HT responses were investigated. Exposure of bundles to solutions containing 3 \times 10^{-6} M glybenclamide did not alter the resting membrane potential, nor the amplitude and frequency of slow potentials. However, the hyperpolarization produced by 5-HT was abolished in the presence of glybenclamide (Fig. 7, A and B). Summed data indicated that the inhibition by glybenclamide of the hyperpolarization produced by 5-HT (Fig. 7E) was produced with no marked change in the decreased frequency (Fig. 7C) and increased amplitude (Fig. 7D) of slow potentials. These data suggested that the hyperpolarization produced by 5-HT was mainly due to the activation of ATP-sensitive K-channels.
Fig. 4. Effects of atropine and nitroarginine on inhibitory responses produced by 5-HT in circular muscle bundles isolated from the stomach antrum. Responses produced by 10^{-6} M 5-HT were recorded in the absence (A) and presence of 10^{-6} M atropine (B) and addition of nitroarginine (L-NA, 10^{-6} M) in the presence of atropine (C). Amplitude of hyperpolarization (D), and the amplitude (E) and frequency (F) of slow potentials produced by 10^{-6} M 5-HT are summarized. In E and F, filled and open bars indicate values obtained before and during application of 5-HT, respectively (mean ± S.D., n=3–6). *, P<0.05.

Discussion

The present experiments revealed that in circular smooth muscle bundles isolated from the guinea-pig antrum, 5-HT produces dual actions on the frequency of slow potentials, with associated increase in amplitude of slow potentials and hyperpolarization. There are many subtypes of 5-HT receptor, and their distribution in gastric tissues is heterogeneous, some distribute on nerve terminals and others distribute on smooth muscle (Gershon, 2004). Distribution of 5-HT4 receptors in the enteric neurons, smooth muscle cells and ICC has been shown in the mouse intestine (Liu et al., 2005). Although the present experiments did not test the effects of selective antagonists of 5-HT receptor subtypes on slow potentials, possible involvement of 5-HT receptors was considered, from the inhibitory actions of methysergide, a known inhibitor of non-selective 5-HT receptors (Bradley et al., 1986).

The hyperpolarization produced by 5-HT may be mediated through activation of ATP-
sensitive K-channels, since it is inhibited by glybenclamide. The evidence that the reversed change in amplitude of 5-HT-induced hyperpolarization in increasing concentrations of $[K^+]_o$ and the reduction of input resistance and time constant of the membrane in the presence of 5-HT, also supports that the hyperpolarization is produced by activation of K-channels. In gastric muscles, excitation of nitrergic nerves hyperpolarizes the membrane and forms a part of the inhibitory junction potential, due to inhibition by NO of the generation of unitary potentials produced in ICC-IM (Suzuki et al., 2003; Teramoto and Hirst, 2003). Unitary potentials may be generated by the increase in Cl$^-$-conductance (Hirst et al., 2002), and may be insensitive to glybenclamide. The present experiments indicated that the hyperpolarizing actions of 5-HT was not altered by L-NA. These results suggest that the hyperpolarization produced by 5-HT is not due to the inhibition of unitary potentials.

The amplitude of slow potentials is related to membrane potential, and it is increased by hyperpolarization and decreased by depolarization of the membrane (Nose et al., 2000; Fukuta et al., 2002; Kito and Suzuki, 2003), possibly because the slow potential is formed by the increased conductance of Cl$^-$ whose equilibrium potential distributes positive to the resting membrane potential (Hirst et al., 2002). The increase in amplitude of slow potentials by 5-HT...
Fig. 6. Effects of potassium ion concentration on 5-HT-induced responses. Electrical responses produced by 5-HT (10^{-5} M) were recorded in different concentrations of potassium ion ([K']_o) (A, 5.9 mM; B, 13.8 mM; C, 3.5 mM). D. Relationship between amplitude of hyperpolarization produced by 10^{-5} M 5-HT and concentration of [K']_o. Mean ± S.D. (n=3-6).

was always associated with hyperpolarization of the membrane, and the amplitude of slow potentials was not altered during the inhibition of hyperpolarization by glybenclamide (Fig. 7). These results suggest that the change in amplitude of slow potentials by 5-HT is mainly indirectly due to the effects of hyperpolarization.

The frequency of slow potentials is also sensitive to the change in membrane potential, and hyperpolarization decreases and depolarization increases the frequency until it reaches to a certain level (about 7 s interval), mainly due to refractory period for the generation of slow potentials (Nose et al., 2000; Kito et al., 2002; Suzuki et al., 2002). The present experiments indicated that the frequency of slow potentials was increased or decreased by 5-HT, with associated hyperpolarization of the membrane. These results indicate that the changes in frequency of slow potentials by 5-HT are not causally related with the membrane hyperpolarization. Experiments using L-NA suggested that 5-HT inhibits the frequency of slow potentials indirectly by the increased production of NO. In antrum muscles, NO decreases the frequency of slow potentials, from evidence that it is increased by preventing the synthesis of NO with L-NA and is decreased by stimulating muscles with SIN-1, an NO donor (Nakamura et al., 2004). In the frequency-increased bundles with 5-HT, application of L-NA further increased the frequency, while in the frequency-inhibited bundles the response to 5-HT was changed to an excitatory response by L-NA. Thus, although the actions of 5-HT are produced in part by the
Fig. 7. Effects of glybenclamide on electrical responses produced by 5-HT in circular smooth muscle bundle of the stomach antrum. Responses produced by 10⁻⁶ M 5-HT were recorded in the absence (A) and presence of 3 × 10⁻⁶ M glybenclamide (B). A and B were recorded from the same cell. Effects of glybenclamide on the frequency (C) and amplitude of slow potentials (D), and the amplitude of hyperpolarization, produced by 5-HT (E) were summarized (mean ± S.D.; n=5 tissues; *, P<0.05).

increased production of NO, this alone cannot interpret the effects of 5-HT on the frequency of slow potentials. Possible involvement of unidentified mechanism in the actions of 5-HT on the frequency of slow potentials is considered.

Some subtypes of the 5-HT receptor are distributed at the cholinergic nerve terminals, and they modulate neuro-muscular transmission (Liu et al., 2005). For example, in small intestine stimulation of 5-HT₄ receptors increases the release of ACh from myenteric cholinergic nerves, while the inhibited release of ACh is produced during stimulation of 5-HT₁ receptors (Taniyama et al., 1991). The present experiments indicated that 5-HT responses were not significantly altered by atropine, suggesting the absence of cholinergic component in the actions of 5-HT on slow potentials. This result did not fit the observation made in antrum muscles with cisapride, a known agonist of 5-HT₁ receptor, which shows that the increase in amplitude and frequency of spontaneous contraction by cisapride is produced indirectly by the increased release of ACh from myenteric nerves due to activation of prejunctional 5-HT₄ receptors (Taniyama et al., 1991; Ohno et al., 1995). Cisapride depolarizes smooth muscle membrane, possibly by direct actions (Ohno et al., 1995), while opposite actions are the case for 5-HT. Comparing the results
obtained from previous and present experiments also allows speculation that the difference appears on the type of tissues examined, i.e., Ohno et al. (1995) examined the effects of cisapride in intact tissues which contained circular and longitudinal muscles and myenteric plexus with ICC-MY and ICC-IM networks, while in the present experiments, the isolated circular muscle bundle contains only ICC-IM and fragments of nerve terminals. Difference in actions of cisapride on circular smooth muscle between intact tissue and isolated bundle could be produced, if this chemical depolarizes the membrane only in ICC-MY, while hyperpolarizes the membrane in smooth muscle or ICC-IM.

It is summarized that in antrum circular smooth muscle tissues, 5-HT hyperpolarizes the membrane by activation of ATP-sensitive K-channels, and increases or decreases the frequency of slow potentials. 5-HT increases production of NO, which causes a part of the inhibitory action of 5-HT on the frequency of slow potentials. No significant contribution of cholinergic mechanism was found in the 5-HT induced increase in the frequency of slow potentials.

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