

SLOW EXCITATORY POST-SYNAPTIC POTENTIALS IN MYENTERIC AH NEURONS OF THE GUINEA-PIG ILEUM ARE REDUCED BY THE 5-HYDROXYTRYPTAMINE₇ RECEPTOR ANTAGONIST SB 269970

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Abstract—Serotonin (5-HT) is a key modulator of neuronal excitability in the central and peripheral nervous system. In the enteric nervous system, 5-HT causes a slow depolarization in the intrinsic sensory neurons, but the receptor responsible for this has not been correlated with known gene products. The aim of this study was to determine whether the newly characterized 5-HT₇ receptor may participate in the 5-HT-mediated depolarization of, and synaptic transmission to, the intrinsic sensory neurons of the guinea-pig ileum.

Intracellular electrophysiological recordings were made from intrinsic sensory neurons identified as myenteric AH neurons from guinea-pig ileum. 5-HT (5 μ M) applied to the cell body evoked both a fast depolarization (5-HT₃ mediated) and/or a slow depolarization (5-HT_{1P}-like). The 5-HT_{15/7} receptor agonist 5-carboxamidotryptamine (5-CT) (5 μ M) evoked only a slow depolarization. When the fast depolarization evoked by 5-HT was blocked with granisetron (1 μ M, 5-HT₃ receptor antagonist), only a slow depolarization remained; this was abolished by the 5-HT₇ receptor antagonist SB 269970 (1 μ M, control: 14 \pm 2 mV, granisetron+SB 269970: -1 \pm 2 mV). The slow depolarization evoked by 5-CT was also significantly reduced by SB 269970 (control: 14 \pm 1 mV, SB 269970: 5 \pm 2 mV) suggesting a 5-HT₇ receptor was activated by exogenous application of 5-CT and 5-HT. Slow excitatory postsynaptic potentials evoked by stimulating descending neural pathways (containing serotonergic fibers) were reduced by SB 269970 (control: 8 \pm 3 mV, SB 269970: 3 \pm 1 mV). However, SB 269970 had no effect on slow excitatory postsynaptic potentials evoked by stimulation of circumferential (tachykinergic) pathways (control: 7 \pm 1 mV, SB 269970: 6 \pm 1 mV).

These data are consistent with the presence on enteric AH neurons of functional 5-HT₇ receptors that participate in slow synaptic transmission. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: electrophysiology, enteric nervous system, synaptic transmission.

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Abbreviations: AH neuron, an enteric neuron with an afterhyperpolarizing potential following an action potential; AHP, afterhyperpolarizing potential; AP, action potential; ENS, enteric nervous system; EPSP, excitatory post-synaptic potential; RMP, resting membrane potential; TTX, tetrodotoxin; 5-CT, 5-carboxamidotryptamine; 5-HT, 5-hydroxytryptamine or serotonin.

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Serotonin (5-HT) is a key modulator of neuronal excitability in the CNS and influences a broad range of behaviors and disease states. Serotonergic projections from the raphé nuclei act on a number of 5-HT receptors located in most brain areas. Recently, the 5-HT₇ receptor has been established as a functional receptor in the CNS (for review see: Hoyer et al., 2002). It causes increases in the excitability of hippocampal neurons (Bacon and Beck, 2000), directs prefrontal cortex development (Beique et al., 2004) and plays a role in thermoregulation (Hedlund et al., 2003).

Neurons of the enteric nervous system (ENS), located in the wall of the intestine, are also strongly modulated by 5-HT (for review see: Galligan, 1996; Gershon, 2004). 5-HT is released from enterochromaffin cells lining the lumen (Bülbring and Lin, 1958) as well as from a class of descending (anally projecting) interneurons in the myenteric plexus (Erde et al., 1985; Takaki et al., 1985; Young and Furness, 1995). The major class of intestinal intrinsic sensory neuron is multipolar with Dogiel type II morphology and AH type electrophysiological characteristics (Furness et al., 1998). They are strongly modulated by exogenous application of 5-HT. Most are depolarized by a 5-HT₃ receptor and/or by a G-protein-coupled receptor termed the 5-HT_{1P} receptor (Mawe et al., 1986; Wang et al., 1996), while about half are also hyperpolarized by a 5-HT_{1A} receptor (Galligan and North, 1991). The role of the 5-HT₇ receptor in regulating enteric neuronal excitability has not been examined. However, it has been localized to intestinal tissue (Hemedah et al., 1999), where its activation depresses peristalsis (Tuladhar et al., 2003), possibly by direct inhibition of the smooth muscle (Carter et al., 1995). In addition, recent findings suggest that 5-HT₇ receptor immunoreactivity is present in cultured myenteric neurons (Cervio et al., 2005).

The aim of this study was to determine if the properties of the 5-HT-mediated slow depolarization in myenteric AH neurons is consistent with mediation by a 5-HT₇ receptor. Further, we wished to determine whether these receptors play a role in synaptic transmission in the ENS.

EXPERIMENTAL PROCEDURES

Tissue preparation

Guinea-pigs of either sex weighing 180–350 g were stunned and killed by severing the carotid arteries and spinal cord in accordance with guidelines of the University of Melbourne Animal Experimentation Ethics Committee. All efforts were made to minimize the number of animals used and their suffering. A 3 cm segment of ileum was removed 10 cm from the ileocaecal junction and placed into physio-

logical salt solution (in mM: NaCl 118, NaHCO₃ 25, D-glucose 11, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, NaH₂PO₄ 1.0) containing nicardipine (1.25 μM) and hyoscine (1 μM) to minimize muscle movement and bubbled with 95% O₂, 5% CO₂. The segment was cut along the mesenteric border, pinned flat in a Petri dish, and the mucosa, submucosa and circular muscle were stripped away. A section of myenteric plexus with attached longitudinal muscle (1 cm by 0.6 cm) was removed and pinned in an organ bath (volume 0.5 ml; Fig. 1A). Oxygenated physiological salt solution was pre-warmed to 36 °C and superfused at a rate of 2–4 ml/min.

Electrophysiology

Myenteric ganglia were visualized at 300× using an inverted microscope (IX-70, Olympus, Tokyo, Japan) with Nomarski differential interference contrast optics. Neurons were impaled with glass microelectrodes containing 1 M KCl and 2% biocytin (*w/v*; tip resistance 100–200 MΩ) and characterized electrophysiologically as either S or AH type (Hirst et al., 1974; Bornstein et al., 1994; Schutte et al., 1995). Voltage recordings were made using an Axoclamp 2A (bridge mode) amplifier, digitized at 5–20 kHz (Digidata 1200B) and recorded on a personal computer using Axoscope 9 (all from Axon Instruments, Foster City, CA, USA). Once each experiment was complete, the cells were filled with biocytin (current pulses, 500 ms every 2 s, ±50 pA) for 5 min, with a period of 15 min for transportation of the dye before the preparation was placed into fixative. The position of the neuron within the preparation was noted for later identification following immunohistochemical processing (described in detail in Monro et al., 2004).

Slow synaptic potentials

The mucosa, submucosa, and circular muscle were left intact on the oral half of the preparation for these experiments and recordings were made from the myenteric plexus exposed on the anal half. Silver wires (250 μm diameter), running circumferentially, were placed above and below the preparation ~1 cm oral to impaled neurons. These were connected to an ISO-flex stimulus isolation unit (AMPI, Jerusalem, Israel) and 0.6 ms, 5 mA pulses were applied (10 Hz for 3 s) to stimulate descending pathways. A bipolar, stainless steel electrode (insulated, 114 μm diameter; MedWire, Mt. Vernon, VT, USA) was used to stimulate fiber tracts circumferential to impaled neurons. The electrode was 1–2 mm away from the impaled neuron and was connected to a stimulus isolation unit (1 mA, 20 Hz for 1 s). These stimulus parameters were used as previous studies have shown they yield robust and reproducible slow excitatory post-synaptic potentials (EPSPs) (e.g. Mawe et al., 1989; Bertrand and Galligan, 1995).

Drug solutions and application

The receptor agonists 5-HT (from Sigma-Aldrich Fine Chemicals, Sydney, Australia), 5-CT (5-carboxamidotryptamine; Tocris, Australian Laboratory Services, NSW, Australia; Adham et al., 1998) and senktide (Auspep, Melbourne, Australia; Hanani et al., 1988) were prepared as stock solutions in distilled water and diluted in HEPES (10 mM)-buffered saline (120 mM NaCl, pH 7.2) on the day of use.

They were applied to the cell body by pressure ejection (15 p.s.i., 50–150 ms) from a micropipette (tip diameter approximately 10 μm) positioned near the cell body (Picospritzer III, Parker Instrumentation, Hadland Photonics Pty Ltd, VIC, Australia). Agonists were applied at 2–3 min intervals to avoid rundown of the responses.

Receptor antagonists were prepared as stock solutions (1000-fold concentration) in distilled water and kept at 4 °C. They included SB 269970 (Tocris; Hagan et al., 2000), granisetron and SB 204070 (the kind gifts of SmithKline Beecham, Middlessex, UK; Wardle et al., 1994), SR 142801 (a kind gift from Dr. Emonds-Alt, Sanofi-Recherche, Montpellier, France; Emonds-Alt et al.,

1995; Patacchini et al., 1995) and the voltage-gated sodium channel blocker, tetrodotoxin (TTX; Alomone Laboratories, Jerusalem, Israel). Drugs were diluted to the final concentration in physiological salt solution before addition to the organ bath.

Measurements and statistics

Data were analyzed using Axoscope 9. Receptor agonist responses were measured as amplitude, number of APs, and duration (from onset to >80% return to baseline). Slow EPSP amplitude, time-to-peak and duration were measured. Where agonist responses or slow EPSPs had APs and AHPs, the amplitude was measured as the maximum depolarization detected immediately prior to the AP takeoff. This provided a conservative estimate of amplitude and, where a decrease in amplitude of a depolarization or slow EPSP was examined, would cause an underestimate of the magnitude of this depression. The average of two to three repetitions was obtained and used for comparisons between neurons; one '*n*' refers to the average responses from a single neuron. In all, 90 neurons were recorded from 60 preparations (one preparation per animal). Unless otherwise stated, numbers given are mean ± S.E.M. Student's *t*-test was used to compare data for significant differences with a *P* value of <0.05 taken as the cutoff for significance. All *t*-tests were one-tailed and paired unless otherwise noted.

RESULTS

Intracellular recordings were made from 67 myenteric AH neurons and 23 myenteric S neurons. AH neurons (Fig. 1B) were included in the study if they had a long lasting AHP following one or more APs, or if the APs had an inflection on the falling phase, while S neurons (Fig. 1C) were included if they responded to electrical stimulation of a fiber tract with a large amplitude fast EPSP (Bornstein et al., 1994). Of those that were filled with biocytin, all AH type were later found to have Dogiel type II morphology, while S type had a variety of uniaxonal morphologies. The resting membrane potentials (RMP) were: AH neurons 66 ± 1 mV, S neurons 59 ± 2 mV.

5-HT evokes fast and/or slow depolarizations in AH neurons

Exogenous 5-HT (5 μM in the spritz pipette) was applied by pressure ejection to the cell body of 53 myenteric AH neurons. Ten responded with a fast depolarization alone, nine with a slow depolarization alone (Fig. 2A), and 34 responded with both (Fig. 3A). The fast depolarization had an average amplitude of 24 ± 5 mV evoking 10 ± 6 APs (*n*=6). The slow depolarization had a duration of 34 ± 4 s, an average amplitude of 18 ± 3 mV and evoked 6 ± 5 APs (*n*=8) and caused a reduction in the size of the AHP (not quantified). Hyperpolarizations were also seen in about half the neurons, either without a slow depolarization, between the fast and the slow depolarization or once the depolarizations were inhibited.

To test whether the slow depolarization evoked by 5-HT was secondary to the activation of neighboring neurons, TTX was used to block sodium channel dependent neural conduction. TTX (1 μM) did not reduce the amplitude (control: 17 ± 7 mV, TTX: 13 ± 5 mV, *P*>0.05, two-tailed test, *n*=4) or the duration (control: 30 ± 3 s, TTX: 34 ± 5 s, *P*>0.05, two-tailed test, *n*=4) of the 5-HT-medi-

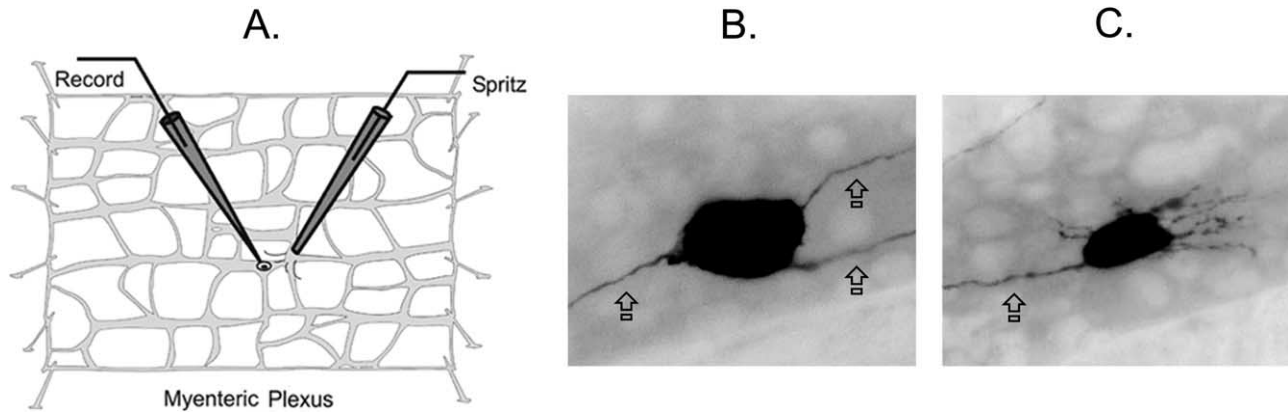


Fig. 1. Schematic of the experimental arrangement, and types of neurons recorded from. (A) The preparation consists of the myenteric plexus attached longitudinal muscle that had been dissected away from the mucosa, submucosa and circular muscle. It was then pinned out mucosal side up in the organ bath. AH and S type neurons were impaled (left) and characterized using electrical stimulation (not shown). Receptor agonists were applied to the cell body by pressure ejection (spritz, right). (B) Example of an AH-type neuron that has been filled with the intracellular marker biocytin (present in the recording electrode) and processed immunohistochemically to reveal its morphology. AH neurons are typically characterized as being multipolar. (C) S-type neuron that has been filled with biocytin and processed immunohistochemically. S neurons are characterized as being uniaxonal. The arrows point to axons, the field of view=65 μm wide.

ated slow depolarization indicating the depolarization was due to a direct action of 5-HT on the impaled AH neuron.

5-CT evokes a slow depolarization that is inhibited by the selective 5-HT₇ receptor antagonist SB 269970

5-CT has been used as a tool to help define 5-HT₇ receptors (Lucchelli et al., 2000). It is a 5-HT_{1/5/7} receptor agonist. 5-CT (5 μM in the spritz pipette) evoked only a slow depolarization when applied to the cell bodies of nine of 12 AH neurons tested (Fig. 2B). 5-HT, at the same concentration, was applied to seven of these AH neurons and evoked a larger depolarization (5-HT: 21 ± 3 mV, 5-CT: 9 ± 2 mV, $P < 0.05$; duration 5-HT: 31 ± 3 s, 5-CT: 35 ± 4 s, $P > 0.05$; Fig. 2). The times to peak amplitude were also different (5-HT: 5 ± 1 s, 5-CT: 11 ± 2 s, $P < 0.05$, two-tailed test). The selective 5-HT₇ receptor antagonist SB 269970 (1 μM) reduced the 5-CT evoked depolarization from 14 ± 1 mV to 5 ± 2 mV (36% of control, $P < 0.05$; Fig. 3B) and this recovered to 18 ± 1 mV upon washout ($n=3$) of the antagonist.

The 5-HT evoked slow depolarization is reduced by SB 269970

In eight of eight experiments, the 5-HT₇ receptor antagonist SB 269970 (1 μM) reduced the 5-HT-evoked slow depolarization in AH neurons from 19 ± 3 mV to 10 ± 2 mV (51% of control, 2 ± 2 APs, duration of 28 ± 3 s, $P < 0.05$) (Fig. 3A). The fast depolarization recorded in the same neurons (Fig. 3A inset) was not affected by SB 269970 (control: 24 ± 5 mV, SB 269970: 29 ± 8 mV, $P > 0.05$). Conversely, the 5-HT-evoked fast depolarization was abolished in five neurons tested with the 5-HT₃ receptor antagonist granisetron (1 μM ; control: 13 ± 3 mV, granisetron: 0 ± 0 mV, $P < 0.05$). In these same neurons, granisetron also produced a small, but non-significant, reduction of the 5-HT-evoked slow depolarization (control: 12 ± 2 mV, granisetron: 9 ± 3 mV, $P > 0.05$).

To address whether the residual slow depolarization was due to an incomplete blockade of the 5-HT₇ receptor, a higher concentration of SB 269970 (2 μM) was tested, but it did not have any greater effect (control: 20 ± 2 mV, SB 269970: 12 ± 3 mV, $n=4$). Similarly, a higher concentration of 5-HT (1 mM in the spritz pipette) applied to the cell body did not evoke a larger depolarization, yet the response was still reduced to about half by SB 269970 (1 μM ; $n=5$).

To test whether the residual response was due in some way to the unblocked fast depolarization, the 5-HT₃ receptor antagonist granisetron was used. In five of five experiments, granisetron (1 μM) blocked the fast depolarization leaving only a 5-HT-evoked slow depolarization in AH neurons. With granisetron present, SB 269970 (1 μM) abolished the slow depolarization (control: 14 ± 2 mV, granisetron+SB 269970: -1 ± 2 mV, $P < 0.05$; Fig. 4). In three AH neurons, the slow depolarization was reduced to less than 2 mV and it was reversed to a hyperpolarization of -5 mV in the two remaining neurons. This hyperpolarization may have been mediated by 5-HT_{1A} receptors as described by Galligan and North (1991).

To ensure that SB 269970 was acting only on 5-HT receptors and not non-specifically interfering with the intracellular pathways that produce the slow depolarization, the tachykinin NK₃ (neurokinin) receptor agonist senktide was used. Both tachykinin- (Guard and Watson, 1987; Baidan et al., 1992) and 5-HT- (Florica-Howells et al., 1993; Pan et al., 1997) mediated slow depolarizations depend on similar second messenger systems, PKC and PKA. If SB 269970 was having a non-specific intracellular effect, instead of binding to the 5-HT₇ receptor, it would be expected that a depolarization evoked by senktide would also be attenuated by SB 269970. Senktide (3 μM in the spritz pipette) was pressure applied to the cell bodies of five AH neurons and caused a slow depolarization (Fig. 5B). SB 269970 (1 μM) had no effect on the amplitude, duration or number of APs evoked by senktide. In the same AH neurons, the slow depolarization to 5-HT was

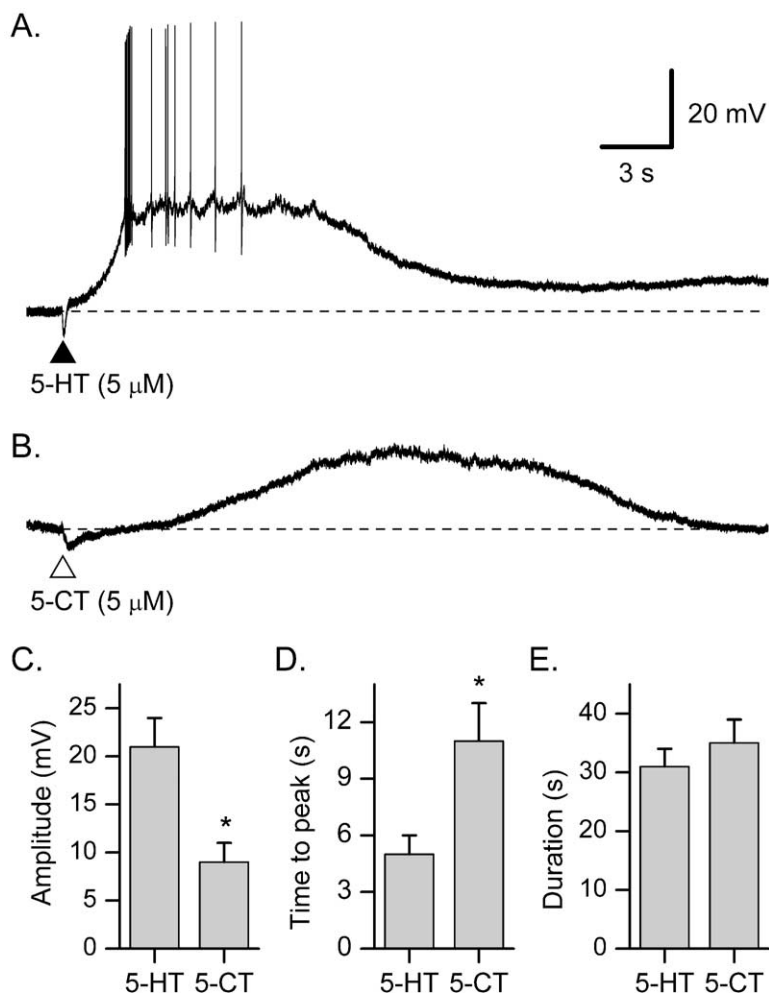


Fig. 2. Slow depolarizations evoked by 5-HT and 5-CT. Voltage traces taken from a single AH neuron. (A) 5-HT (5 μ M) applied to the cell body (at the triangle, 50 ms, 15 p.s.i.) evoked a slow depolarization that evoked many APs (RMP = -86 mV). (B) 5-CT (5 μ M) applied to the cell body (at the open triangle, 150 ms, 15 p.s.i.) evoked a slow depolarization that was smaller than that evoked by 5-HT (RMP = -82 mV). (C–E) Histograms comparing the amplitudes (C) time-to-peak amplitude (D) and durations (E) of slow depolarizations evoked by 5-HT and 5-CT in seven neurons ($*P < 0.05$).

blocked by SB 269970 (Fig. 5A). Conversely, the selective NK₃ receptor antagonist SR 142801 (1 μ M) blocked the effects of senktide, but had no effect on the 5-HT-evoked slow depolarization.

Stimulation of circumferential or descending fiber tracts evokes slow EPSPs

In the myenteric plexus, the 5-HT-containing neurons are in descending (anally projecting) pathways (Furness and Costa, 1982). Stimulation of circumferential pathways primarily evokes slow EPSPs through release of tachykinin (Alex et al., 2002; Johnson and Bornstein, 2004). Therefore, we predicted that selective stimulation of the descending pathway would evoke primarily a serotonergic slow EPSP, while slow EPSPs evoked by stimulation of circumferentially directed nerve trunks would be insensitive to blockade of 5-HT receptors.

Trains of electrical stimuli were applied at the orally positioned stimulating electrodes (30 pulses, 10 Hz) and

evoked slow EPSPs of 7 ± 1 mV (at RMP of -70 ± 3 mV) in 12 of 14 AH neurons tested (Fig. 6A). The time to peak was 34 ± 7 s and the duration was 62 ± 14 s. Trains of stimuli applied via the circumferentially positioned electrode (10 pulses, 20 Hz) evoked slow EPSPs in all 14 of these same AH neurons (Fig. 6B). The amplitude was similar at 9 ± 2 mV (at RMP of -71 ± 2 mV), but the time to peak (25 ± 6 s) and duration (47 ± 7 s) were both significantly shorter ($P < 0.05$, $n = 12$; Fig. 6C–E). Both slow EPSPs were able to evoke AP discharge (e.g. Fig. 7) and caused a reduction in the size of the AHP (not quantified).

SB 269970 (1 μ M) was tested on the descending and circumferential slow EPSPs. SB 269970 did not affect the input resistances or RMPs of six neurons tested. Slow EPSPs evoked by stimulation of descending fibers were reduced in nine neurons tested to 43% of control (control: 8 ± 3 mV, SB 269970: 3 ± 1 mV, $P < 0.05$; Fig. 7A) and the firing of APs was largely prevented. In contrast, in eight of the same neurons and in one additional neuron, the slow

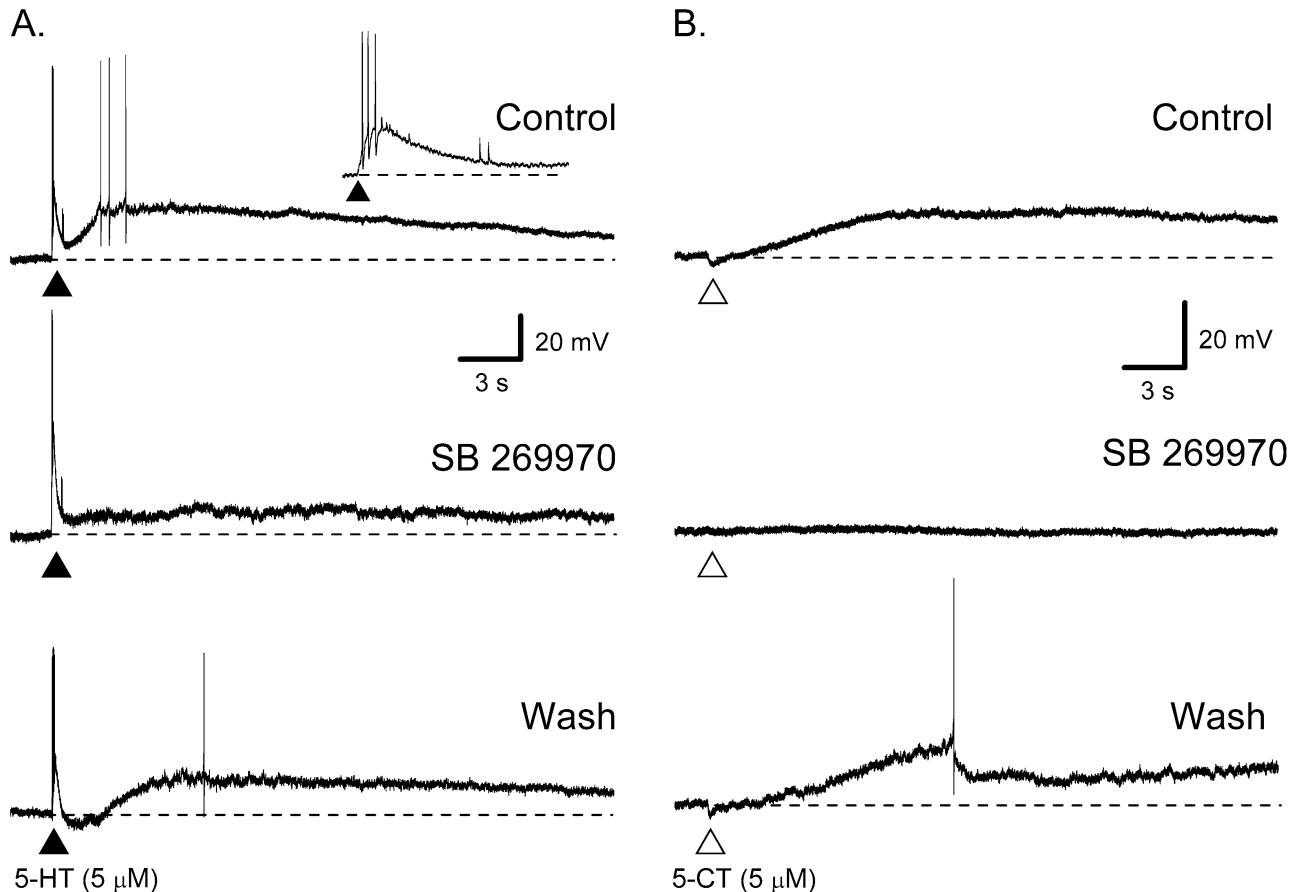


Fig. 3. Effect of 5-HT₇ receptor blockade on the slow depolarization evoked by 5-HT and 5-CT in AH neurons. Voltage traces taken from two AH neurons (A, B). (A) 5-HT (5 μM) was applied to the cell body (at the triangle, 50 ms, 15 p.s.i.) and evoked both a fast and slow depolarization that evoked multiple APs (upper trace, RMP = -58 mV). Inset, an enlargement of the fast depolarization with APs (1 s trace). Middle trace, addition of SB 269970 (1 μM) to the bath significantly reduced the slow depolarization while leaving the fast depolarization intact (RMP = -57 mV, $P < 0.05$); this effect washed out (lower trace, RMP = -59 mV). (B) 5-CT (5 μM) was applied to the cell body (at the open triangle, 150 ms, 15 p.s.i.) and only evoked a slow depolarization (upper trace, RMP = -84 mV). Middle trace, addition of SB 269970 (1 μM) to the bath blocked the slow depolarization (RMP = -86 mV, $P < 0.05$). Lower trace, recovery upon washout (RMP = -85 mV).

EPSP evoked by circumferential stimulation was not affected by SB 269970 (control: 7 ± 1 mV, SB 269970: 6 ± 1 mV, $P > 0.05$; Fig. 7B).

SB 269970 does not inhibit the slow depolarization in S neurons

Several classes of interneuron and motor neuron (electrophysiologically classed as S neurons) have been reported to receive 5-HT containing terminals (Young and Furness, 1995). Stimulation at the orally positioned electrodes (30 pulses, 10 Hz) evoked a slow EPSP of 8 ± 2 mV (at RMP of -51 ± 3 mV) in seven of eight S neurons tested. The time to peak was 9 ± 1 s and the duration was 24 ± 4 s. SB 269970 (1 μM) was tested on three of these descending slow EPSPs and did not reduce their amplitudes (control: 8 ± 3 mV, SB 269970: 12 ± 2 mV, $P > 0.05$, $n = 3$).

It is possible that the S neurons tested above either did not receive serotonergic input or were not sensitive to 5-HT. We used exogenous application of 5-HT in an attempt to reveal any 5-HT₇-like receptor activity in S neu-

rons. 5-HT (5 μM in the spritz pipette) evoked a slow depolarization when applied to the cell bodies of 13 of 15 S neurons tested. Application of TTX (300 nM) to the bath did not effect the slow depolarization (control: 9 ± 2 mV, TTX: 8 ± 2 mV, $P > 0.05$, $n = 4$). SB 269970 (1 μM) had no effect on the amplitude of the slow depolarization (control: 11 ± 1 mV, SB 269970: 10 ± 1 mV, $P > 0.05$, $n = 6$). In contrast, when the selective 5-HT₄ receptor antagonist SB 204070 (1 μM) was tested, it inhibited the slow depolarization (control: 8 ± 1 mV, SB 204070: 2 ± 1 mV, $P > 0.05$, $n = 4$; Fig. 8).

DISCUSSION

The main finding of this study is that, in the myenteric AH neurons of the guinea-pig ileum, 5-HT activates a receptor with many of the properties of a 5-HT₇ receptor. These receptors were activated by exogenous application of 5-HT and 5-CT, which caused slow depolarizing potentials associated with the generation of APs. Electrical stimulation of descending fiber tracts caused endogenous release of

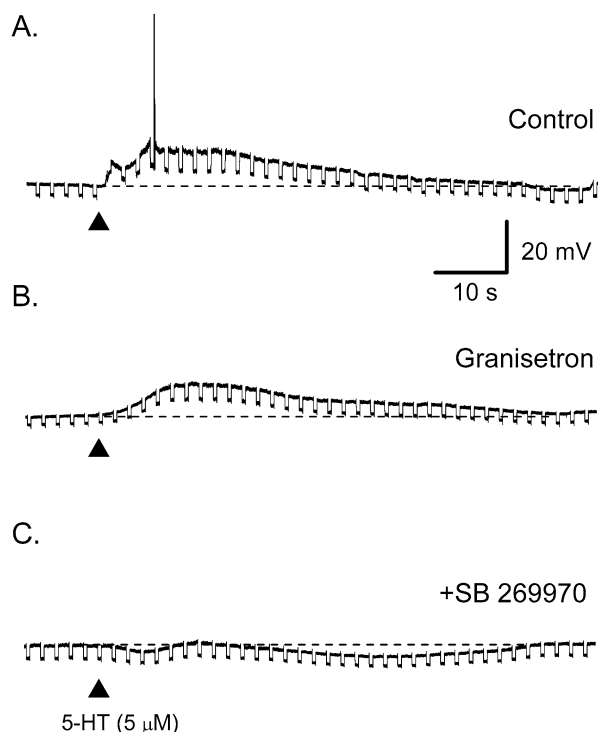


Fig. 4. Effect of combined 5-HT₃ and 5-HT₇ receptor blockade on depolarizations evoked by 5-HT. Voltage traces from a single AH neuron. 5-HT (5 μM) was applied to the cell body (at the filled triangle, 50 ms, 10 p.s.i.) and evoked both a fast and slow depolarization (*upper trace*, RMP = -67 mV). *Middle trace*, addition of granisetron (1 μM) to the bath blocked the fast depolarization while leaving the slow depolarization intact (RMP = -70 mV). *Lower trace*, a combination of granisetron and SB 269970 (1 μM) added to the bath blocked the slow depolarization and revealed what is likely to be a small 5-HT_{1A}-mediated hyperpolarization (RMP = -71 mV). Voltage deflections in all traces are due to hyperpolarizing current pulses passed through the recording electrode to monitor input resistance. The increase in pulse size during the depolarizations indicates there has been an increase in resistance (i.e. a closure of membrane channels) from 80 to 160 MOhm (A), from 60 to 120 MOhm (B) or no significant change (C).

5-HT and generation of a slow synaptic potential. The slow depolarization in response to 5-HT, 5-CT and to stimulation of descending fiber tracts was inhibited or attenuated by the selective 5-HT₇ receptor antagonist SB 269970. Slow depolarizations evoked by tachykinins and slow EPSPs elicited from circumferential pathways containing tachykinergic fibers were not blocked by SB 269970. This suggests that there are functional 5-HT₇ receptors on the AH neurons that can respond to serotonergic input and, importantly, that the AH neurons receive pharmacologically distinct inputs from circumferential and descending pathways.

5-HT₇ receptors are expressed in intestinal tissues

Since the 5-HT₇ receptor was first cloned much work has gone into defining its localization and function. Four isoforms (5-HT_{7A-D}) have been described (Heidmann et al., 1997). In the CNS it has been found in thalamic, hippocampal, cortical, hypothalamic, basal ganglia, amygdala, midbrain and hindbrain regions (see Vanhoenacker et al.,

2000). Several lines of evidence suggest 5-HT₇ receptors are present on intestinal tissues. Binding studies show that 5-HT₇ receptors are expressed in the guinea-pig ileum (Hemedah et al., 1999). They can cause relaxation of pre-contracted smooth muscle (Carter et al., 1995; Lucchelli et al., 2000) or a contraction in the rat jejunum (McLean and Coupar, 1996). Because the effects of agonists are not changed during blockade of neuronal conduction, the 5-HT₇ receptor identified in these studies is thought to be located on the smooth muscle. However, these studies did not localize the receptor and, thus, it may be expressed on both muscles and neurons. This idea is consistent with a recent finding that 5-HT₇ receptor immunoreactivity is present in cultured myenteric neurons (Cervio et al., 2005).

Enteric neurons express receptors with the properties of 5-HT₇ receptors

In the present study, we found evidence for functional 5-HT₇ receptors expressed on the AH neurons in the myenteric plexus. When 5-HT was applied to the cell bodies it caused a fast depolarization and/or a slow depolarization. The slow depolarization was reduced by the selective 5-HT₇ receptor antagonist SB 269970 while the fast depolarization was unaffected. When the 5-HT₃ receptor antagonist granisetron was first used to block the fast depolarization (and thus remove this confounding influence) the slow depolarization was abolished by SB 269970 and a small hyperpolarization was revealed in some cases. The nature of the influence that the 5-HT₃ receptor has is unclear. One idea is that, like the nicotinic receptor (Schneider and Galligan, 2000), presynaptic 5-HT₃ receptors can mediate the release of tachykinin resulting in a slow depolarization of the post-synaptic neuron. The hyperpolarization is presumably due to the activity of a 5-HT_{1A} receptor (Galligan and North, 1991). SB 269970 is typically used to block 5-HT₇ receptors, but also has a lesser affinity for 5-HT₁ (Hoyer et al., 2002) and 5-HT₅ receptors. At concentrations used in this study, it would not be expected to affect 5-HT₁ receptors and would be marginally effective at 5-HT₅ receptors. The presence of a 5-HT_{1A}-mediated hyperpolarization supports the idea that these receptors, at least, were not affected. Further pharmacological studies utilizing a larger range of SB 269970 concentrations are needed to rule out the participation of these other receptors and show definitively that a 5-HT₇ receptor is involved.

The agonist 5-CT has been used extensively in the CNS and ENS to localize 5-HT₇ receptors (e.g. Lucchelli et al., 2000). 5-CT has an affinity for 5-HT₁ and 5-HT₅ receptors in addition to 5-HT₇ receptors (Galligan, 1996; Hoyer et al., 2002; Nelson, 2004). In this study 5-CT caused a slow depolarization similar to that produced by 5-HT though of a lower amplitude and slower onset. These differences could have been due, as noted above, to a small 5-HT₃-mediated depolarization contributing to the 5-HT response. The 5-CT-mediated depolarization was largely inhibited by SB 269970, consistent with the involvement of a 5-HT₇ receptor.

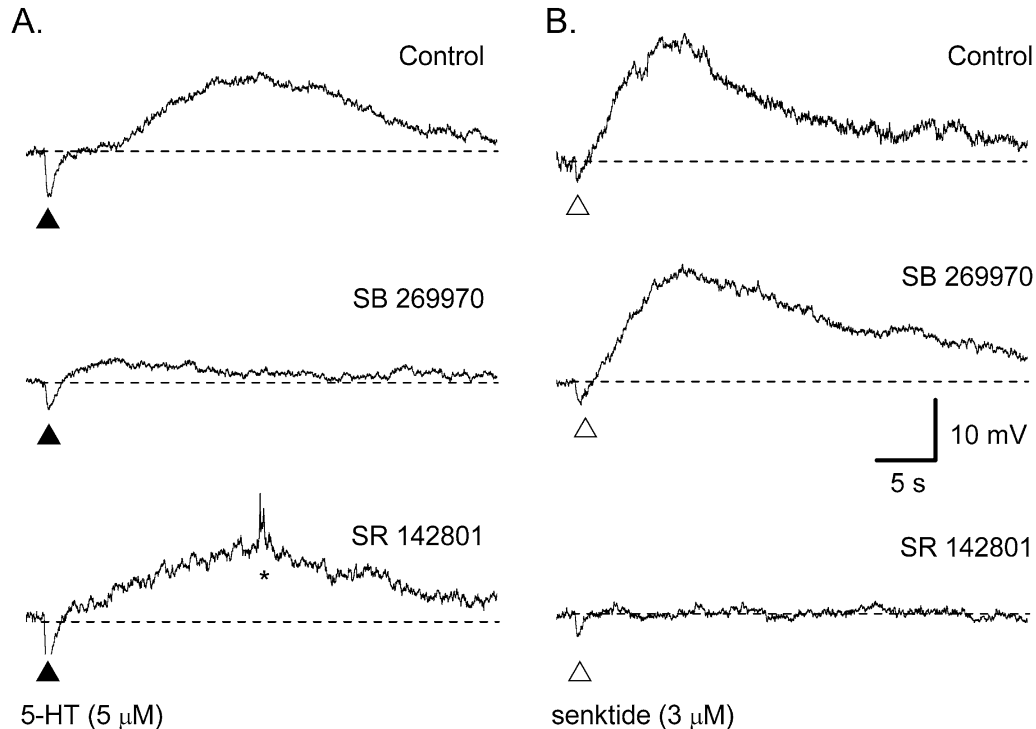


Fig. 5. Effect of SB 269970 on the NK3-mediated slow depolarization. Voltage traces from a single AH neuron. (A) 5-HT (5 μ M) was applied to the cell body (at the filled triangle, 50 ms, 10 p.s.i.) and evoked a slow depolarization (upper trace, RMP = -67 mV). The addition of the 5-HT₇ receptor antagonist SB 269970 (1 μ M) blocked this (middle trace) while addition of the NK₃ receptor antagonist SR 142801 (1 μ M) had no effect. * Transient depolarization of unknown origin. (B) The selective NK₃ receptor agonist senktide (3 μ M) was applied to the cell body (at the open triangle, 50 ms, 10 p.s.i.) and evoked a slow depolarization (upper trace, RMP = -67 mV). The addition of SB 269970 had no effect (middle trace) while addition of SR 142801 blocked it (lower trace).

In the CNS and ENS, 5-HT₁ receptors are coupled to increases in potassium conductance and decreases in adenylyl cyclase activity (Saxena, 1995; Galligan, 1996); it is the 5-HT_{1A} receptor that is probably responsible for the small hyperpolarization seen in this study (e.g. Fig. 4C). Other types of 5-HT₁ receptor exist, but only a preliminary account of the 5HT_{1B/D} receptors in the ENS has been published (Liu et al., 2004) while enteric 5-HT_{1E} and 5-HT_{1F} receptors have not been characterized. Nonetheless, all 5-HT₁ receptors appear to cause an inhibition of adenylyl cyclase. 5-HT₅ receptors have not been characterized in the ENS, but when expressed in other systems they also appear to cause an inhibition of adenylyl cyclase (Nelson, 2004). In contrast, the 5-HT₇ receptor is positively coupled to adenylyl cyclase and, through production of cAMP, can activate PKA (Adham et al., 1998). In the CNS, one consequence of 5-HT₇ receptor activation is closure of potassium channels and a reduction of the AHP (Bacon and Beck, 2000; Tokarski et al., 2003). In the ENS, increases in cAMP are coupled to these same changes (Palmer et al., 1987; Bertrand and Galligan, 1995) as is the slow depolarization evoked by 5-HT.

Thus, these studies show that 5-HT₁ and 5-HT₅ receptors are negatively coupled and 5-HT₇ receptors are positively coupled to adenylyl cyclase and increases in cAMP levels. Together with the evidence that increased cAMP leads to depolarization in myenteric AH neurons, this implies that the 5-HT-evoked depolarization is due to 5-HT₇

receptors. This conclusion is consistent with a recent finding that, in cultured myenteric neurons, 5-HT₇ receptor immunoreactivity colocalizes with NeuN (a marker for the AH neurons; Cervio et al., 2005). The same is true of whole-mount preparations where the 5-HT₇ receptor has been colocalized with NeuN and calbindin (M. Tonini, personal communication).

Some properties of the 5-HT_{1P} receptor may be due to the 5-HT₇ receptor

There is strong evidence in the literature for a unique, and enteric specific, high affinity 5-HT receptor historically called the 5-HT_{1P} receptor. The slow depolarization evoked by 5-HT in myenteric AH neurons is the one response that has been fully characterized as being due to a 5-HT_{1P} receptor (Takaki et al., 1985; Mawe et al., 1986); identification of other 5-HT_{1P}-mediated responses has depended on the pharmacological selectivity of the compounds identified in these original studies. The 5-HT_{1P} receptor is a G-protein-coupled receptor that reportedly acts through G_o, protein kinase C and finally accumulation of cAMP and activation of PKA (Fiorica-Howells et al., 1993; Pan et al., 1997). The 5-HT_{1P} receptor has a novel pharmacology (Takaki et al., 1985; Mawe et al., 1986, 1989) but has not been correlated with known 5-HT receptor gene products (Hoyer et al., 2002). There have been suggestions that the observed pharmacology of 5-HT_{1P}

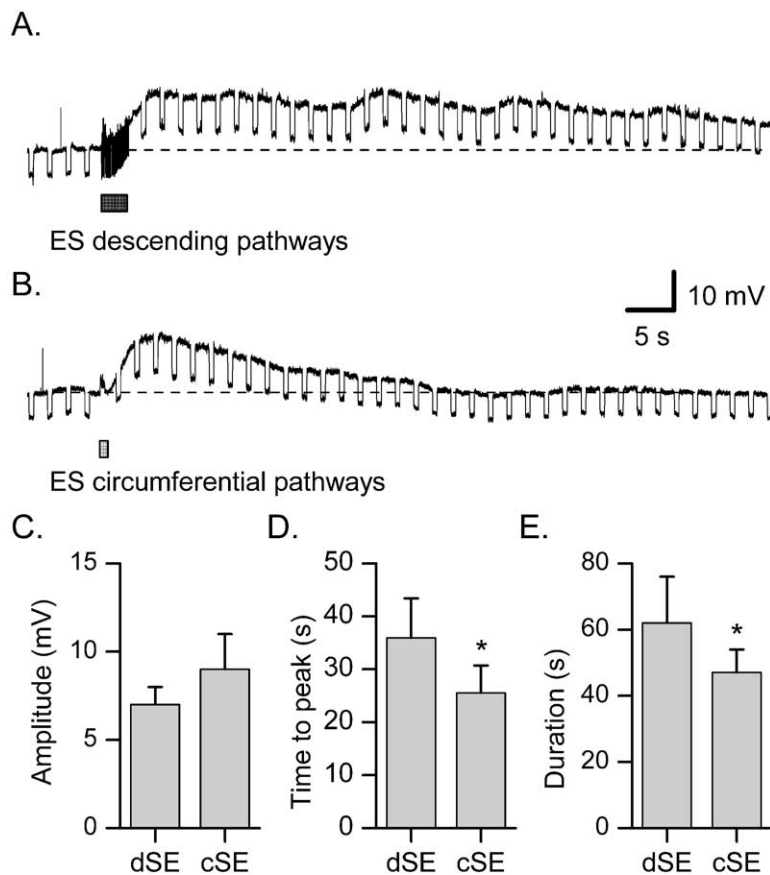


Fig. 6. Comparison of orally and circumferentially evoked slow EPSPs. Voltage traces from a single AH neuron (A, B). (A) A train of electrical stimuli (10 Hz, 3 s, at the black bar) was applied transmurally 1 cm oral of the recording site to stimulate descending pathways. A slow EPSP was evoked that was associated with an increase in input resistance (voltage deflections are due to hyperpolarizing current pulses, RMP = -57 mV, input resistance from 60 to 180 MOhms). (B) In the same neuron, stimulation of circumferential fiber tracts (20 Hz, 1 s, at the gray bar) elicited a slow EPSP with a faster time-to-peak and a shorter duration (RMP = -56 mV, input resistance from 60 to 200 MOhms). (C–E) Histograms comparing the amplitudes (C) time-to-peak amplitude (D) and durations (E) of slow EPSPs evoked by stimulation of descending (dSE) or circumferential (cSE) pathways ($n=11$, * $P<0.05$).

receptor can be accounted for by splice variants of the 5-HT₄ receptor. 5-HT₄ receptors couple to G_s, are on enteric neurons and may mediate some 5-HT_{1P}-like responses (Grider et al., 1996). However, they appear to be located presynaptically rather than on the cell bodies of AH neurons (Pan and Galligan, 1994, 1995; Fiorica-Howells et al., 2004). Another idea, supported by preliminary reports, is that dimers of the 5-HT_{1B/1D} receptors and the dopamine D₂ receptor may account for some properties of the 5-HT_{1P} receptor (Liu et al., 2004; Liu and Gershon, 2005). As SB 269970 shows some affinity for the 5-HT₁ receptors, the possibility that any such dimer was also blocked in our study cannot be excluded.

The slow depolarization characterized here is identical to the 5-HT-evoked depolarization seen in previous studies (Erde et al., 1985; Takaki et al., 1985; Mawe et al., 1989). Both consist of a slow onset, long lasting depolarization. Both are associated with a decrease in conductance (e.g. Fig. 4), a reduction in the AHP and AP generation. Both are seen in the same sub-type of guinea-pig myenteric neuron—the AH neuron with Dogiel type II morphology. Our data strongly suggest that either

SB 269970 blocks 5-HT_{1P} receptors, or that the 5-HT_{1P} receptor is one of the previously characterized receptors that SB 269970 blocks, such as the 5-HT₇ receptor. It remains to be seen whether 5-HT₇ receptors have a similar pharmacological profile to 5-HT_{1P} receptors in the ENS. Whether or not the receptor is really a 5-HT₇ receptor, SB 269970 is clearly a useful tool for investigating the G protein-coupled receptor mediating the slow depolarization and for probing effects that have been ascribed to 5-HT_{1P} like responses to mechanical stimulation of the mucosa (Sidhu and Cooke, 1995).

5-HT₇ receptors may mediate enteric slow synaptic potentials

To date, no 5-HT₇-mediated synaptic potentials have been recorded in the CNS or the ENS. In this study, stimulation of descending fiber tracts in the myenteric plexus containing 5-HT fibers evoked slow EPSPs that were substantially reduced by the selective 5-HT₇ receptor antagonist SB 269970. The 5-HT containing neurons are descending interneurons that connect with each other and with other functional classes

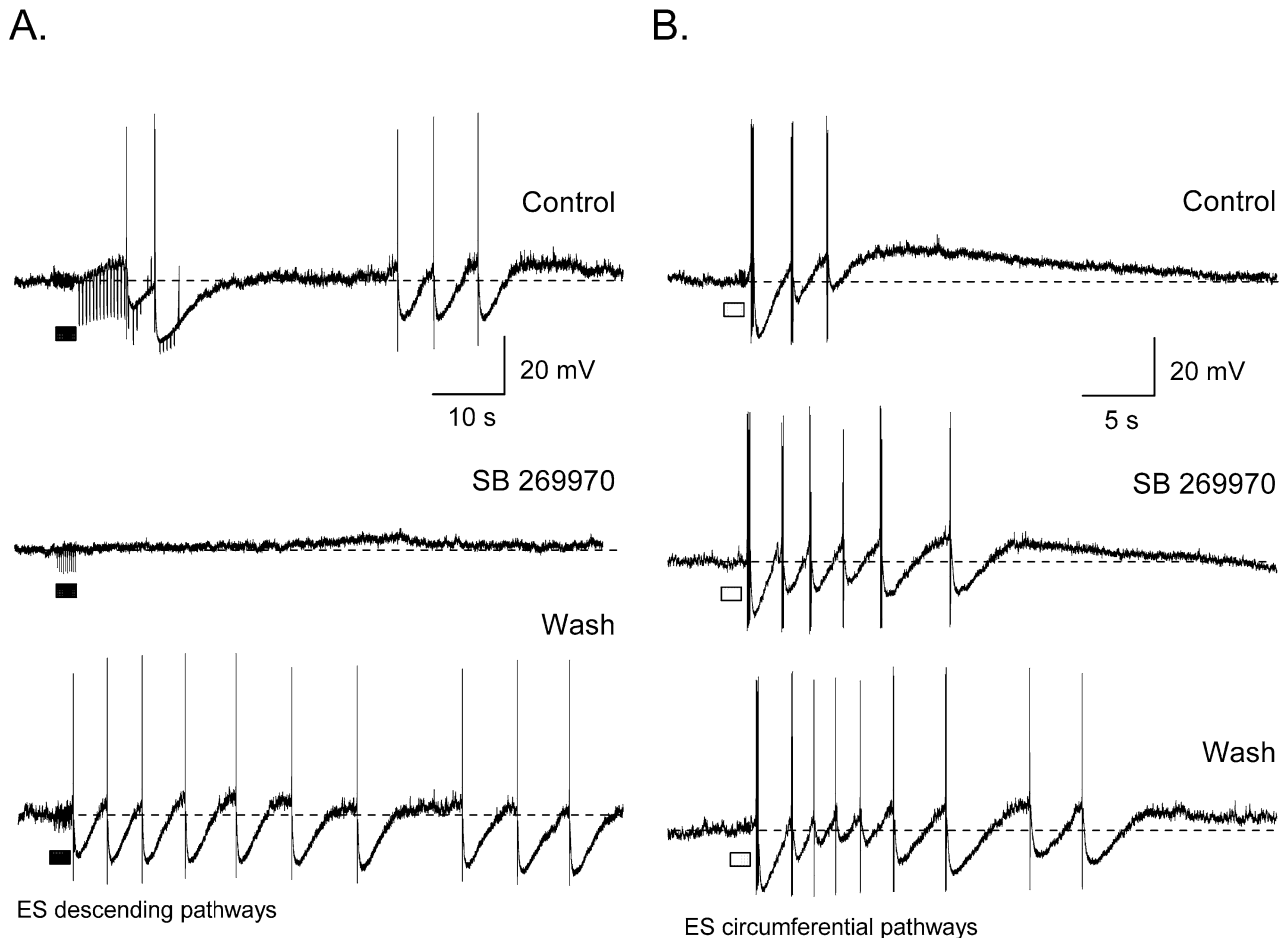


Fig. 7. Effect of 5-HT₇ receptor blockade on orally and circumferentially evoked slow EPSPs. Voltage traces from a single AH neuron (A, B). (A) A train of electrical stimuli (10 Hz, 3 s, at the gray bar) was applied transmurally 1 cm oral of the recording site to stimulate descending fiber tracts and evoke a slow EPSP. *Upper trace*, in control conditions the slow EPSP is associated with an increase in input resistance that evokes APs (RMP = -50 mV). Each AP is followed by a large AHP. Note, second AP evoked by a depolarizing current pulse. *Middle*, SB 269970 (1 μ M) added to the bath reduced the slow EPSP (RMP = -55 mV). *Lower*, upon washout the slow EPSP recovered (RMP = -56 mV). (B) In the same neuron, stimulation of circumferential fiber tracts (20 Hz, 1 s, at the gray bar) elicited a slow EPSP that generated APs (RMP = -49 mV). *Upper*, slow EPSP in control conditions. *Middle*, SB 269970 (1 μ M) had no effect on the slow EPSP (RMP = -50 mV). *Lower*, washout (RMP = -55 mV).

of neurons. Electron microscopy studies show there are some (Young and Furness, 1995) or many (Erde et al., 1985) 5-HT terminals near AH neuron cell bodies, though these contacts may not be true synapses and not all AH neurons have such contacts (Young and Furness, 1995). The present results provide further evidence for 5-HT terminals near to AH neuron cell bodies and suggest that 5-HT-mediated slow EPSPs seen in previous studies (Erde et al., 1985; Takaki et al., 1985; Mawe et al., 1989) could have been mediated by 5-HT₇ receptors.

Slow EPSPs evoked by stimulation of circumferential pathways were unaffected by SB 269970, but these slow EPSPs are known to be strongly inhibited by a combination of NK₁ and NK₃ tachykinin receptor antagonists (Johnson and Bornstein, 2004). Both tachykinin and 5-HT-mediated slow EPSPs depend on similar second messenger systems and similar populations of potassium channels. As mentioned above, the 5-HT_{1P} receptor appears to couple through both PKC and PKA. The tachykinin receptors have a similar profile with both PKC and PKA dependent path-

ways activated (Guard and Watson, 1987; Baidan et al., 1992). SB 269970 did not inhibit the tachykinin slow EPSP and so did not affect either the release or actions of tachykinins at NK receptors, the second messenger pathways activated or the potassium channels closed. Thus, we can conclude that SB 269970 does not non-selectively interfere with slow EPSP generation. The slow EPSP evoked in the same neurons by stimulation of descending pathways was attenuated by SB 269970. As SB 269970 inhibited the response to exogenous 5-HT, this suggests that the descending pathway slow EPSP is a mixed response. Most likely the electrical stimulus triggered release of some tachykinins from long descending projections of AH neurons (Brookes et al., 1995).

S neurons do not express functional 5-HT₇ receptors

5-HT applied to S neurons causes both a fast and slow depolarization. Surprisingly, blocking 5-HT₇ receptors with SB 269970 had no effect in these neurons. This indicates that

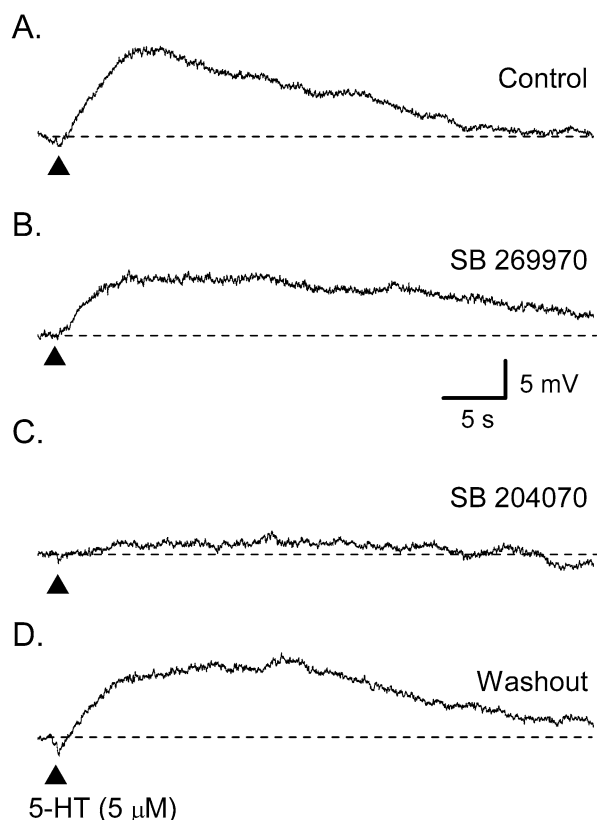


Fig. 8. Effect of SB 269970 on 5-HT-evoked slow depolarizations in a myenteric S neuron. Voltage traces from a single S neuron (A–D). (A) 5-HT (5 μ M) was applied to the cell body (at the filled triangle, 50 ms, 10 p.s.i.) and evoked a slow depolarization (RMP = -67 mV). (B) The addition of the 5-HT₇ receptor antagonist SB 269970 (1 μ M) had no significant effect. (C) In contrast, addition of the 5-HT₄ receptor antagonist SB 204070 (1 μ M) blocked the slow depolarization. (D) Washout (RMP = -55 mV).

S neurons do not express the same receptor as the AH neurons and further shows that SB 269970 does not non-specifically interfere with the slow depolarization. Despite the reports that 5-HT₄ receptors are located only presynaptically (Pan and Galligan, 1994, 1995), an antagonist at these receptors was tested because, like the 5-HT₇ receptor, the 5-HT₄ receptor couples to increases in adenylyl cyclase activity. It was found that blockade of 5-HT₄ receptors reduced the 5-HT-evoked slow depolarization. In these experiments TTX was applied before the 5-HT₄ receptor antagonist to ensure that all effects were at the cell body. The original studies of 5-HT-mediated slow depolarizations (Mawe et al., 1986; Fiorica-Howells et al., 1993) blocked 5-HT₄ receptors and found no effect, but they only looked at AH neurons and not at S neurons. It remains for future studies to determine whether the clinically important 5-HT₄ receptor has a role at the S neuron cell body. The main finding from this study was that the 5-HT₇ receptor was not involved in the 5-HT-mediated slow depolarization in S neurons.

CONCLUSIONS

Taken together these data are consistent with a functional 5-HT₇ receptor on the enteric AH neurons. Further, it

shows that a serotonergic component of the descending but not circumferential slow EPSP is likely to be mediated by these 5-HT₇ receptors. The AH neurons function as sensory neurons and are critical for the initiation and propagation of intestinal reflexes (Bornstein et al., 2002). Modulating the excitability of these neurons is thought to be key to controlling intestinal motility and perhaps secretion (Clerc et al., 2002; Wood and Kirchgessner, 2004). Because the pharmacological specificity of this descending pathway differs from circumferential pathways, there must be converging inputs onto the AH neurons. Converging inputs imply integration and this supports the idea that they function both as sensory neurons and interneurons (e.g. Thomas et al., 2004; Wood and Kirchgessner, 2004). If 5-HT₇ receptors are on the enteric AH neurons, then they represent an important new drug target for the modification of intestinal function.

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REFERENCES

- Adham N, Zgombick JM, Bard J, Branchek TA (1998) Functional characterization of the recombinant human 5-hydroxytryptamine_{7(a)} receptor isoform coupled to adenylyl cyclase stimulation. *J Pharmacol Exp Ther* 287:508–514.
- Alex G, Clerc N, Kunze WA, Furness JB (2002) Responses of myenteric S neurones to low frequency stimulation of their synaptic inputs. *Neuroscience* 110:361–373.
- Bacon WL, Beck SG (2000) 5-Hydroxytryptamine₇ receptor activation decreases slow afterhyperpolarization amplitude in CA3 hippocampal pyramidal cells. *J Pharmacol Exp Ther* 294:672–679.
- Baidan LV, Fertel RH, Wood JD (1992) Effects of brain-gut related peptides on cAMP levels in myenteric ganglia of guinea-pig small intestine. *Eur J Pharmacol* 225:21–27.
- Beique JC, Chapin-Pennick EM, Mladenovic L, Andrade R (2004) Serotonergic facilitation of synaptic activity in the developing rat prefrontal cortex. *J Physiol* 556:739–754.
- Bertrand PP, Galligan JJ (1995) Signal-transduction pathways causing slow synaptic excitation in guinea pig myenteric AH neurons. *Am J Physiol* 269:G710–G720.
- Bornstein JC, Furness JB, Kunze WA (1994) Electrophysiological characterization of myenteric neurons: how do classification schemes relate? *J Auton Nerv Syst* 48:1–15.
- Bornstein JC, Furness JB, Kunze WA, Bertrand PP (2002) Enteric reflexes that influence motility. In: *Nervous control of the gastrointestinal tract* (Costa M, Brookes SH, eds), pp 1–55. Taylor and Francis, London, UK.
- Brookes SJ, Song ZM, Ramsay GA, Costa M (1995) Long aboral projections of Dogiel type II, AH neurons within the myenteric plexus of the guinea pig small intestine. *J Neurosci* 15:4013–4022.
- Bülbring E, Lin RCY (1958) The effect of intraluminal application of 5-hydroxytryptamine and 5-hydroxytryptophan on peristalsis: the local production of 5-HT and its release in relation to intraluminal pressure and propulsive activity. *J Physiol (Lond)* 140:381–407.
- Carter D, Champney M, Hwang B, Eglen RM (1995) Characterization of a postjunctional 5-HT receptor mediating relaxation of guinea-pig isolated ileum. *Eur J Pharmacol* 280:243–250.
- Cervio E, De Ponti F, De Giorgio R, Barbara G, Stanghellini V, Dellabianca A, Sternini C, Tonini M (2005) Expression and role of 5-HT₇ receptors in modulating peristalsis and accommodation in the

- guinea pig ileum. 20th International Symposium on Neurogastroenterology and Motility.
- Clerc N, Gola M, Vogalis F, Furness JB (2002) Controlling the excitability of IPANs: a possible route to therapeutics. *Curr Opin Pharmacol* 2:657–664.
- Emonds-Alt X, Bichon D, Ducoux JP, Heaulme M, Miloux B, Poncelet M, Proietto V, Van Broeck D, Vilain P, Neliat G, Soubri P, Le Fur G, Breliere JC (1995) SR 142801, the first potent nonpeptide antagonist of the tachykinin NK₃ receptor. *Life Sci* 56:27–32.
- Erde SM, Sherman D, Gershon MD (1985) Morphology and serotonergic innervation of physiologically identified cells of the guinea pig's myenteric plexus. *J Neurosci* 5:617–633.
- Florica-Howells E, Li Z, Hen R, Gingrich JA, Holick KA, Gershon MD (2004) Location and function of 5-HT₄ receptors in the murine ENS. *Soc Neurosci Abstr* 826.1, Online.
- Florica-Howells E, Wade PR, Gershon MD (1993) Serotonin-induced increase in cAMP in ganglia isolated from the myenteric plexus of the guinea pig small intestine: mediation by a novel 5-HT receptor. *Synapse* 13:333–349.
- Furness JB, Costa M (1982) Neurons with 5-hydroxytryptamine-like immunoreactivity in the enteric nervous system: their projections in the guinea-pig small intestine. *Neuroscience* 7:341–349.
- Furness JB, Kunze WA, Bertrand PP, Clerc N, Bornstein JC (1998) Intrinsic primary afferent neurons of the intestine. *Prog Neurobiol* 54:1–18.
- Galligan JJ (1996) Electrophysiological studies of 5-hydroxytryptamine receptors on enteric neurons. *Behav Brain Res* 73:199–201.
- Galligan JJ, North RA (1991) Opioid, 5-HT_{1A} and alpha 2 receptors localized to subsets of guinea-pig myenteric neurons. *J Auton Nerv Syst* 32:1–11.
- Gershon MD (2004) Review article: serotonin receptors and transporters: roles in normal and abnormal gastrointestinal motility. *Aliment Pharmacol Ther* 20(Suppl 7):3–14.
- Grider JR, Kuemmerle JF, Jin JG (1996) 5-HT released by mucosal stimuli initiates peristalsis by activating 5-HT₄/5-HT_{1p} receptors on sensory CGRP neurons. *Am J Physiol* 270:G778–G782.
- Guard S, Watson SP (1987) Evidence for neurokinin-3 receptor-mediated tachykinin release in the guinea-pig ileum. *Eur J Pharmacol* 144:409–412.
- Hagan JJ, Price GW, Jeffrey P, Deeks NJ, Stean T, Piper D, Smith MI, Upton N, Medhurst AD, Middlemiss DN, Riley GJ, Lovell PJ, Bromidge SM, Thomas DR (2000) Characterization of SB-269970-A, a selective 5-HT(7) receptor antagonist. *Br J Pharmacol* 130:539–548.
- Hanani M, Chorev M, Gilon C, Selinger Z (1988) The actions of receptor-selective substance P analogs on myenteric neurons: an electrophysiological investigation. *Eur J Pharmacol* 153:247–253.
- Hedlund PB, Danielson PE, Thomas EA, Slanina K, Carson MJ, Sutcliffe JG (2003) No hypothermic response to serotonin in 5-HT₇ receptor knockout mice. *Proc Natl Acad Sci U S A* 100:1375–1380.
- Heidmann DE, Metcalf MA, Kohen R, Hamblin MW (1997) Four 5-hydroxytryptamine₇ (5-HT₇) receptor isoforms in human and rat produced by alternative splicing: species differences due to altered intron-exon organization. *J Neurochem* 68:1372–1381.
- Hemedah M, Coupar IM, Mitchelson FJ (1999) [3H]-Mesulergine labels 5-HT₇ sites in rat brain and guinea-pig ileum but not rat jejunum. *Br J Pharmacol* 126:179–188.
- Hirst GDS, Holman ME, Spence I (1974) Two types of neurones in the myenteric plexus of duodenum in the guinea-pig. *J Physiol (Lond)* 236:303–326.
- Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 71:533–554.
- Johnson PJ, Bornstein JC (2004) Neurokinin-1 and -3 receptor blockade inhibits slow excitatory synaptic transmission in myenteric neurons and reveals slow inhibitory input. *Neuroscience* 126:137–147.
- Liu M, Gershon MD (2005) Slow excitatory ("5-HT_{1p}"-like) responses of mouse myenteric neurons to 5-HT: mediation by heterodimers of 5-HT_{1B/1D} and DRD2 receptors. *Gastroenterology*, In Press.
- Liu GM, Wen Y, Gershon MD (2004) 5-HT_{1p} receptor activity in the mouse enteric nervous system. *Soc Neurosci Abstr*. 620.19, Online.
- Lucchelli A, Santagostino-Barbone MG, D'Agostino G, Masoero E, Tonini M (2000) The interaction of antidepressant drugs with enteric 5-HT₇ receptors. *Naunyn Schmiedebergs Arch Pharmacol* 362:284–289.
- Mawe GM, Branchek TA, Gershon MD (1986) Peripheral neural serotonin receptors: identification and characterization with specific antagonists and agonists. *Proc Natl Acad Sci U S A* 83:9799–9803.
- Mawe GM, Branchek TA, Gershon MD (1989) Blockade of 5-HT-mediated enteric slow EPSPs by BRL 24924: gastrokinetic effects. *Am J Physiol* 257:G386–G396.
- McLean PG, Coupar IM (1996) Characterisation of a postjunctional 5-HT₇-like and a prejunctional 5-HT₃ receptor mediating contraction of rat isolated jejunum. *Eur J Pharmacol* 312:215–225.
- Monro RL, Bertrand PP, Bornstein JC (2004) ATP participates in three excitatory postsynaptic potentials in the submucous plexus of the guinea pig ileum. *J Physiol* 556:571–584.
- Nelson DL (2004) 5-HT₅ receptors. *Curr Drug Targets CNS Neurol Disord* 3:53–58.
- Palmer JM, Wood JD, Zafirov DH (1987) Transduction of aminergic and peptidergic signals in enteric neurones of the guinea-pig. *J Physiol* 387:371–383.
- Pan H, Galligan JJ (1994) 5-HT_{1A} and 5-HT₄ receptors mediate inhibition and facilitation of fast synaptic transmission in enteric neurons. *Am J Physiol* 266:G230–G238.
- Pan H, Galligan JJ (1995) Effects of 5-HT_{1A} and 5-HT₄ receptor agonists on slow synaptic potentials in enteric neurons. *Eur J Pharmacol* 278:67–74.
- Pan H, Wang HY, Friedman E, Gershon MD (1997) Mediation by protein kinases C and A of Go-linked slow responses of enteric neurons to 5-HT. *J Neurosci* 17:1011–1024.
- Patacchini R, Bartho L, Holzer P, Maggi CA (1995) Activity of SR 142801 at peripheral tachykinin receptors. *Eur J Pharmacol* 278:17–25.
- Saxena PR (1995) Serotonin receptors: subtypes, functional responses and therapeutic relevance. *Pharmacol Ther* 66:339–368.
- Schneider DA, Galligan JJ (2000) Presynaptic nicotinic acetylcholine receptors in the myenteric plexus of guinea pig intestine. *Am J Physiol* 279:G528–G535.
- Schutte IW, Kroese AB, Akkermans LM (1995) Somal size and location within the ganglia for electrophysiologically identified myenteric neurons of the guinea pig ileum. *J Comp Neurol* 355:563–572.
- Sidhu M, Cooke HJ (1995) Role for 5-HT and ACh in submucosal reflexes mediating colonic secretion. *Am J Physiol* 269:G346–G351.
- Takaki M, Branchek T, Tamir H, Gershon MD (1985) Specific antagonism of enteric neural serotonin receptors by dipeptides of 5-hydroxytryptophan: evidence that serotonin is a mediator of slow synaptic excitation in the myenteric plexus. *J Neurosci* 5:1769–1780.
- Thomas EA, Sjoval H, Bornstein JC (2004) Computational model of the migrating motor complex of the small intestine. *Am J Physiol Gastrointest Liver Physiol* 286:G564–G572.
- Tokarski K, Zahorodna A, Bobula B, Hess G (2003) 5-HT₇ receptors increase the excitability of rat hippocampal CA1 pyramidal neurons. *Brain Res* 993:230–234.
- Tuladhar BR, Ge L, Naylor RJ (2003) 5-HT₇ receptors mediate the inhibitory effect of 5-HT on peristalsis in the isolated guinea-pig ileum. *Br J Pharmacol* 138:1210–1214.

- Vanhoenacker P, Haegeman G, Leysen JE (2000) 5-HT₇ receptors: current knowledge and future prospects. *Trends Pharmacol Sci* 21:70–77.
- Wang HY, Fiorica-Howells E, Pan H, Gershon MD, Friedman E (1996) Myenteric ganglionic 5-hydroxytryptamine(1P) signal transmission is mediated via G_o protein. *J Pharmacol Exp Ther* 277:518–524.
- Wardle KA, Ellis ES, Baxter GS, Kennett GA, Gaster LM, Sanger GJ (1994) The effects of SB 204070, a highly potent and selective 5-HT₄ receptor antagonist, on guinea-pig distal colon. *Br J Pharmacol* 112:789–794.
- Wood JD, Kirchgessner A (2004) Slow excitatory metabotropic signal transmission in the enteric nervous system. *Neurogastroenterol Motil* 16(Suppl 1):71–80.
- Young HM, Furness JB (1995) Ultrastructural examination of the targets of serotonin-immunoreactive descending interneurons in the guinea pig small intestine. *J Comp Neurol* 356:101–114.

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