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Controversies involving the role of 5-hydroxytryptamine (5-HT) in generating colonic migrating motor complexes: what is spontaneous?

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It is important to understand and elucidate the mechanisms underlying the colonic migrating motor complex (CMMC), since it is a primary motor event in the large bowel that assists in the movement of fecal contents.¹ Recently, Keating and Spencer² have used tension recordings and real time amperometry to determine the role of 5-hydroxytryptamine (5-HT) in generating CMMCs in the isolated mouse colon. They make a number of conclusions that contradict our recent study.

In our study we concluded that the neural circuitry required for generating the CMMC is within the myenteric plexus. However, under normal circumstances, enterochromaffin cells (ECC) in the mucosa release 5-HT to activate the mucosal endings of sensory neurons in order to bring them to threshold, thereby initiating the CMMC. These conclusions were based on the following observations: 1) Spontaneous CMMCs are blocked by ondansetron (1-5 μ M; 5-HT₃ antagonist) or following removal of the mucosa. To date, we have examined 52 preparations without the mucosa and have not observed a single spontaneous CMMC. 2) In preparations with the mucosa, CMMCs can be evoked by either mucosal stimulation or circumferential stretch. 3) A fecal pellet regulates the direction of CMMCs by activating local reflexes. 4) CMMCs are readily evoked in preparations without the mucosa by electric field stimulation (EFS). Ondansetron reduces the preceding hyperpolarization but does not affect the fast oscillations or slow depolarizing phase of the CMMC. In support of our conclusions, Keating & Spencer² demonstrated that “*high concentrations of 5-HT were being secreted, at least in vitro, from the mucosa*” and that “*most CMMCs (93%) could be temporally correlated with the release of 5-HT*”. Also, they stated that “*5-HT is released by mucosal distortion.....thus it might be expected that a moving fecal pellet would induce 5-HT release.....where it might modulate the firing of the intrinsic pacemaker, which must lie in the myenteric plexus*”.

In contrast to our study, Keating and Spencer found that “spontaneous” CMMCs occurred in preparations either with or without the mucosa; CMMCs in both preparations were completely blocked by ondansetron (3 μ M). This lead them to conclude that “*the intrinsic pacemaker and pattern generator underlying CMMC generation lies within the myenteric plexus and/or muscularis externa and does not require any release of 5-HT from enterochromaffin cells. Endogenous release of 5-HT from enterochromaffin cells plays a modulatory role, not an essential role in CMMC generation.*”

Keating & Spencer implied that the reason we didn't see any spontaneous CMMCs following the removal of the mucosa was because our dissection technique damaged the myenteric plexus.

This is unlikely for a number of reasons: 1) The low resting membrane potential and spontaneous inhibitory junction potentials in the circular muscle are preserved in these preparations, implying that the neural circuits mediating tonic inhibition are intact. 2) CMMCs were readily evoked by EFS, suggesting that myenteric nerve pathways are undamaged. In fact, the evoked CMMC in preparations without the mucosa is blocked by a 5-HT₇ antagonist³ but not by ondansetron (3 μ M), which only blocks the preceding inhibition, suggesting that descending 5-HT interneurons are also viable.

We believe that the differences in pharmacology and spontaneous CMMC activity in preparations devoid of the mucosa could be attributed to: 1) dissection technique and 2) circumferential stretch. 1) During our dissection we always used ice cold Krebs' solution in order to slow the release of 5-HT from ECC in the mucosa in order to minimize the uptake of 5-HT into descending "accumulating" interneurons.^{4,5} It's unclear in their manuscript whether Keating & Spencer dissected away their mucosa in ice cold or warm Krebs' solution. If they used warm Krebs', then the mucosa would be expected to release copious amounts of 5-HT as it was being stripped away that would likely load 5-HT "accumulating" interneurons,^{4,5} perhaps accounting for the differences in pharmacology. 2) Their preparations appear to be under significant circumferential stretch. They used a rake system (~10mm long) at several different sites along the colon. Although they didn't mention their resting tension it appears that their preparations were under considerable amounts of circumferential stretch, since some of their CMMC contractions are enormous (~150mN/15g). We used a minimal amount of tension to record our spontaneous CMMCs, since we did not want to unduly perturb the system from its normal physiological state by activating stretch reflexes.¹ Our contractions were ~10-30mN, which were similar to most in the existing literature. Also, in preparations with the mucosa, their control CMMCs were abnormally frequent (1/min as compared to the accepted 0.3/min); whereas in preparations without the mucosa they were considerably reduced in frequency. Also, in both their preparations the conduction velocities of CMMCs were very rapid (4-5mm/s) compared to what we have reported. In fact, our conduction velocity for the CMMC of 0.8mm/s corresponds to the velocity of propagation of a fecal pellet, which is not surprising since we demonstrated that the CMMC is necessary for fecal pellet propulsion.¹

Therefore, we suggest that Keating & Spencer are not studying a physiologically normal preparation, but are largely evoking CMMCs by circumferential stretch in preparations where the descending interneurons are heavily loaded with 5-HT. We suggest that due to the stretch sensitivity of the colon that the resting conditions be accurately reported to avoid confusion regarding the conditions that result in so called "spontaneous" activity.

In conclusion, we agree with Keating & Spencer that the myenteric plexus generates and propagates CMMCs, however we believe that the release of 5-HT from the colonic mucosa is normally integral in the initiation of CMMCs rather than a modulator, as proposed by Keating & Spencer.

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References

1. Heredia DJ, Dickson EJ, Bayguinov PO, Hennig GW, Smith TK. Localized release of serotonin (5-hydroxytryptamine) by a fecal pellet regulates migrating motor complexes in murine colon. *Gastroenterology* 2009 Apr;136(4):1328–38. Epub 2008 Dec 10. [PubMed: 19138686]

2. Keating DJ, Spencer NJ. Release of 5-hydroxytryptamine from the mucosa is not required for the generation or propagation of colonic migrating motor complexes. *Gastroenterology*. 2009 e-pub In Press.
3. Dickson EJ, Heredia DJ, Hennig GW, Smith TK. The importance of 5-HT 1A and 5-HT 7 receptors during murine colonic migrating motor complex. *Neurogastroenterol Motil* 2009;21 1:48. Abstract 151.
4. Meedeniya ACB, Brookes SJH, Costa M. Sources of 5-hydroxytryptamine immunoreactivity in the myenteric plexus of the guinea-pig small intestine. *Proc Aust Neurosci Soc* 1994a;5:176.
5. Meedeniya ACB, Brookes SJH, Costa M. Identification of 5- hydroxytryptamine handling neurones and their distribution in the myenteric plexus of the guinea-pig small intestine. *Proc Aust Physiol Pharmacol Soc* 1994b;25:157.