5-HT receptors on interstitial cells of Cajal, smooth muscle and enteric nerves

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Abstract The majority of the body's serotonin (5-HT) is produced by the gastrointestinal tract. 5-HT has several functions in the gastrointestinal tract. 5-HT is a paracrine signalling molecule released from enterochromaffin cells, a survival and proliferating factor and a neurotransmitter. The actions of 5-HT are transduced by a large family of 5-HT receptors, several of which are expressed on different gastrointestinal cell types including enteric nerves, smooth muscle and interstitial cells of Cajal (ICC). This review will summarize recent advances in understanding the role of 5-HT in regulating function of ICC, and the expression and function of 5-HT receptors on muscle and enteric nerves in human tissue. Rodent ICC express several 5-HT receptors including 5-HT_{2B} receptors which regulate ICC survival and proliferation. Human smooth muscle and enteric neurons also express several 5-HT receptor subtypes. Expression and function of these receptors is significantly different from small laboratory animals. 5-HT₇ receptor activation causes relaxation of muscle, whereas 5-HT_{2B} receptors increase muscle activity. The 5-HT₄ receptor appears to mediate both inhibition and activation of smooth muscle involving myogenic as well as neural actions. Despite the abundant expression of 5-HT₃ receptors in the human enteric nervous system no functional correlate has been as yet demonstrated.

Keywords 5-HT receptor, human enteric nervous system, human smooth muscle, serotonin.

SEROTONIN AND INTERSTITIAL CELLS OF CAJAL

The majority of the body's serotonin (5-HT) is produced by the gastrointestinal tract.¹ Ninety per cent of 5-HT is produced by enterochromaffin cells (EC), found in the gastrointestinal epithelium.² The other major source of 5-HT in the gastrointestinal tract are enteric neurons, in particular interneurons.^{3,4} In some species, mast cells contain high levels of 5-HT^{5–7} and platelets avidly take up and deliver 5-HT.^{8,9} The actions of 5-HT are transduced by a large family of 5-HT receptors divided in seven classes (5-HT₁–5-HT₇). 5-HT has several distinct functions in the gastrointestinal tract. 5-HT acts as a paracrine factor transducing information from EC to intrinsic primary afferent neurons¹ and to adjacent cells in the mucosa and submucosa.¹⁰ Furthermore, 5-HT is a neurotransmitter¹¹ and is also increasingly recognized as a survival factor.^{12,13} Control of gastrointestinal motility requires the coordinated activity of several cell types including nerves, smooth muscle cells and interstitial cells of Cajal (ICC). ICC are now known to be essential for normal motility.¹⁴ Several receptors are expressed on ICC¹⁵ including 5-HT receptors.^{16–18}

An understanding of the potential role that 5-HT receptors expressed on ICC may play in the control of ICC function and survival requires an understanding of the different roles that ICC play in the control of gastrointestinal motility. ICC are a specialized mesenchymal cell type in the gastrointestinal tract that are neither neurons nor smooth muscle cells. The best studied function of ICC is the generation of spontaneous, rhythmic electrical oscillations called slow waves (the pacemaker function of ICC).¹⁹ In stomach and small intestine, ICC surrounding the neuronal myenteric plexus (ICC-MP) and in conjunction with intramuscular ICC (ICC-IM) generate and propagate

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slow waves.^{20,21} In the colon, slow waves appear to originate from ICC at the level of the submucosa (ICC-SMP).²² Also, in colon, ICC-MP may drive the cyclic depolarizations responsible for high-amplitude contractions.²³ Slow waves are conducted passively to neighbouring smooth muscle where they produce rhythmical membrane potential changes.^{24,25} As contractions only occur during the depolarization phase of the slow wave, slow waves determine the maximal frequency of gastrointestinal musculature contractions. Subclasses of ICC such as ICC-IM and ICC at the level of the deep muscular plexus (ICC-DMP) are in close contact with varicose endings of enteric motor neurons.²⁶ These ICC are involved in the conduction and amplification of neuronal signals from excitatory cholinergic and inhibitory nitrergic motor neurons.^{27,28} Together with the dual roles of ICC in pacing the gastrointestinal tract and amplifying neurotransmission, ICC also release carbon monoxide (CO)^{29–31}, a critical hyperpolarizing factor required for the maintenance of intestinal smooth muscle membrane potential and its gradient.³² Finally, ICC act as mechanosensors through mechanosensitive ion channels expressed on the ICC cell membrane.^{33,34}

THE EXPRESSION AND ROLE OF SEROTONIN RECEPTORS ON RODENT ICC

Of the seven classes of 5-HT receptors known, 5-HT₁, 5-HT₂, 5-HT₃, 5-HT₄ and 5-HT₇ receptors are expressed in the gastrointestinal tract and affect gastrointestinal motor function.³⁵ So far, of these, only two 5-HT receptors have been described on rodent ICC; 5-HT₃ and 5-HT₄ with recent evidence for a third, 5-HT₂. Data regarding the expression and function of 5-HT receptors on human ICC are lacking and therefore this part of the review will focus on the rodent literature.

THE 5-HT₃ RECEPTOR

5-HT₃ receptors are ligand-gated cation channels.³⁶ Evidence for expression of 5-HT₃ receptors in ICC comes from work carried out in the rat small intestine.¹⁷ In rat intestine, 5-HT₃ receptors are expressed by different cell types including functionally distinct classes of neurons, ICC and endocrine cells.¹⁷ 5-HT₃ receptors are found in both ICC-MP and ICC-DMP. However not all ICC appeared to express 5-HT₃ receptors, that is some ICC at both the ICC-MP and ICC-DMP did not appear to express 5-HT₃ by immunohistochemistry.

The role of the 5-HT₃ receptor on ICC is currently unknown. 5-HT₃ receptors are ligand-gated cation channels and allow entry of several cations including Ca²⁺ in some but not all types of 5-HT₃ receptors. 5-HT₃ receptor activation results in neurotransmitter release, including acetylcholine, from presynaptic terminals and neuronal excitation of postsynaptic cells.³⁷ Given the known function of 5-HT₃ receptors it can be hypothesized that activation of 5-HT₃ receptors on ICC would result in rapid depolarization and therefore alteration or initiation of the slow wave.

THE 5-HT₄ RECEPTOR

5-HT₄ receptors are G protein-coupled metabotropic receptors. Evidence for expression of 5-HT₄ receptors in ICC comes from work carried out in the mouse and guinea pig small intestine.^{16,18} Studies using an antibody to 5-HT₄ showed that together with the presence of 5-HT₄ receptors in subsets of enteric neurons and smooth muscle cells, the 5-HT₄ receptor was also expressed in ICC-MP. The expression of 5-HT₄ in ICC-DMP appeared to be much less robust.^{16,18}

Similar to the 5-HT₃ receptor, the role of 5-HT₄ receptors in ICC is not known as no functional studies have been carried out. 5-HT₄ receptor agonists acting presynaptically enhance neurotransmitter release at neuron-neuronal synapses³⁸ while 5-HT₄ receptor agonists acting on smooth muscle cause relaxation.³⁹ The 5-HT₄ receptor is also thought to be coupled by G_s to the stimulation of adenylyl cyclase, resulting in an increase in cyclic AMP (cAMP) and activation of protein kinase A.⁴⁰ cAMP has been shown to mediate slow wave amplitude and frequency in murine ICC suggesting a potential role for the 5-HT₄ receptor in modulating slow waves.⁴¹ The 5-HT₄ receptor also appears to be involved in neuronal development and survival.⁴² Whether activation of the 5-HT₄ receptor does in fact play a role in the survival of ICC or is involved in the generation and propagation of the electric slow wave by increasing cAMP levels remains to be elucidated.

OTHER 5-HT RECEPTORS EXPRESSED ON ICC

Recent data suggest that 5-HT_{2B} receptors are expressed in ICC. ICC networks are not fixed in number, rather the ICC networks turnover in health. ICC can disappear under certain circumstances by programmed cell death, necrosis or transdifferentiation, and in more permissive conditions they can reappear through proliferation or differentiation. For example, partial obstruction of the murine ileum by a clip leads to

disrupted ICC networks oral to the site of obstruction. Removal of the obstruction results in the redevelopment of ICC networks within 30 days.⁴³ *Trichinella spiralis* infection to the mouse small intestine results in disrupted ICC networks 10–15 days after infection with complete ICC recovery by 40 days after infection.⁴⁴ In the human, a patient with hypertrophic pyloric stenosis did not have any ICC in the pylorus at the time of myotomy but a full network of ICC was present 4 months after the first surgery.⁴⁵ The mechanisms underlying ICC disappearance and recovery are unknown.

Evidence, mostly from outside the gastrointestinal tract and also in studies on enteric nervous system (ENS) development, indicates that 5-HT can regulate cell survival and proliferation.^{12,46} Four serotonin receptors have been implicated in transduction of the 5-HT signal to regulate cell survival and proliferation, namely 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2B} and 5-HT_{2C} receptors.^{12,46–56} Immunohistochemistry on human intestine and single cell PCR on mouse derived intestinal cells revealed that 5-HT_{2B} receptors are expressed on neurons, smooth muscle cells and neonatal and adult ICC.^{57–60} Activation of the 5-HT_{2B} receptor *in vitro*, resulted in increased neonatal ICC numbers through proliferation⁶⁰ suggesting a potential role of the 5-HT_{2B} receptor in regulating ICC numbers.

SOURCE OF 5-HT FOR ICC

5-HT receptors are abundantly expressed in the gastrointestinal tract. However, the source of 5-HT that can activate the different receptors found is still not clear for all the cell types that express 5-HT receptors, including ICC. 5-HT is found in significant quantities in EC,² serotonergic neurons,⁴ platelets⁸ and mast cells.⁵

In non-diseased tissue, it is unlikely that ICC and myenteric neurons respond to 5-HT released by the EC in the mucosa because the 5-HT transporters present in serotonergic neurons, mucosal and submucosal cells will rapidly take up 5-HT.^{61,62} However, a subset of intrinsic primary afferent neurons located in the submucosal plexus project to the myenteric plexus and are activated by mucosal 5-HT.^{63,64} These submucosal to myenteric projections may result in mucosal 5-HT activating myenteric neurons and, indirectly, ICC.

In inflammation, 5-HT transporters have been shown to be downregulated^{65,66} and 5-HT production increased⁶⁵ making it more likely that mucosally generated 5-HT can reach ICC and activates the expressed receptors. Another possible source of 5-HT are serotonergic interneurons. Serotonergic interneu-

rons represent about 2% of the total number of enteric neurons.⁴ However, while in the myenteric plexus they may represent a possible source of 5-HT for other myenteric neurons, interneurons do not make direct contact with ICC and therefore would be unlikely to provide sufficient 5-HT to activate ICC 5-HT receptors. Mast cells can represent a source for 5-HT. Mast cells are known to be in close apposition to ICC⁶⁷ and therefore may be one of the sources of 5-HT. Murine, but not human, mast cells contain storage proteins that can bind 5-HT in order to release it to the appropriate target cells.^{5,7} Finally, platelets are a rich source of 5-HT^{8,9} and may serve as a significant 5-HT source. It is not currently known if platelet derived 5-HT can activate ICC 5-HT receptors.

5-HT RECEPTORS ON ENTERIC NEURONS AND SMOOTH MUSCLE: HUMAN STUDIES

5-HT can be released in response to several stimuli like mechanical distortion, mucosal stroking or electrical stimulation of enteric neurons. The crucial role of 5-HT for transmission within enteric circuits and initiation of normal reflex activity in animal models has been expanded by studies which suggested impairment of serotonergic mechanisms as one of the factors in the pathogenesis of gut diseases. Indeed, 5-HT receptor modulating substances are effective in relieving symptoms associated with irritable bowel syndrome (IBS), such as abdominal pain or altered stool frequency and consistency.⁶⁸ The increased number of EC cells in some IBS patients⁶⁹ further implicates a role for 5-HT in IBS pathogenesis.

In this section, we summarize the current knowledge on 5-HT receptor expression in human enteric nerves and smooth muscle (see Fig. 1) by focusing on 5-HT₃, 5-HT₄, 5-HT₇ and 5-HT_{2B} receptors.

THE 5-HT₃ RECEPTOR

There are three evolutionary arms of 5-HT₃ receptors which are 5-HT_{3A}, 5-HT_{3B} and 5-HT_{3C-E}. RT-PCR expression analysis revealed that all known 5-HT₃ receptor subunits are expressed in the human gut.^{70,71} However, there is a striking region specific expression of the different subunits.^{70,71} 5-HT_{3C} and in particular 5-HT_{3C1.3} is mainly expressed in the small intestine. 5-HT_{3D} is only expressed in the colon, kidney and liver, whereas the 5-HT_{3E} subunit is exclusively expressed in the small intestine and colon but not in other organs. There is a low expression of 5-HT_{3A} and 5-HT_{3B} subunits and a total lack of 5-HT_{3D} and 5-HT_{3E}

Figure 1 Summary of current knowledge on 5-HT receptor localization and their putative functions in the human intestine.

subunits in the human stomach.⁷⁰ The functional relevance of the expression of different 5-HT₃ subunits in the human gut remains unknown. It has been known that the properties of 5-HT₃ receptors change with different combinations of the subunits.⁷² Homomeric 5-HT_{3A} receptors reveal low conductance while heteromeric 5-HT_{3A/B} receptors display large conductance. However, there are only very minor differences in their respective pharmacological profiles.⁷³ Co-expression of 5-HT_{3A} with 5-HT_{3C} reduces biological function of 5-HT_{3A} receptors as the magnitude of 5-HT induced currents is reduced.⁷⁴ It is intriguing to speculate that, if 5-HT_{3C} is involved in serotonin-responsiveness of the gut, 5-HT_{3C}-specific agents with less activity at 5-HT_{3A} may be more desirable agents for treatment of diseases such as IBS.

5-HT mediates rapid excitatory responses in human submucosal neurons that are mediated by 5-HT₃ receptors which comprise both 5-HT_{3A} and 5-HT_{3B} receptor subunits.⁷⁵ Only a relatively small component of 5-HT evoked action potential discharge may be mediated by other 5-HT receptors none of which have been electrophysiologically identified today. Although 5-HT₃ receptors are present in the majority of human submucosal and myenteric neurons⁷⁵, their functional relevance is not clear. For example, the 5-HT₃ agonist 2-methyl-5-HT does not evoke secretion in human mucosa-submucosa preparations.⁷⁵ Likewise, while 5-HT₃ receptors are involved in the initiation and/or transmission of muscle and secretory reflex activity in the guinea-pig gut, they do not appear to be crucial for the peristaltic reflex in the human colon.^{76,77} This lack of demonstration for the function of 5-HT₃ receptors in the isolated human gut together with the clinical observation that 5-HT₃ antagonists improve symptoms in IBS patients⁷⁸ raises the question of whether this

beneficial effect is rather due to actions on non-ENS targets or whether there is a disease associated alteration in the properties of enteric 5-HT₃ receptors.

THE 5-HT₄ RECEPTOR

5-HT₄ receptor mRNA is expressed throughout the human gut at 2–12 fold higher levels as in the liver.⁷⁹ The A, B, C, D, G and I isoforms of the 5-HT₄ receptor are all present in the human intestine.^{80,81} The 5-HT_{4D} splice variant appears to be expressed only in the gut and not in other organs. Activation of 5-HT₄ receptors results in inhibition and activation of muscle activity in the human colon. While the inhibition appears to be a direct effect on the muscle, the motility enhancing effect is due to the activation of neural excitatory cholinergic pathways.⁸² A recent study by Celtek *et al.* showed that the 5-HT₄ evoked inhibition of muscle activity may not be a purely myogenic response but involves the activation of inhibitory nitrenergic pathways in the ENS, too.⁸³ Although these functional data would suggest 5-HT₄ receptor expression in the ENS, 5-HT₄ receptor immunoreactivity on enteric nerves has not yet demonstrated in human tissue. In analogy to guinea-pig and mouse ENS, presynaptic 5-HT₄ receptors which facilitate acetylcholine release have also been postulated in human tissue. A preliminary report describes 5-HT₄-IR on smooth muscle cells, blood vessels and mast cells but neurons appeared to be negative.⁸⁴ Therefore, one has to consider that the pharmacologically identified neural component of 5-HT₄ mediated muscle activity may be secondary and due to release from mast cell of mediators that are known to activate human enteric neurons.⁸⁵

Application of 5-HT₄ agonists onto human intestinal mucosa evokes ascending contraction and descending relaxation together with release of the excitatory transmitter substance P proximal to the stimulation and release of the inhibitory transmitter vasoactive intestinal peptide distal to the stimulation.⁸⁶ This would suggest that activation of 5-HT₄ receptors at the mucosal level is sufficient to activate the polarized components of the peristaltic reflex. Mucosal stroking also evokes ascending contraction and descending relaxation involving 5-HT₄ and 5-HT_{1P} but not 5-HT₃ receptors in the human jejunum.⁷⁶ Intriguingly, none of the receptors are involved in stretch evoked muscle responses.⁷⁶

THE 5-HT₇ RECEPTOR

A number of 5-HT₇ splice variants were described in the human gut.⁸⁷ The functional and pharmacological

properties of human 5-HT₇ splice variants are virtually indistinguishable.⁸⁷ Functional studies indicate that 5-HT induced muscle relaxation in the human colon is mediated by 5-HT₇ receptors.⁸⁸ It is not entirely clear whether nerves are involved in this effect. Since the relaxation is not affected by 0.3 $\mu\text{mol L}^{-1}$ tetrodotoxin, it has been concluded that the 5-HT₇ receptor is very likely localized on the muscle⁸⁸ although presynaptic actions at the neuromuscular junction cannot be totally excluded. An earlier study revealed a neural component as 5-HT-evoked inhibition of spontaneous activity was sensitive to 1.5 $\mu\text{mol L}^{-1}$ tetrodotoxin.⁸⁹

THE 5-HT_{2B} RECEPTOR

5-HT induced activation of muscle is mediated by 5-HT_{2B} receptors in the human colon.⁹⁰ In the muscularis externa, immunoreactivity is highest in the longitudinal muscle layer and in the myenteric plexus while lower expression is found in the circular muscle layer.⁹⁰

SUMMARY

Rodent ICC express different classes of 5-HT receptors. The source for the receptor ligand is presently unclear, however the differential distribution of the different receptors suggests an active functional role for the receptors, a role that may be amplified during inflammation. ICC 5-HT₃ and 5-HT₄ receptors may be involved in regulation of gastrointestinal motility while ICC 5-HT_{2B} receptors may regulate ICC survival and proliferation.

Smooth muscles as well as enteric neurons in the human gut also express several 5-HT receptor subtypes (Fig. 1). 5-HT₇ receptor activation causes relaxation of the muscle, whereas 5-HT_{2B} receptors are involved in increased muscle activity. The 5-HT₄ receptor appears to mediate both inhibition and activation of the smooth muscle. Despite the abundant expression of 5-HT₃ receptors in the human ENS no functional correlate has been demonstrated yet. Both the cellular expression as well as the function of 5-HT receptors in the human gut is significantly different from those in small laboratory animals.

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CONFLICT OF INTEREST STATEMENT

None declared.

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