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CROSSTALK

CrossTalk proposal: 5-HT is necessary for peristalsis

Terence K. Smith[1]
and Michael D. Gershon[2]
[1]*Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV, USA*
[2]*Department of Pathology and Cell Biology, Columbia University, New York, NY, USA*
Email: tksmith@medicine.nevada.edu

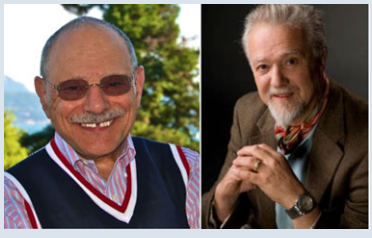
The overwhelming preponderance of the 5-HT of every known mammal is in the gut (Erspamer, 1966; Gershon & Tack, 2007; Gershon, 2013). Enteric 5-HT must be an important signalling molecule to be so conserved. Most enteric 5-HT is in enterochromaffin (EC) cells, but smaller amounts are present in myenteric neurons. Despite their small numbers, serotonergic neurons project widely throughout the enteric nervous system (ENS) and also innervate interstitial cells of Cajal (ICC) (Okamoto *et al.* 2014). The abundance and variety of enteric 5-HT receptors (5-$HT_{1\text{-}7}$ and subtypes) also suggest that 5-HT plays a significant role in GI physiology (Smith *et al.* 2014). The efficacy of therapies targeting enteric 5-HT or its receptors against GI motility disorders supports this idea (Gershon & Tack, 2007; Gershon, 2013). Beyond motility and secretion, putative roles that 5-HT plays include metabolism, osteogenesis, immunity, neurogenesis and neuroprotection (Gershon, 2013). The multiplicity of enteric 5-HT targets and receptors complicates ascertaining the physiological roles of 5-HT. Controversy is thus to be expected and has appeared in recent papers, which question whether EC or neuronal 5-HT has anything to do with peristalsis (Keating & Spencer, 2010; Spencer *et al.* 2011; Sia *et al.* 2013; Spencer *et al.* 2013). These papers are important not because enteric 5-HT is vestigial (it is not) or that its roles in normal and abnormal GI motility can be ignored (they cannot). Instead, they highlight common misunderstandings about peristaltic reflexes and 5-HT cellular biology. The papers focus on colonic migrating motor complexes (CMMCs), which are aborally propagating propulsive contractile complexes, essentially peristaltic reflexes. The authors assert that they can evoke CMMCs after mucosal removal or depleting 5-HT with reserpine; therefore, they conclude that neither EC cells, nor neuronal 5-HT is necessary for CMMCs. To comprehend what is misunderstood, it is necessary to discuss basic information about 5-HT and GI motility.

Two tryptophan hydroxylase isoforms, TPH1 and TPH2, are rate limiting in 5-HT biosynthesis, TPH1 in EC cells, and TPH2 in serotonergic neurons (Gershon, 2013). 5-HT is synthesized in the cytosol but stored in vesicles. Reuptake terminates actions of 5-HT. Because 5-HT is charged, two transporters are required for transmembrane transport, a vesicular monoamine transporter (VMAT1 in EC cells and VMAT2 in neurons) (Henry *et al.* 1998) and a plasmalemmal serotonin reuptake transporter (SERT) (Blakely, 2001). Reserpine inhibits VMAT (Henry *et al.* 1998). Intracellular 5-HT is thus reduced due to enhanced catabolism; but reserpine does not prevent 5-HT biosynthesis or constitutive release. That requires deletion or inhibition of TPH, which when isoform-selective, distinguishes mucosal from neuronal 5-HT (Li *et al.* 2011; Gershon, 2013). SERT deletion amplifies 5-HT effects. Because GI motility is abnormal after deletion of SERT (Chen *et al.* 2001), either isoform of TPH (Li *et al.* 2011; Gershon, 2013), or exposure to 5-HT antagonists/agonists (Monro *et al.* 2002; Smith *et al.* 2014), 5-HT clearly influences GI motility.

Peristalsis is a general term applied to enteric motile behaviour that should not be conflated with the ENS-mediated peristaltic reflex that can drive propulsion (Gershon & Tack, 2007; Gershon, 2013; Furness *et al.* 2014). That reflex, first called the 'law of the intestine' (Bayliss & Starling, 1899), is an oral contraction and anal relaxation; it is evoked by increased intraluminal pressure and involves polarized neural pathways within the ENS (Furness *et al.* 2014; Smith *et al.* 2014). Many enteric cells, not just neurons and muscle, participate in peristaltic reflexes (Smith *et al.* 2014). The peristaltic reflex is only one of many activity patterns encoded within the ENS (Furness *et al.* 2014).

Mucosal pressure/distortion or chemical stimuli release 5-HT from EC cells and evoke peristaltic reflexes (Bülbring & Lin, 1958; Bertrand *et al.* 2008). Luminally applied 5-HT mimics pressure (Bülbring & Crema, 1958; Bülbring & Lin, 1958). Fecal pellets apply pressure to the mucosa and thereby release 5-HT, which entrains CMMCs (Heredia *et al.* 2009). Mucosal removal, anaesthesia, or asphyxiation all abolish mucosally evoked peristaltic reflexes (Bülbring & Crema, 1958; Bayguinov *et al.* 2010; Dickson *et al.* 2010). Mucosally released 5-HT acts on 5-HT_3 and/or 5-HT_{1P} (or 5-HT_7) receptors to stimulate intrinsic primary afferent neurons (IPANs) (Pan & Gershon, 2000; Bertrand *et al.* 2008; Dickson *et al.* 2010), which engage the ENS (Kirchgessner *et al.* 1992; Bayguinov *et al.* 2010; Okamoto *et al.* 2014). IPANs are found in both plexuses (Kirchgessner *et al.* 1992; Bayguinov *et al.* 2010; Okamoto *et al.* 2014), and appear to link mucosal and neuronal 5-HT pools together (Okamoto *et al.* 2014; Smith *et al.* 2014). 5-HT_3 antagonists can block CMMCs when applied around

Terence Smith (right) is Professor in the Department of Physiology and Cell Physiology at the University of Nevada-Reno, USA, where he is the Director of the Dynamic Imaging Core. After working for several years in solid state physics, he received his PhD in neuropharmacology/electrophysiology from Monash University, Victoria, Australia under Professors Mollie Holman and David Hirst. His interests have focused on the circuitry in the enteric nervous system and how this affects gut pacemakers. **Michael Gershon** (left) is Professor of Pathology and Cell Biology at Columbia University, College of Physicians and Surgeons. He received his MD degree from Cornell University, did post-doctoral research with Professor Edith Bülbring in the Department of Pharmacology of Oxford University, and chaired the Department of Anatomy and Cell Biology at Columbia until 2006. His interests are in enteric neuronal development, cell biology, and function as well as the roles serotonin plays in the bowel.



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fecal pellets, as do intraluminal 5-HT_3 and 5-HT_7 antagonists, suggesting that these antagonists act locally on the EC cell-to-IPAN junction (Heredia *et al.* 2009; Smith *et al.* 2014).

Evidence suggests that neuronal, as well as mucosal, 5-HT is critical for peristaltic reflexes. Serosally applied 5-HT desensitizes ENS receptors, thereby inhibiting peristaltic reflexes (Bülbring & Crema, 1958; Smith *et al.* 2014). 5-HT antagonism interferes with transmission in ENS pathways, CMMCs, and tonic inhibition in the colon (Monro *et al.* 2002; Dickson *et al.* 2010). Neuronal 5-HT can mediate intestinal slow excitatory postsynaptic potentials (sEPSPs): 5-HT_7 antagonists inhibit sEPSPs in IPANs (Monro *et al.* 2005), as well as CMMCs (Dickson *et al.* 2010). Tryptamine, which first releases and then depletes endogenous 5-HT, initially induces but then abolishes sEPSPs without affecting similar responses to exogenous 5-HT (Takaki *et al.* 1985). Anti-idiotypic antibodies, which bind selectively to all 5-HT receptors, also mimic sEPSPs before blocking them irreversibly (Wade *et al.* 1994).

Radial stretch of the bowel wall activates high threshold mechanosensitive interneurons that activate CMMCs (Heredia *et al.* 2009). Mucosal stimuli and radial stretch evoke similar reflex responses because nerve pathways from each converge on final common neurons (Smith *et al.* 1992, 2007). Mucosal reflexes alone propel small fecal pellets that do not produce radial stretch down the colon (Heredia *et al.* 2013); fluid or larger pellets that stretch the gut can be propelled in the absence of the mucosa or mucosal 5-HT (Spencer *et al.* 2011; Heredia *et al.* 2012, 2013). Because stimuli that short circuit mucosal activation evoke CMMC-like responses does not mean the mucosa and its 5-HT are not physiologically critical. Stimuli restricted to the mucosa cannot evoke CMMCs in the TPH1KO colon and thus are 5-HT-dependent (Heredia *et al.* 2013). If the TPH1KO bowel is stretched, CMMC-like responses are evoked; however, they do not propagate and thus are not CMMCs. In an analogy, the lower leg can be made to move involuntarily through the patellar reflex or voluntarily. Voluntary leg movement does not obviate the need for quadriceps muscle spindles to evoke patellar reflexes. Circuits in the myenteric plexus can be engaged in the absence of mucosal 5-HT to give rise to contractile activity; however, under physiological circumstances the mucosa is present and, when pressed, secretes 5-HT. When the gut is intact, therefore, 5-HT will do what it does when the mucosa releases it, initiate peristaltic reflexes.

The argument (Spencer *et al.* 2013) that because reserpine-induced 5-HT depletion fails to prevent CMMCs, 5-HT is not needed is invalid. Because reserpine only inhibits VMAT, it cannot drive tissue 5-HT to zero. Reserpine lowers intracellular 5-HT to levels that may be difficult to detect (Bülbring & Crema, 1959; Spencer *et al.* 2013); however, the 5-HT that remains activates receptors. In fact, constitutive 5-HT release in reserpine-treated animals enhances intestinal motility (Bülbring & Crema, 1959). The continued secretion of 5-HT thus explains the ability of 5-HT_3 and 5-HT_4 antagonists to block responses in reserpinized preparations (Sia *et al.* 2013; Spencer *et al.* 2013). Importantly, 5-HT_3 antagonists do not affect the CMMC-like activity in TPH1KO mice (Heredia *et al.* 2013). The abnormality of CMMCs in TPH1KO mice establishes that physiologically meaningful peristaltic reflexes are 5-HT dependent (Heredia *et al.* 2013).

Total GI transit and colonic motility are slowed in TPH2KO mice but gastric emptying is accelerated (Li *et al.* 2011; Gershon, 2013). Normal GI motility thus requires neuronal 5-HT; however, because neuronal 5-HT is a growth factor, the TPH2KO ENS is severely hypoplastic (Li *et al.* 2011), which could be responsible for defective GI motility. Accelerated gastric emptying probably occurs because serotonergic activation of gastric inhibitory motor neurons is impaired in mice lacking neuronal 5-HT (Li *et al.* 2011). Patterns of GI motility other than peristaltic reflexes evidently compensate for the defective peristaltic reflex/CMMC of TPH1KO mice (Li *et al.* 2011; Gershon, 2013). Compensatory mechanisms, such as enhanced prostaglandin synthesis may also be mobilized to generate CMMC-like activity in the absence of mucosal 5-HT (Heredia *et al.* 2012; Smith *et al.* 2014).

In conclusion, 5-HT has satisfied all of the criteria needed to identify it a mediator of peristaltic reflexes. EC cells and enteric neurons synthesize 5-HT. Exogenous 5-HT and stimuli that release endogenous 5-HT evoke peristaltic reflexes, which are lost or impaired when mucosal and/or neuronal 5-HT is depleted or 5-HT receptors are antagonized. Mucosal and neuronal 5-HT are thus essential for physiological manifestation of peristaltic reflexes.

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Additional information

Competing interests

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