Neural control mechanisms underlying motility in guinea pig and human intestine

A thesis submitted in total fulfillment of the requirements of the degree of Doctor of Philosophy

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Summary

The patterns of motor activity in the lower gastrointestinal tract of mammals and the mechanisms underlying their generation are incompletely understood. In this thesis, experiments were performed to provide greater insight into the role of the enteric nervous system in the generation of different propulsive motor patterns in the isolated guinea pig and human lower gastrointestinal tract.

In Chapter 2, we revealed the presence of a novel form of colonic peristalsis that was surprisingly preserved despite complete blockade of major excitatory neurotransmitters (acetylcholine, tachykinins) at both enteric neuro-neuronal and neuro-muscular junctions. It was also shown that following blockade of major excitatory neuro-neuronal and neuro-muscular transmitters an intrinsic oral-aboral polarity underlying neurogenic propulsive motor patterns was always preserved.

In Chapters 3-4, the role of endogenous serotonin in the generation and propagation of colonic peristalsis was investigated. It was found that in preparations acutely depleted of all endogenous serotonin, peristalsis was still preserved with remarkably few deficits. We also demonstrated that selective antagonists of 5-HT3 and 5-HT4 receptors could still exert a temporary blockade of peristalsis despite the absence of any detectable endogenous serotonin. These raise support for the notion that 5-HT3 and 5-HT4 receptors can display constitutive activity and the antagonists can behave as inverse agonists.

Experiments in Chapters 5 were conducted on isolated segments of human bowel and the patterns of motor activity characterised in terminal ileum and colon *ex vivo*. Long segments of bowel were preserved *ex vivo* which allowed us to preserve enteric neural activity and record propulsive neurogenic motor patterns. From our small bowel studies, we report in this thesis that propagating motor patterns are only preserved in longer segments of bowel tissue, suggesting that an intact neural circuitry is vital for their generation.

Experiments on the human colon *ex vivo* allowed for characterisation of the motor activity in what we considered "experimental control tissue" and compared these activities with those obtained from colonic specimens from patients with slow transit constipation (STC) (Chapter 6). We have recorded similar motor activities and contractile patterns, even in specimens from patients with STC. The presence of an underlying contractile activity that appeared similar to that seen in healthy controls raises the possibility that the aetiology underlying slow transit constipation may be induced by alterations in extrinsic neural inputs, rather than any overt dysfunction of the ENS. These experiments pave the way for an exciting future of experimentation.

Declaration

I declare that the contents of this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university and to the best of my knowledge it does not contain any material previously published or written by another person except where due reference is made in the text.

Tiong Cheng Sia, August 2014

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Publications

Sia, TC; Brookes, S; Dinning, P; Wattchow, D; Spencer, NJ. 'Peristalsis and propulsion of colonic content can occur after blockade of major neuro-neuronal and neuro-muscular transmitters in isolated guinea pig colon' **American Journal of Physiology** (2013) 305: G933-G939

Sia, TC; Whiting, M, Kyloh, M; Nicholas, S; Brookes, S; Oliver, J; Dinning, P; Wattchow, D; Spencer, NJ. '5-HT3 and 5-HT4 antagonists inhibit peristaltic contractions in guinea pig distal colon by mechanisms independent of endogenous 5-HT' **Frontiers in Neuroscience** (2013) 7:136

Sia, TC; Flack, N; Robinson, L; Kyloh, M; Nicholas, S; Brookes, SJ; Wattchow, DA; Dinning, P; Oliver, J; Spencer, NJ. 'Is Serotonin in enteric nerves required for distension-evoked peristalsis and propulsion of content in guinea pig distal colon?' **Neuroscience** (2013) 240: 325-335

Sia, TC; Kuizenga, M; Dodds, K; Wiklendt, L; Arkwright, J; Thomas, T; Brookes, S; Spencer, NJ; Wattchow, DA; Dinning, P; Costa, M. 'Neurally mediated discrete clustered contractions propagating in *ex vivo* segments of human ileum'. **American Journal of Physiology** (2014) (electronically published ahead of print)

Raghupathi, R; Duffield, M; Zelkas, L; Meedeniya, A; Brookes, S; Sia, TC; Wattchow, D; Spencer, NJ; Keating, D. 'Identification of unique release kinetics of serotonin from guinea pig and human enterochromaffin cells' **Journal of Physiology** (2013) 591: 5959-5975

Spencer, NJ; Nicholas, SJ; Sia, TC; Staikopoulos, V; Kyloh, M; Beckett, EA. 'By what mechanism does ondansetron inhibit colonic migrating motor complexes: Does it require endogenous serotonin in the gut wall?' **Neurogastroenterology & Motility** (2013) 25:677-685

Spencer, NJ; Kyloh, M; Wattchow, DA; Sia, TC; Nicholas, S; Brookes, SJ. Characterization of motor patterns in isolated human colon: are there differences in patients with slow-transit constipation? **American Journal of Physiology** (2011) 302: G34-43

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Conference proceedings

2014 Royal Australasian College of Surgeons Paper Day

- "Motility recordings *in vivo* and *ex vivo* shed insight into the etiology of slow transit constipation" (Oral)

Special Seminar, Centre for Neuroscience, Flinders University, SA

- "Investigations in the neural mechanisms that govern peristalsis in the guinea pig and human lower gastrointestinal tract" (Oral)

Australian Neuroscience Society Annual Conference, Adelaide, SA

- "Mechanisms underlying hexamethonium and atropine resistant peristalsis in the guinea pig distal colon" (poster)

2013 Royal Australasian College of Surgeons (SA) Paper Day

- Is there a functional role for serotonin neurotransmission in peristalsis (Oral)

- Evidence of peristalsis in the *ex vivo* human small bowel (Oral)

Royal Australasian College of Surgeons Annual Scientific Meeting Tripartite- WA, SA, NT

- "Investigations into the mechanisms underlying peristalsis in the human small bowel" (Oral)

Flinders Medical Centre Grand Round

- *Ex vivo* Peristalsis in the Human Lower Gastrointestinal Tract: Where are we now? (Oral)

Australian Neuroscience Society Annual Scientific Conference (ANS ASC), Melbourne, VIC

- A human model for understanding small bowel motility (Oral)

Royal Australasian College of Surgeons Annual Scientific Congress, Auckland, New Zealand

- Comparison of human *ex vivo* and *in vivo* ileal motor patterns; is myogenic activity preserved in an organ bath? (Oral)

2012 Surgical Research Society of Australasia Annual Scientific Meeting, Adelaide, SA

- An *ex vivo* model for studying human small bowel and colonic motor activity (Oral)

Australian Neuroscience Society Annual Scientific Conference, Gold Coast, QLD

- Endogenous Serotonin is Not required in the Generation of Colonic Peristalsis (Oral)

- *Ex vivo* characterization of the human colon: a pioneering effort

Royal Australasian College of Surgeons (SA) Paper Day

- Insights into serotonergic pathways in colonic motility (Oral)

- Behaviour of the human small bowel and colon in vitro: some early findings (Oral)

Neurogastroenterology and Motility Annual Convention, Bologna, Italy

- Identification of different patterns of propagating motor activity in the isolated human colon (Poster)

Digestive Diseases Week, San Diego, CA, USA

- *Ex vivo* Characterisation of the Human Colon (Poster)

Royal Australasian College of Surgeons Annual Scientific Congress, Kuala Lumpur, Malaysia

- Is there a need to target serotonergic pathways in colonic dysmotility? (Poster)

- *Ex vivo* analysis of colonic motility from extended lengths of resected human colon (Poster)

2011 Oral- Royal College of Surgeons (SA) Paper Day

- Role of serotonin in Colonic Motility (Oral)