CROSSTALK

CrossTalk opposing view: 5-HT is not necessary for peristalsis

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Contention

Our contention is that endogenous 5-hydroxytryptamine (5-HT) is not necessary for colonic peristalsis, nor other neurogenic motor patterns in the lower gastrointestinal (GI) tract, such as colonic migrating motor complexes (CMMCs).

Background

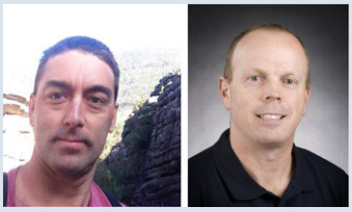
Large quantities of 5-hydroxytryptamine (5-HT) are synthesized in the gut, in enterochromaffin (EC) cells of the mucosa (via tryptophan hydroxylase-1) and in a small proportion in enteric neurons (via tryptophan hydroxylase-2). It has been well established that 5-HT can be released from the mucosa by mechanical stimulation, including contractile activity of the gut and compression of the gut wall (Bertrand, 2006). Numerous 5-HT receptors are expressed on enteric neurons and extrinsic afferent endings, and exogenous 5-HT has potent effects on neuronal excitability and transmitter release (Gershon, 2000). Despite this, the evidence that 5-HT is necessary for peristalsis or CMMCs in the lower GI-tract is all circumstantial. Extensive evidence now suggests that these motor patterns occur independent of 5-HT.

Three lines of disputed evidence

High quantities of 5-HT are synthesized and released from the mucosa. Release of endogenous 5-HT can occur during peristalsis and CMMCs and exogenous 5-HT can evoke peristalsis or CMMCs. The observation that mucosal compression causes release of *endogenous* 5-HT at the same time as the onset of peristalsis or CMMCs does not reflect a causal relationship between these events. Mucosal compression distorts multiple cell types in the gut wall, including the underlying myenteric plexus – which is itself exquisitely sensitive to mechanical deformation (Kunze *et al.* 2000). Compression applied to the myenteric plexus still evokes peristaltic reflexes, even after the mucosa has been removed (Spencer *et al.* 2003) and CMMCs also persisted in these preparations (Keating & Spencer, 2010; Zagorodnyuk & Spencer, 2011). Thus, while mucosal stimulation can release large amounts of *endogenous* 5-HT, we contend that this release is not a *necessary* step in initiating peristalsis or CMMCs. The observation that *exogenous* 5-HT (and many other agonists) can initiate peristalsis and CMMCs does not reveal any information about the functional role of *endogenous* 5-HT.

It has been suggested that *endogenous* 5-HT release from the mucosa initiates the colonic peristaltic reflex (Foxx-Orenstein *et al.* 1996; Grider *et al.* 1996) and is 'critical' for spontaneous CMMCs (Heredia *et al.* 2009). It was reported that 'removing the mucosa appeared to abolish spontaneous CMMCs, suggesting that the mucosa is normally critical for their generation' (Heredia *et al.* 2009) – a conclusion based on four mice. It was further stated that 'the trigger for the CMMC appears to be spontaneous or evoked (i.e. a fecal pellet) release of 5-HT from EC cells to stimulate AH neurons'. Although, 5-HT release from the mucosa was not measured, nor were recordings made from neurons to confirm this hypothesis. If 5-HT release from the mucosa was essential for peristalsis or CMMCs, then both these motor patterns would be expected to cease when the mucosa is removed. They don't. With practice it was demonstrated that the entire mucosa can be reliably removed from an isolated specimen of colon and still record peristalsis (Spencer *et al.* 2011) and *spontaneous* and *evoked* CMMCs (Keating & Spencer, 2010). In these mucosa-free preparations, release of 5-HT that is normally associated with peristaltic or CMMC contractions was abolished. Importantly, when removing the mucosa we observed that if the dissection technique was too vigorous, the delicate neural circuitry required for peristalsis or CMMCs is disrupted. This may explain why spontaneous CMMCs were not recorded after removal of the mucosa in the four mice studied in Heredia *et al.* (2009). We consider it inconceivable that dissection damage in our preparations could have created a whole new motor pattern that mimicked normal peristalsis and CMMCs.

In other studies, electrochemical detection of 5-HT release from the mucosa showed that 5-HT release was only associated with some, *but not all*, contractions underlying peristalsis (Bertrand, 2006) or CMMCs (Keating & Spencer, 2010), again arguing against a causal link between 5-HT release and these colonic motor patterns. If 5-HT release from the mucosa was critical for peristalsis and CMMCs, then deleting the gene responsible for synthesizing mucosal 5-HT (TPH-1) should block CMMCs and affect transit. It doesn't. The laboratory that concluded 5-HT release from the mucosa



Nick Spencer completed his PhD in 1998 in the field of electrophysiology of intestinal smooth muscle, at Monash University. Since then, his primary interests involve the mechanisms that activate intrinsic and extrinsic neural circuits in the gastrointestinal tract. After his PhD, he spent 10 years at the University of Nevada School of Medicine, investigating neuronal mechanisms underlying gastrointestinal motility. The past 7 years, he has been at Flinders University, where he is currently an Associate Professor. **Damien Keating** is Associate Professor in the Discipline of Human Physiology at Flinders University. He received his PhD in cell physiology from the University of Adelaide in 2003 and is currently an R. D. Wright Biomedical Research Fellow with the NHMRC. His research focuses on the mechanisms controlling cell signalling and the release of hormones and neurotransmitters. He currently undertakes a large amount of this work using primary cultures of serotonin-secreting human enterochromaffin cells.

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was critical for CMMCs (Heredia *et al.* 2009) also showed that CMMCs still occurred when mucosal 5-HT synthesis had been prevented (Heredia *et al.* 2013). Furthermore, conscious mice lacking mucosal 5-HT synthesis have *no* inhibitory deficits in GI-transit (Yadav *et al.* 2010; Li *et al.* 2011).

Endogenous 5-HT is synthesized in enteric neurons and numerous 5-HT receptors are expressed in the gut. There is some evidence that 5-HT may be a neurotransmitter in a small population of myenteric (Zhou & Galligan, 1999; Galligan *et al.* 2000) and submucosal neurons (Monro *et al.* 2004) of the small intestine, despite only ∼1% of enteric neurons expressing 5-HT (Costa *et al.* 1982; Wardell *et al.* 1994; Costa *et al.* 1996). For a substance to be a neurotransmitter, it must be 'present in the presynaptic terminal and is released in amounts sufficient to exert a defined action on the postsynaptic neuron or effector organ' (Gardner *et al.* 2000). Based on this criterion, we are not aware of any evidence that *endogenous* 5-HT participates in any synaptic potential in the colon of the mouse (Furukawa *et al.* 1986; Nurgali *et al.* 2004) and rarely (Nurgali *et al.* 2003) or never (Wade & Wood, 1988; Spencer & Smith, 2004) in the colon of the guinea-pig, which are the species we have been studying CMMCs and peristalsis with. In our experience in guinea-pig colon (Spencer & Smith, 2004) and in mouse (Furukawa *et al.* 1986; Nurgali *et al.* 2004), human (Brookes *et al.* 1987) and rat colon (Brookes *et al.* 1988), all fast synaptic potentials are blocked by hexamethonium.

5-HT antagonists can block peristalsis and CMMCs and retard transit. Perhaps the strongest evidence that endogenous 5-HT is necessary for peristalsis is based on the finding that 5-HT antagonists can substantially inhibit or block colonic peristalsis (Foxx-Orenstein *et al.* 1996; Grider *et al.* 1996; Kadowaki *et al.* 1996). However, when these experiments were repeated with the same antagonists (SDZ-205-557 and ondansetron) they were only found to cause a temporary blockade of peristalsis. In the continued presence of both antagonists peristalsis reappeared (Sia *et al.* 2013*a, b*). Importantly, these antagonists also blocked peristalsis in colonic preparations from mucosa-free, reserpine-treated animals, where endogenous 5-HT had been depleted. This showed that endogenous 5-HT may not be necessary for activation of 5-HT3 and 5-HT4 receptors. We suggest that 5-HT3 and 5-HT4 antagonists bind to 5-HT3 and 5-HT4 receptors which are constitutively active even in the absence of any 5-HT. Indeed, ligand-gated 5-HT3 receptors (Hu & Peoples, 2008) and G-protein coupled 5-HT4 receptors (Berthouze *et al.* 2005) both show constitutive activity. These

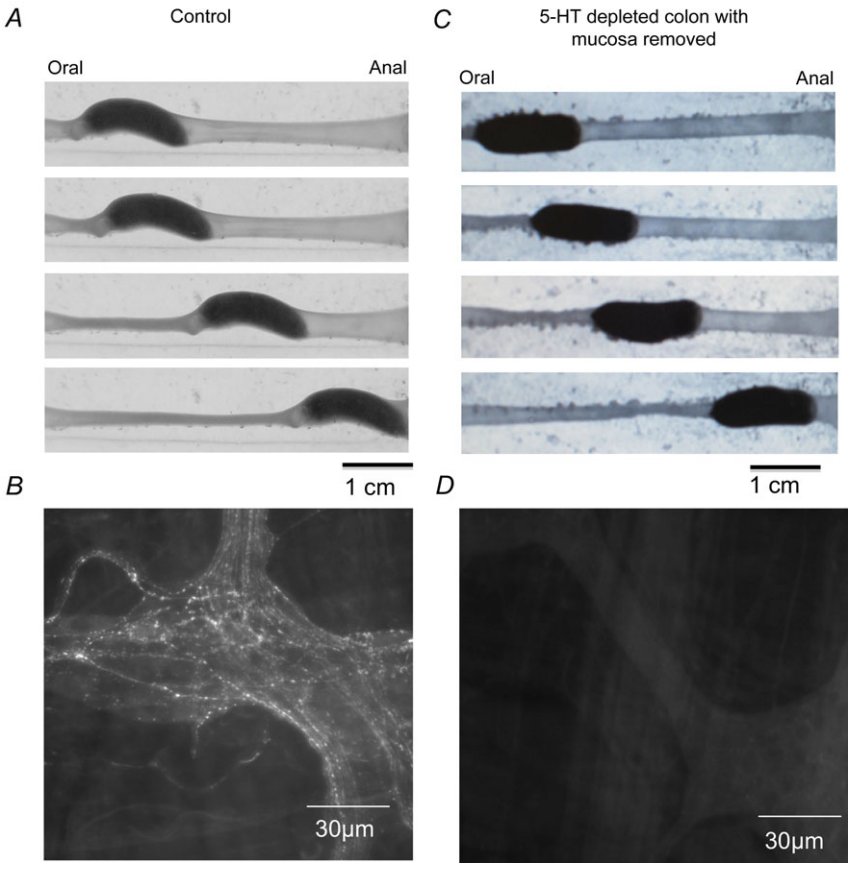

Figure 1. Colonic Paristalsis occurs without endogenous 5-HT
A, peristalsis in the distal colon. *B*, 5-HT immunoreactivity. *C*, in mucosa-free colon also depleted of neuronal 5-HT, peristalsis still occurs. *D*, confirmation of depletion of neuronal 5-HT after reserpine.

antagonists could work as inverse agonists, reducing the constitutive activity of the receptors, rather than blocking the effects of endogenous 5-HT.

At present, we are unsure of the role of 5-HT in the lower GI tract. We do know that 5-HT can have many effects and it *may* modulate enteric circuits and motility. However, recent evidence from a number of laboratories, using a number of techniques, shows that neither mucosal nor neuronal 5-HT is necessary for colonic peristalsis or CMMCs.

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Additional information

Competing interests

None declared.

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