The pacemaker and pattern generator underlying colonic migrating motor complexes does not require release of serotonin from the mucosa

D.J. Keating and N.J. Spencer, Department of Human Physiology, School of Medicine, Flinders University, SA 5001, Australia.

Introduction. Colonic migrating motor complexes (CMMCs) are cyclical contractions of the colonic smooth muscle layers which occur *in vivo* and *in vitro* and propagate over large distances along the large bowel, facilitating the propulsion of colonic contents. The location of the pacemaker that generates CMMC rhythmicity is unknown, but must lie within the colon itself, since CMMCs occur in an isolated whole colon. Data obtained from a number of laboratories strongly suggests that the activity of the CMMC pacemaker involves release of serotonin (5-HT) from the intestinal epithelium, since antagonists of the 5-HT_3 receptor slow the CMMC pacemaker and prolong intestinal transit, making these drugs effective in relieving the symptoms of diarrhea-predominant irritable bowel syndrome (D-IBS). What is not clear, is how or where the release of 5-HT from EC cells acts to control the CMMC pacemaker, or whether EC cells themselves even form part of the CMMC pacemaker mechanism. Since 5-HT is known to be released specifically from EC cells, we have correlated, in real time, the release of serotonin from EC cells, whilst recording CMMCs in isolated whole preparations of mouse colon.

Aim. To determine the role of endogenous serotonin release from EC cells in the generation and propagation of CMMCs.

Methods. Carbon fibre electrodes were used to record the dynamic release of 5-HT from a population of ECs in the mid colon, whilst at the same time, recordings were made of spontaneously occurring CMMCs propagating from the proximal to distal colon using isometric mechanical recording techniques. The whole colon was isolated from male C57BL/6 mice and a longitudinal incision made along the whole length. Two types of preparation were used: one type where the entire colon was intact and measurements of 5-HT release were made from EC cells, and a second type of preparation where the mucosa, submucosa and submucosal plexus were removed from the whole colon, and measurements of 5-HT release were made from the circular muscle.

Results. It was found that each CMMC contraction was commonly found to temporally correlate with a simultaneous release of 5-HT from the mid colon. To test whether the CMMC pacemaker required release of 5-HT from EC cells, we dissected off the mucosa, submucosa and submucosal plexus from the entire full length of colon. Removal of these structures abolished all cyclical rises in 5-HT release from the mucosa, but did not prevent the cyclical generation of CMMCs. Specifically, it was found that removal of the mucosa, submucosa and submucosal plexus caused a slight, but insignificant decrease in CMMC rhythmicity (control interval: 1.5 ± 0.27min; *c.f.* after mucosal removal: 2.0 ± 0.3min; n =4: $p = 0.26$), but no significant differences in CMMC amplitudes (control: 38.8 ± 11.1mN, *c.f.* without mucosa: 44.9 ± 7.6mN; n = 4: $p = 0.66$), or half durations (control: 14.7 ± 4.3 *c.f.* without mucosa: 18.3 ± 2.9s; n = 4; $p = 0.52$). All CMMC activity that persisted in preparations consisting of only myenteric ganglia and smooth muscle were abolished by hexamethonium 200μM; n = 3.

Conclusions. The pacemaker and pattern generator underlying the cyclical generation and propagation of CMMCs is located within the myenteric plexus, and does not require release of serotonin, nor any other endogenous substances from the colonic epithelium. Also, no evidence was found to suggest that intrinsic neural inputs from the submucosal plexus were required for CMMC generation or propagation.