Identification of the pacemaker mechanism underlying migrating motor complexes in mouse small intestine

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Introductions: Migrating motor complexes (MMCs) are spontaneous contractions of the small intestine, which occur cyclically and propagate over large lengths of intestine, leading to the propulsion of intestinal content. Although the mechanism underlying MMC generation is unknown, there has been a resurgence of interest in the mechanisms underlying the pacemaker or "clock" that generates spontaneous MMCs. This is because in human patients with diarrhea-predominant IBS (D-IBS) the frequency of MMC is altered and thought to be responsible for the abdominal pain and discomfort underlying the disease. Data obtained from a number of laboratories strongly suggests that the activity of the MMC pacemaker involves release of serotonin (5-HT) from the intestinal epithelium (mucosa), since antagonists of the 5-HT3 receptor slow the MMC pacemaker and prolong intestinal transit, making these drugs effective in relieving the symptoms of 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 MMC pacemaker mechanism. Greater than 90% of all the 5-HT in the body is made and released specifically by EC cells in the intestinal mucosa, so an understanding of whether EC cells and 5-HT release from EC cells is involved in the MMC pacemaker mechanism is of supreme importance to clinical treatment of D-IBS. Although the location of the pacemaker that generates MMC rhythmicity is unknown, it must lie within the small intestine itself, since MMCs still occur in an isolated ileum.

Aim: To determine the role of the mucosa and submucosal plexus (and hence endogenous serotonin release from EC cells) in the generation and propagation of spontaneous MMCs.

Methods: Spontaneously occurring MMCs were recorded from isolated intact segments of mouse ileum at 36°C. Simultaneous mechanical recordings were made from three site along the ileum to determine the propagation velocity and direction of velocity of MMCs. The primary aim was to determine whether MMCs would still occur if the mucosa and submucosal plexus were removed from the ileum and if so, how are their properties different from control preparations with mucosa present.

Results: In intact preparations of ileum, regular MMCs were recorded, with a mean interval of 4.1 ± 0.1 min (n=5). To test whether the mechanisms underlying MMC generation required the mucosa, we sharp dissected off the mucosa, submucosa and submucosal plexus from the entire full length of ileum. Removal of these structures did not prevent the cyclical generation of MMCs. Surprisingly, the pacemaker frequency actually increased, such that the mean interval between MMCs after the removal of the mucosa, submucosa and submucosal plexus was 2.6 ± 0.2 min; n=5 ($p<0.05$). There were no significant differences in MMC amplitudes (control: 0.5 ± 0.06mN, *c.f.* without mucosa: 0.1 ± 0.01mN; n=4: $p<0.05$), or half durations (control: 29.7 ± 3.1s *c.f.* without mucosa: 16.8 ± 1.3s; n=4; $p<0.05$). MMCs that persisted in dissected preparations consisting of only myenteric ganglia and smooth muscle were abolished by hexamethonium 200μM; n=3.

Conclusions: These results lead to the inescapable conclusion that in the small intestine, the pacemaker and pattern generator underlying the cyclical generation and propagation of MMCs is located within the myenteric plexus and/or *muscularis externa* and do not require the release of substances from the mucosa or neuronal activity within the submucosal plexus for their spontaneous activation.