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Effects of 5-HT_{1A} and 5-HT_4 receptor agonists on slow synaptic potentials in enteric neurons

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Abstract

Intracellular electrophysiological methods were used to examine the effects of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeOT), 4-amino-5-chloro-2-methoxy-*N*-(4-[1-azabicyclo[3,3,1]nonyl]) benzamide hydrochloride (renzapride), *cis*-4-amino-5-chloro-*N*[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidiny]-2-methoxybenzamide monohydrate (cisapride) and endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-(1-methyl)ethyl-2-oxo-1*H*-benzimidazole-1-carboxamidehydrochloride (BIMU 8) on noncholinergic slow excitatory postsynaptic potentials (slow EPSPs) in myenteric afterhyperpolarization (AH) neurons of guinea pig ileum. 5-HT (0.01–1 μM) and 5-CT (0.001–0.1 μM) produced a concentration-dependent inhibition of slow EPSPs. The 5-HT_{1A} receptor antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimidobutyl]piperazine (NAN-190) produced rightward shifts in 5-HT and 5-CT concentration-response curves; facilitation of slow EPSPs was never observed. 5-MeOT caused a depolarization and inhibited spike afterhyperpolarizations in a concentration-dependent manner but this effect was not blocked by the 5-HT_3/5-HT_4 receptor antagonist, tropisetron (1 μM). Renzapride (0.01–0.3 μM), cisapride (0.01–1.0 μM) and BIMU 8 (0.01–1.0 μM) did not change the membrane potential of any neuron tested. Renzapride and BIMU 8 did not change the amplitude of slow EPSPs. In 13 of 19 neurons cisapride did not change the amplitude of slow EPSPs; in 6 neurons cisapride (1 μM) reversibly inhibited the slow EPSP. Responses to substance P which mimicked the slow EPSP were not affected by cisapride. These data indicate that excitatory 5-HT_4 receptors are not localized to cell bodies of AH neurons and are not coupled to presynaptic facilitation of noncholinergic slow EPSPs in AH neurons.

Keywords: Enteric nerve; Noncholinergic slow synaptic potential; 5-HT receptor; Gastrointestinal pharmacology

1. Introduction

5-Hydroxytryptamine (5-HT) has multiple actions on gastrointestinal motility which can be attributed to an action at several 5-HT receptor subtypes localized to myenteric nerves. In nerve-muscle preparations in vitro, 5-HT causes contractions by activating 5-HT_3 and 5-HT_4 receptors (Craig and Clarke, 1990; Craig et al., 1990; Turconi et al., 1991). These excitatory 5-HT receptors are localized to cholinergic (Kilbinger and Pfeuffer-Friederich, 1985; Kilbinger and Wolf, 1992) and noncholinergic neurons (Buchheit et al., 1985). 5-HT also inhibits electrically evoked contractions by acting at presynaptic 5-HT_{1A} receptors localized to cholinergic and noncholinergic excitatory neurons (Fozard and Kilbinger, 1985; Small et al., 1991; Galligan, 1992). In guinea pig isolated ileum, 5-HT facilitates and inhibits distention-evoked peristalsis (Bülbring and Crema, 1957). Facilitation of the peristaltic reflex is due to a decrease in threshold pressures required to elicit the emptying phase of peristalsis and 5-HT_4 receptor agonists enhance peristalsis (Tonini et al., 1989; Tonini and Costa, 1990; Craig and Clarke, 1991; Buchheit and Buhl, 1991; Rizzi et al., 1992). Inhibition of the peristaltic reflex by 5-HT has been attributed to block of ganglionic transmission (Bülbring and Crema, 1957).

Intracellular electrophysiological studies have demonstrated several types of myenteric neuron in guinea pig ileum. The 'synaptic' (S) neuron exhibits fast excitatory postsynaptic potentials (fast EPSPs) following stimulation of presynaptic nerves (Hirst et al., 1974). The fast EPSPs are mediated largely by acetyl-

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choline acting at nicotinic receptors (Hirst et al., 1974). A second type of neuron is the 'afterhyperpolarization' (AH) neuron. Most AH neurons exhibit slow excitatory postsynaptic potentials (slow EPSPs) when presynaptic nerves are stimulated and action potentials in AH neurons are followed by a spike afterhyperpolarization that lasts from 1 to 10 s (Hirst et al., 1974; Morita et al., 1982). Some neurons with a long-lasting spike afterhyperpolarization also receive fast synaptic input (Wood, 1994). Data from combined electrophysiological, morphological and immunohistochemical studies indicate that S neurons are likely to be interneurons and motorneurons (Costa and Furness, 1989; Steele et al., 1991; Bornstein et al., 1984,1991) while AH neurons may be sensory neurons and interneurons (Hendriks et al., 1990; Kunze et al., 1993; Bornstein et al., 1991; Wood, 1994). Electrophysiological studies have shown that 5-HT_{1A} receptors mediate presynaptic inhibition of fast EPSPs (Galligan et al., 1988; Pan and Galligan, 1994). Inhibition of synaptic input to motorneurons (S neurons) could account for inhibition of the peristaltic reflex caused by 5-HT. It has also been shown that 5-HT_4 receptor agonists facilitate fast EPSPs (Tonini et al., 1989; Pan and Galligan, 1994) and this effect could contribute to facilitation of the peristaltic reflex. However, distention-evoked reflexes are only partly blocked by nicotinic antagonists and noncholinergic neurotransmission may play a role in peristalsis (Bartho et al., 1987; Holzer, 1989; Waterman et al., 1994). Facilitation of noncholinergic neurotransmission by 5-HT_4 receptor agonists has not been demonstrated.

5-HT mediates some slow EPSPs in the myenteric plexus (Wood and Mayer, 1979; Johnson et al., 1980,1981; Takaki et al., 1985). 5-HT acts at 5-HT_{1P} receptors to cause slow depolarizations which mimic the slow EPSP and antagonists of 5-HT_{1P} receptors block some slow EPSPs (Mawe et al., 1986). Some drugs which act as 5-HT_4 receptor agonists also interact with 5-HT_{1P} receptors. For example, the substituted benzamides, cisapride and renzapride, facilitate peristalsis via an agonist action at 5-HT_4 receptors but cisapride and renzapride block slow depolarizations caused by 5-HT (Nemeth et al., 1985; Mawe et al., 1989). Substituted benzamides block 5-HT_{1P}-mediated responses by acting at a modulatory benzamide binding site on the 5-HT_{1P} receptor (Mawe et al., 1989; Wade et al., 1991). These observations suggest that there are several potential opposing actions of 5-HT receptor agonists and antagonists on slow EPSPs in myenteric AH neurons. Drug action on noncholinergic slow EPSPs in AH neurons is an important issue as AH neurons (putative sensory neurons and interneurons) can communicate with other neurons via slow EPSPs (Kunze et al., 1993). Excitation and enhancement of noncholinergic neurotransmission from sensory neurons or between interneurons are mechanisms by which 5-HT_4 receptor agonists could facilitate peristalsis.

The purpose of the present study was to examine the actions 5-HT_4 receptor agonists on noncholinergic slow EPSPs. We also studied the actions of 5-HT_4 receptor agonists on membrane potential of AH neurons to determine if 5-HT_4 receptors are present on cell bodies and if 5-HT alters the excitability of these cells via an action at 5-HT_4 receptors.

2. Materials and methods

Male guinea pigs (weight 300–450 g, Michigan Department of Public Health) were anesthetized using halothane inhalation and killed by a blow to the head and exsanguination. A small piece (5 mm^2) longitudinal muscle-myenteric plexus was pinned out flat in a silastic elastomer-lined recording chamber and superfused with warmed (36° C), oxygenated (95% O_2, 5% CO_2) Krebs-bicarbonate solution at a flow rate of 3.5–7 ml/min. The composition of the Krebs solution was (millimolar): NaCl, 117; KCl 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2, glucose 11, $NaHCO_3$ 2.5. The Krebs solution contained nifedipine (1 μM) to minimize longitudinal muscle contractions and scopolamine (1 μM) to block muscarinic receptors. A glass electrode (tip diameter 40–60 μm) filled with Krebs solution was used to evoke slow EPSPs. The stimulating electrode was positioned on an interganglionic nerve strand and slow EPSPs were evoked using trains of stimuli (10–60 V 0.5 ms pulse duration, 10–20 Hz for 0.5 s) provided by a Grass S44 stimulator and stimulus isolation unit.

Intracellular recordings were obtained using an amplifier that allowed membrane potential measurement and intracellular current injection through a single electrode (Axoclamp 2A, Axon Instruments, Foster City, CA, USA). The spike afterhyperpolarization in AH neurons was evoked using depolarizing current pulses of sufficient strength to elicit calcium-dependent action potentials in the presence of tetrodotoxin (0.3 μM). The current pulse was passed through the recording microelectrode and the peak amplitude of the slow afterhyperpolarization was measured. Membrane potential changes were displayed on a digital oscilloscope (Gould 400 Series) and recorded on a chart recorder (Gould 2400S).

In most experiments drugs were added in a known concentration to the superfusing Krebs solution. Concentration-responses curves were done using cumulative addition of all agonists except BIMU 8. BIMU 8 concentration-response curves were done noncumulatively with a 10 min interval between successive concentrations. In some experiments, drugs were applied by pressure ejection from a micropipette (10 μm tip diameter) positioned within 200 μm of the impaled

neuron. Substance P, cisapride, renzapride and BIMU 8 were applied in this manner and the pipette contained 0.1 mM of each drug. Pressure pulse (N_2) duration was varied between 50–200 ms and pressure pulses of 5–15 psi supplied by a Picospritzer (General Valve Co., Fairfield, NJ, USA) were used.

5-HT, 5-methoxytryptamine (5-MeOT) and tetrodotoxin were purchased from Sigma Chemical Co., St. Louis, MO, USA. 1-(2-Methoxyphenyl)-4-[4-(2-phthalimidobutyl]piperazine (NAN-190) was purchased from Research Biochemicals, Natick, MA, USA. 4-Amino-5-chloro-2-methoxy-*N*-(4-[1-azabicyclo[3,3,1]-nonyl]) benzamide hydrochloride (renzapride) was a gift from Smithkline Beecham Pharmaceuticals, Betchworth, Surrey, UK. *cis*-4-Amino-5-chloro-*N*[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidiny]-2-methoxybenzamide monohydrate (cisapride) was a gift from Janssen Research Foundation, Beerse, Belgium. Endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-(1-methyl)ethyl-2-oxo-1*H*-benzimidazole-1-carboxamidehydrochloride (BIMU 8) was a gift from Boehringer Ingelheim Italia, Milan Italy. (3α-Tropanyl)-1*H*-indole-3-carboxylic acid ester (tropisetron) was a gift from Sandoz, Basel, Switzerland. 5-Carboxamidotryptamine (5-CT) was a gift from Glaxo Group Research (Greenford, Middlesex, UK).

Data are expressed as the mean ± S.E.M. Inhibition of the slow EPSP is expressed as percent change from pre-drug amplitude. The IC_{50} (agonist concentration causing half-maximal inhibition of slow EPSPs or slow afterhyperpolarization) was determined from average concentration-response curves. Analysis of variance, Newman-Keuls test and Student's *t*-test for paired data were used to establish significant differences among treatment groups.

3. Results

Data were obtained from 100 AH neurons with an initial resting membrane potential of -78 ± 1 mV and input resistance of 150 ± 7 MΩ.

3.1. 5-HT and 5-CT inhibit slow EPSPs

5-HT (0.01–1 μM) produced a concentration-dependent inhibition of slow EPSPs in 20 of 24 neurons tested (Fig. 1A and B); the IC_{50} was 0.02 μM. Previous studies have shown that under control conditions, 5-HT inhibited fast EPSPs recorded from myenteric neurons but when 5-HT_{1A} receptors were blocked, 5-HT facilitated fast EPSPs (Pan and Galligan, 1994). Similar experiments were done here for 5-HT effects on slow EPSPs. The 5-HT_{1A} receptor antagonist, NAN-190 (0.3 μM) (Rydelek-Fitzgerald et al., 1990), caused a rightward shift in the 5-HT concentration-response curve

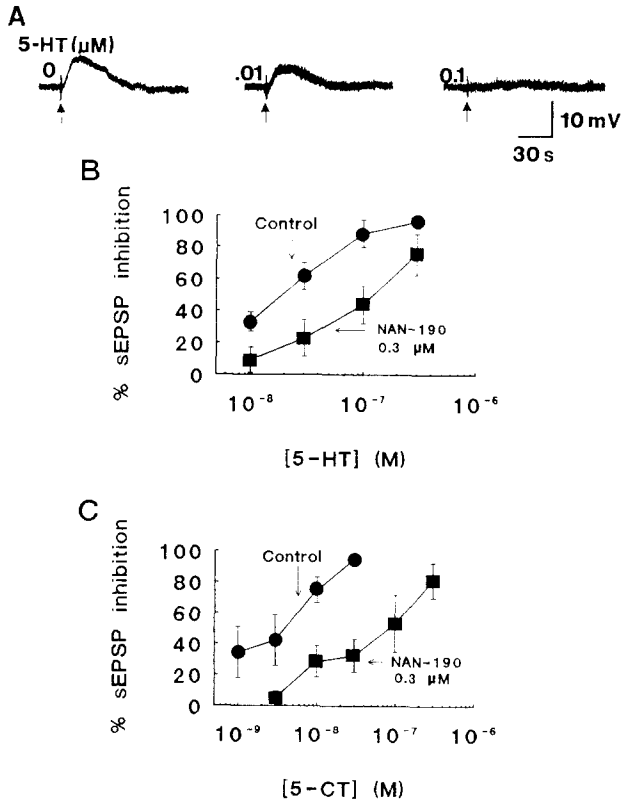

Fig. 1. 5-HT and 5-CT inhibit slow EPSPs. A: Slow EPSP is inhibited by 5-HT at the indicated concentrations. B: Concentration-response curves for inhibition of slow EPSP by 5-HT in the absence and presence of NAN-190. C: Concentration-response curves for inhibition of slow EPSPs by 5-CT in the absence and presence of NAN-190.

(Fig. 1B). In the presence of NAN-190, the 5-HT IC_{50} was 0.12 μM and 5-HT never increased the amplitude of slow EPSPs.

The 5-HT_1 receptor agonist, 5-CT (0.001–0.3 μM) produced a concentration-dependent inhibition of the slow EPSP in 13 of 15 neurons tested; the 5-CT IC_{50} was 0.004 μM (Fig. 1C). NAN-190 (0.3 μM) caused a rightward shift in the 5-CT concentration-response (Fig. 1C).

3.2. 5-HT and 5-MeOT depolarize AH neurons

5-HT (0.01–1 μM) did not change membrane potential in 17 of 24 neurons while in 7 neurons, 5-HT (0.3 μM) caused a membrane depolarization that ranged between 5–20 mV in amplitude and was associated with an increase in membrane input resistance. 5-MeOT (0.1–3 μM) was applied to 9 neurons and caused a membrane depolarization in 6 neurons. The depolarization was associated with an increased input resistance and inhibition of the spike afterhyperpolarization. The 5-MeOT-induced depolarization was concentration-dependent but the amplitude of the depolarization caused by a given concentration varied on successive applications of agonist. Inhibition of the

spike afterhyperpolarization by 5-MeOT was reproducible and was used to study interactions between 5-MeOT and the 5-HT_3/5-HT_4 receptor antagonist, tropisetron. 5-MeOT inhibited the spike afterhyperpolarization in a concentration-dependent manner; the IC_{50} was 0.4 μM. Tropisetron (1 μM) did not change the 5-MeOT concentration-response curve (Fig. 2A and B).

3.3. Effects of BIMU 8, renzapride and cisapride on slow EPSPs

Cisapride, renzapride and BIMU 8 were applied by superfusion to determine if these drugs would alter slow EPSPs or membrane potential in AH neurons. It was found that neither BIMU 8 (0.01–1 μM) ($n = 7$, Fig. 3C) nor renzapride (0.01–0.3 μM) ($n = 16$, Fig. 3A) altered slow EPSP amplitude or the resting membrane potential in any neuron tested. These concentrations of agonist were chosen for study as they had been shown previously to be effective in facilitating fast EPSPs (Pan and Galligan, 1994).

Cisapride (0.01–1 μM) was applied by superfusion to 19 neurons. In 13 neurons cisapride had no effect on slow EPSP amplitude (Fig. 3B) while in 6 neurons, cisapride (1 μM) reversibly inhibited the slow EPSP (Fig. 4A and B). In these same 6 neurons, lower concentrations (0.01 and 0.1 μM) of cisapride did not change slow EPSP amplitude. In order to determine if

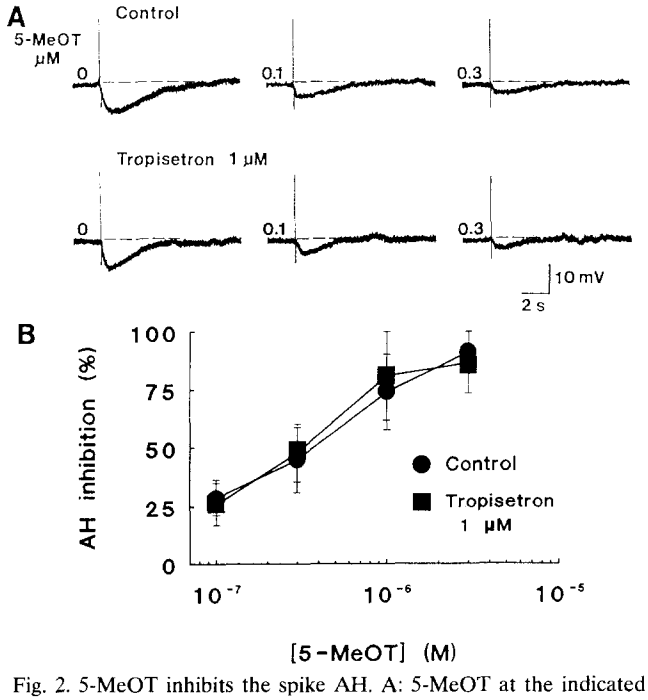

Fig. 2. 5-MeOT inhibits the spike AH. A: 5-MeOT at the indicated concentrations inhibits the spike AH and this effect is not blocked by tropisetron. B: Concentration-response curves from experiments similar to that shown in A showing that 5-MeOT-induced inhibition of the AH is not blocked by tropisetron.

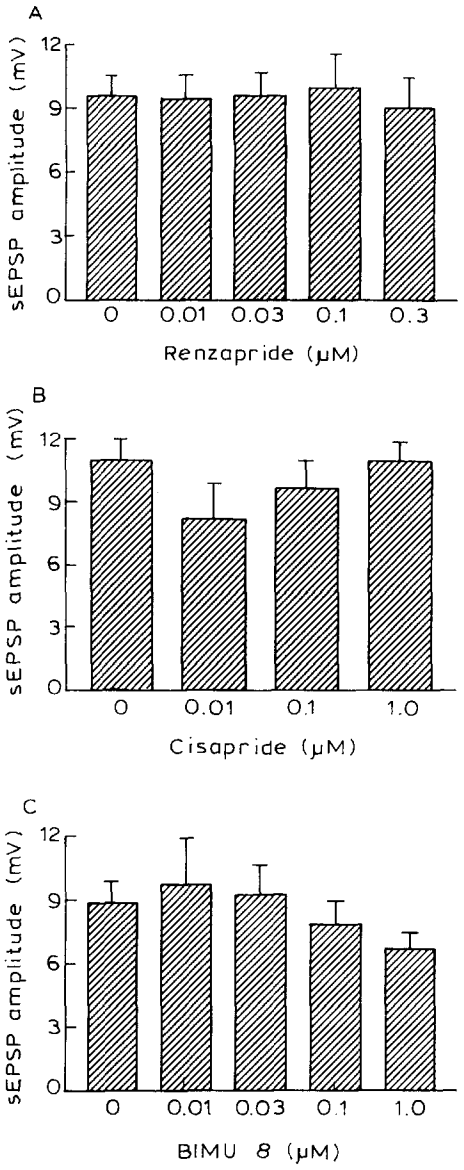

Fig. 3. Amplitude of slow EPSPs is unaffected by the 5-HT_4 receptor agonists renzapride (A), cisapride (B) and BIMU 8 (C).

cisapride acted postsynaptically to disrupt slow EPSP signal transduction, substance P was applied to 6 neurons. Substance P applied by pressure ejection from a micropipette mimicked the slow EPSP but cisapride did not alter the depolarization caused by substance P (Fig. 4B). Cisapride did not change the membrane potential in any neuron.

3.4. Local application of cisapride, renzapride and BIMU 8

The data above indicate that cisapride, renzapride and BIMU 8 applied by superfusion did not change the membrane potential of AH neurons. However, it is possible that when applied by superfusion these agonists cause receptor desensitization (Pan and Galligan, 1994). In order to address this possibility cisapride,

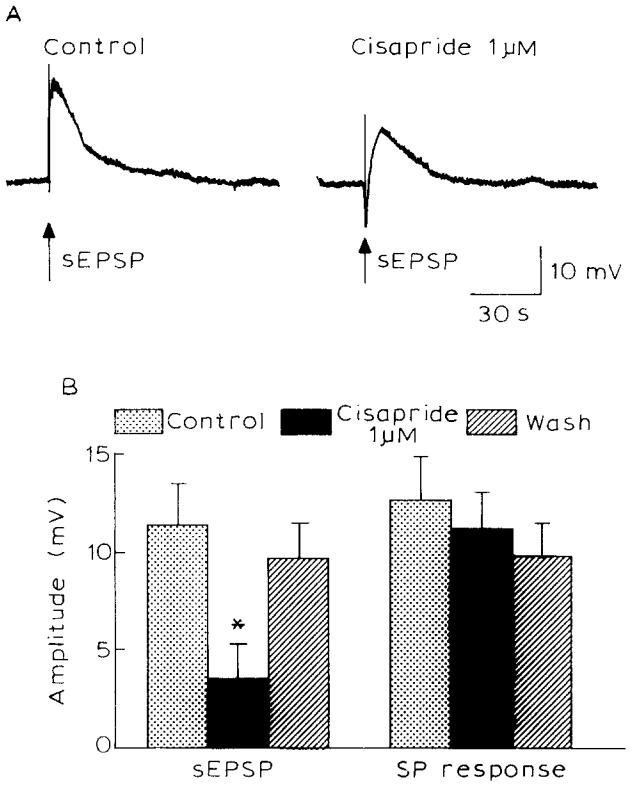

Fig. 4. Slow EPSP inhibited by cisapride in a subpopulation of neurons. A: Slow EPSP in control and in the presence of cisapride. B: Data from experiments similar to A showing that the slow EPSP amplitude is reversibly reduced by cisapride. Responses to substance P (SP) are unaffected by cisapride ($n = 7$).

renzapride and BIMU 8 were applied by pressure ejection from a micropipette (pipette concentration, 0.1 mM) positioned near the impaled neuron. Neither renzapride ($n = 6$), cisapride ($n = 5$) or BIMU 8 ($n = 7$) changed the membrane potential of any neuron tested. However, presynaptic nerve stimulation evoked a slow EPSP in each of these neurons (Fig. 5). 5-HT applied by pressure ejection elicited a 10–15 mV membrane depolarization in 3 neurons tested (Fig. 5D).

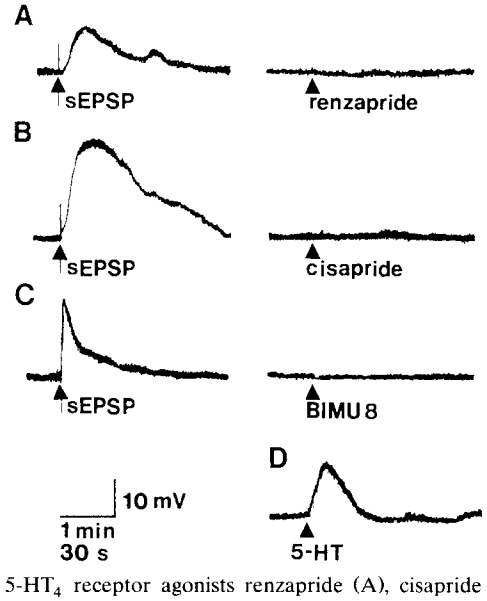

Fig. 5. 5-HT_4 receptor agonists renzapride (A), cisapride (B), and BIMU 8 (C) applied by pressure ejection from a micropipette do not cause membrane depolarizations. A slow EPSP could be evoked in the same cells. D: In some cells, 5-HT applied by pressure ejection caused a slow depolarization similar to the slow EPSP.

4. Discussion

4.1. 5-HT_4 receptor agonists do not facilitate noncholinergic slow EPSPs

Previous work has shown that 5-HT_4 receptor agonists facilitate fast EPSPs in the myenteric plexus and when inhibitory 5-HT_{1A} receptors are blocked, 5-HT causes a concentration-dependent facilitation of fast EPSPs (Pan and Galligan, 1994). 5-HT acting at presynaptic 5-HT_{1A} receptors also inhibits noncholinergic slow EPSPs (Galligan et al., 1988) but when 5-HT_{1A} receptors were blocked using NAN-190, 5-HT did not facilitate slow EPSPs. The 5-HT_1 selective agonist, 5-CT, inhibits slow EPSPs and the 5-CT concentration-response curve was shifted to the right by NAN-190. Renzapride, cisapride and BIMU 8, which can act as 5-HT_4 receptor agonists (Sanger, 1987; Taniyama et al., 1991; Turconi et al., 1991; Bockaert et al., 1992; Rizzi et al., 1992), facilitate fast EPSPs (Pan and Galligan, 1994) but none of these drugs increased slow EPSP amplitude. Taken together, these data indicate that nerve terminals releasing mediators of noncholinergic slow EPSPs express inhibitory 5-HT_{1A} but not facilitatory 5-HT_4 receptors.

At 1 μM, cisapride reversibly inhibited slow EPSPs recorded from some neurons. Cisapride blocks slow depolarizations caused by 5-HT (Nemeth et al., 1985) and 5-HT is a mediator of some slow EPSPs in the myenteric plexus (Wood and Mayer, 1979; Johnson et al., 1980, 1981; Takaki et al., 1985; Mawe et al., 1986). Cisapride acts at a benzamide binding site on the 5-HT_{1P} receptor to block responses mediated at this receptor (Mawe et al., 1986; Wade et al., 1991). As cisapride did not block responses to substance P, also a mediator of some slow EPSPs (see below), it is possible that 5-HT mediated the cisapride-sensitive slow EPSPs. Renzapride can also act at the benzamide binding site to block 5-HT_{1P}-mediated responses (Mawe et al., 1986; Frieling et al., 1991; Tack et al., 1992). However, in the present study the maximum concentration of renzapride used (0.3 μM) was less than that needed (1 μM) to block the benzamide site (Mawe et al., 1986).

4.2. 5-HT_4 receptors are not localized to the cell body of AH neurons

5-MeOT can desensitize 5-HT_4 receptors in nerve muscle preparations (Craig et al., 1990). When applied

by superfusion 5-MeOT caused a slow depolarization identical to the response caused by 5-HT. Although the 5-MeOT-induced depolarization was generally concentration-dependent, the amplitude of the depolarization caused by a given concentration of 5-MeOT was variable on successive applications of agonist. This precluded measurement of 5-MeOT-induced depolarizations in the absence and presence of antagonist as a means of receptor characterization. However, 5-MeOT inhibited the spike afterhyperpolarization in a concentration-dependent and reproducible manner. The spike afterhyperpolarization is due to a K^+ conductance activated by calcium entering the cell during the action potential (Morita et al., 1982). The calcium-activated K^+ channel is active at rest and contributes to the resting membrane potential in AH neurons (Grafe et al., 1980; North and Tokimasa, 1987). The calcium-activated K^+ channel is a target for many treatments, including 5-HT, which depolarize myenteric AH neurons (Grafe et al., 1980; Galligan, 1993). We used 5-MeOT-induced inhibition of the spike afterhyperpolarization to examine interactions between 5-MeOT and the 5-HT_3/5-HT_4 antagonist, tropisetron. Inhibition of the spike afterhyperpolarization caused by 5-MeOT was not affected by a concentration of tropisetron (1 μM) which blocks 5-HT_4 receptors (Bockaert et al., 1992). These data indicate that 5-MeOT is not acting at 5-HT_4 receptors to depolarize AH neurons and that 5-MeOT must be acting as a 5-HT_{1P} receptor agonist. Furthermore, it was found that cisapride, renzapride and BIMU 8 did not cause a depolarization of AH neurons when these drugs were applied by superfusion or pressure ejection from a micropipette. However, it was possible to elicit a slow EPSP in these neurons or a slow depolarization caused by 5-HT.

4.3. Functional implications

There are two issues to consider regarding the actions of 5-HT_4 receptor agonists on myenteric nerves and intestinal motor reflexes: (1) reflex facilitation by 5-HT_4 receptor agonists and the possible role of noncholinergic slow EPSPs in AH neurons, and (2) the neuronal localization of 5-HT_4 receptors.

It is unlikely that facilitation of slow synaptic input to AH neurons contributes to 5-HT_4-mediated facilitation of peristalsis. We could provide no evidence to indicate that 5-HT_4 receptors regulate the release of the mediators of noncholinergic slow EPSPs in the same way that 5-HT_4 receptors regulate release of acetylcholine as a mediator of fast EPSPs (Kilbinger and Wolf, 1992; Pan and Galligan, 1994). One explanation for selective facilitation of fast EPSPs by 5-HT_4 receptor agonists is that the mediators of fast EPSPs and slow EPSPs are released by different nerves and 5-HT_4 receptors are expressed only by those nerves releasing mediators of fast EPSPs. However, immunohistochemical studies of enteric nerves have shown that choline acetyltransferase, a marker for cholinergic nerves, is co-localized with 5-HT or with substance P in different populations of myenteric interneurons (Costa and Furness, 1989; Costa et al., 1992). Both substance P and 5-HT are mediators of enteric slow EPSPs. Therefore, differential localization of 5-HT_4 receptors to cholinergic and noncholinergic interneurons cannot account for the selective effect of 5-HT_4 receptor agonists on fast EPSPs. It is possible that the mechanisms regulating release of acetylcholine, substance P and 5-HT from these nerves are different and 5-HT_4 receptors couple only to release of acetylcholine.

The second issue is localization of 5-HT_4 receptors on myenteric neurons. In adult hippocampal neurons, 5-HT_4 receptors couple to adenylate cyclase and increase in intracellular cAMP (Bockaert et al., 1990). When intracellular electrophysiological methods are used to study the actions of 5-HT_4 receptor agonists and antagonists on hippocampal neurons it was found that 5-HT_4 receptors mediate inhibition of the spike afterhyperpolarization caused by 5-HT (Chaput et al., 1990; Torres et al., 1994). These data indicate that 5-HT_4 receptors are present on the cell body of hippocampal neurons. In gastrointestinal smooth muscle, 5-HT_4 receptors also couple to adenylate cyclase and increase in cyclic 3′,5′-adenosine monophosphate which causes smooth muscle relaxation (Ford et al., 1992) indicating that stimulation of adenylate cyclase is a general mechanism for signal transduction by 5-HT_4 receptors. In myenteric AH neurons, treatments which increase intracellular cyclic 3′,5′-adenosine monophosphate cause depolarizations and inhibition of the spike afterhyperpolarization (Nemeth et al., 1986; Palmer et al., 1986). Based on data presented here 5-HT_4 receptors coupled to adenylate cyclase are not present on cell bodies of myenteric AH neurons. If this were the case cisapride, renzapride and BIMU 8 would cause depolarizations and increases in excitability. Previous work showed that membrane potential and input resistance in myenteric S neurons were not changed by 5-HT_4 receptor agonists (Pan and Galligan, 1994). Taken together these data indicate that 5-HT_4 receptors are either not localized to the cell bodies of myenteric neurons or do not couple to intracellular pathways which regulate membrane potential. In the myenteric plexus, 5-HT_4 receptors may be localized only to nerve terminals releasing mediators of the fast EPSP.

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