

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**

Dr Tiong Cheng Sia

MBBS/ BMedSci

Discipline of Human Physiology
Flinders Medical Science and Technology
Centre for Neuroscience
School of Medicine, Flinders University
Adelaide, South Australia

August 2014

Table of Contents

TABLE OF CONTENTS	III
SUMMARY	XI
DECLARATION	XIII
ACKNOWLEDGMENTS	XV
PUBLICATIONS	XVII
CONFERENCE PROCEEDINGS	XIX
CHAPTER 1: REGULATION OF MOTILITY IN THE LOWER GASTROINTESTINAL TRACT	1
1.1. Anatomy of the lower gastrointestinal tract	2
1.1.1. Components of the gastrointestinal tract	2
1.1.2. The bowel wall	3
1.1.3. Inter-species correlation	4
1.2. Classification of neurons in the GI tract	5
1.3. Neural control of peristalsis- intrinsic innervation	7
1.3.1. Myenteric plexus	8
1.4. Mediators of excitatory post-synaptic potentials	9
1.4.1. Acetylcholine (ACh)	9
1.4.2. Tachykinins	10
1.4.3. Serotonin	11
1.4.4. Adenosine triphosphate (ATP)	24
1.5. Mediators of inhibitory post-synaptic potentials	25
1.5.1. Non adrenergic, Non cholinergic inhibitory neurotransmission (NANC)	25
1.5.2. Nitric oxide (NO)	26
1.5.3. Vasoactive intestinal peptide (VIP)	27
1.5.4. Opioid peptides	27
1.6. Myogenic factors in gastrointestinal motility	28
1.6.1. Smooth muscles in the gastrointestinal tract	28
1.6.2. Interstitial cells of Cajal (ICC)	29
1.7. Extrinsic innervation of the intestine	32
1.8. Motility patterns in the small intestine	33
1.8.1. Peristalsis and the “Law of the intestine”	33
1.8.2. Segmenting contractions	35

1.8.3. Migrating Motor Complexes (MMC)	36
1.9. Motility patterns in the large intestine	38
1.9.1. The peristaltic reflex and the “Law of the intestine” in the colon	39
1.9.2. Colonic migrating complexes (CMMCs)	40
1.10. Quantification of <i>in vivo</i> motor patterns using different technologies	43
1.10.1. Transit studies	44
1.10.2. Manometry	44
1.10.3. <i>Ex vivo</i> Investigations	47
1.11. Specific aims of this thesis	49
CHAPTER 2: NOVEL INSIGHTS INTO NEURO-NEURONAL AND NEURO-MUSCULAR TRANSMISSION UNDERLYING DISTENSION-EVOKED COLONIC PERISTALSIS IN THE GUINEA PIG COLON	51
2.1. Abstract	52
2.2. Introduction	53
2.3.1. Aims	54
2.4. Methods	55
2.4.1. Experimental protocol	55
2.4.2. Mechanical recordings from the circular muscle during peristalsis and faecal pellet propulsion	56
2.4.3. Video imaging of peristalsis and generation of spatio-temporal maps	56
2.4.4. Measurements and Statistics	57
2.4.5. Drugs and Solutions	57
2.5. Results	58
2.5.1. Hexamethonium resistant peristalsis	58
2.5.2. The effect of atropine on hexamethonium-resistant peristalsis	59
2.5.3. The effects of NK-2 receptor blockade on hexamethonium- and atropine-resistant peristalsis	60
2.5.4. Is there recovery of peristalsis after blockade with TTX?	61
2.5.5. Effects of ω -conotoxin on distension-induced peristalsis	61
2.5.6. An intrinsic polarity underlying peristalsis in the presence of neuro-neuronal and neuro-muscular antagonists	62
2.6. Discussion	63
2.6.1. Presence of a novel form of peristalsis	63
2.6.2. Is hexamethonium, atropine and NK-2 receptor resistant-peristalsis mediated by a neural phenomenon?	64
2.6.3. Why is there an intrinsic polarity underlying peristalsis in the presence of hexamethonium, atropine, NK-2 receptor antagonists and ω -conotoxin?	65
2.7. Conclusion	66
FIGURES: CHAPTER 2	67
Figure 1. Preservation of peristalsis in antagonists of major neuro-neuronal and neuro-muscular junctions	68
Figure 2. Preservation of peristalsis in the presence of combinations of antagonists	70

Figure 3. Proportional breakdown of the effects of various antagonists used independently and in combination	72
Figure 4. Velocities of propagation in presence of major neuro-neuronal and neuro-muscular junction antagonists	74
Figure 5. Preservation of an intrinsic polarity in the presence of major neuro-neuronal and neuro-muscular junction blockade	76
CHAPTER 3: IS SEROTONIN IN ENTERIC NERVES REQUIRED FOR DISTENSION-EVOKED PERISTALSIS AND PROPULSION OF CONTENT IN GUINEA PIG DISTAL COLON?	79
3.1. Abstract:	80
3.2. Introduction	81
3.3. Methods	83
3.3.1. Preparation of tissues	83
3.3.2. Technique to deplete endogenous 5-HT from the enteric nervous system	84
3.3.3. Terminology used to define different types of preparations	84
3.3.4. Protocol for measuring faecal pellet propagation	85
3.3.5. Mechanical recordings of circular muscle contractility during peristalsis evoked by a fixed faecal pellet	85
3.3.6. Fluid-infused peristalsis	86
3.3.7. Video imaging of peristalsis and generation of spatio-temporal maps	86
3.3.8. Measurements and Statistics	87
3.3.9. Immunohistochemistry	87
3.3.10. Drugs and Solutions	88
3.4. Results	88
3.4.1. Effects of reserpine pretreatment on endogenous 5-HT in myenteric ganglia	88
3.4.2. Effects of depletion of endogenous 5-HT on faecal pellet distension induced peristalsis	88
3.4.3. Effects of 5-HT ₃ and 5-HT ₄ receptor antagonists on peristalsis evoked by natural faecal pellets	90
3.4.4. Effects of depletion of endogenous neuronal 5-HT on fluid-distension induced peristalsis	91
3.4.5. Effects of 5-HT ₃ and 5-HT ₄ receptor antagonists on peristalsis evoked by slow constant fluid infusion	92
3.5. Discussion	93
3.5.1. Peristalsis is reliably reproduced following complete depletion of endogenous serotonin	93
3.5.2. The role of endogenous 5-HT in synaptic transmission and its receptors in peristalsis	95
3.6. Conclusion	97
FIGURES: CHAPTER 3	99
Figure 1. Immunohistochemical evidence of 5-HT depletion in the myenteric plexus of guinea pig distal colon	100
Figure 2. Evidence of peristalsis in serotonin depleted guinea pig distal colon <i>ex vivo</i>	102
Figure 3. Characteristics of peristaltic events unchanged in 5-HT depleted guinea pig distal colon <i>ex vivo</i>	104

Figure 4. Characteristics of velocity of peristalsis and proportion of continuous or staggered peristaltic runs before and after exposure to antagonists of 5-HT3 and 5-HT4 receptors.	106
Figure 5. Fluid distension evoked peristaltic events unaffected by 5-HT depletion	108
Figure 6. Effect of 5-HT3 and 5-HT4 antagonists on frequency of peristaltic events	110
Figure 7. Spatio-temporal mapping showing peristalsis unaffected in the presence of 5-HT3 and 5-HT4 antagonists	112
CHAPTER 4. 5-HT3 AND 5-HT4 ANTAGONISTS INHIBIT PERISTALTIC CONTRACTIONS IN GUINEA PIG DISTAL COLON BY MECHANISMS INDEPENDENT OF ENDOGENOUS 5-HT	115
4.1. Abstract	116
4.2. Introduction	117
4.3. Methods	119
4.3.1. Preparation of tissues	119
4.3.2. Technique to deplete endogenous 5-HT from the enteric nervous system	120
4.3.3. Terminology used to define different preparations	120
4.3.4. Mechanical recordings of circular muscle contractility during peristalsis evoked by a fixed faecal pellet	121
4.3.5. Measurements and Statistics	121
4.3.6. Immunohistochemistry	122
4.3.7. Drugs and Solutions	122
4.3.8. Chemical Analysis of Tissue Extracts for 5-HT by Tandem Mass Spectrometry	123
4.4. Results	123
4.4.1. Peristaltic contractions in control environments	123
4.4.2. Effects of 5-HT3 or 5-HT4 receptor antagonist on peristaltic contractions	124
4.4.3. Confirmation of depletion of 5-HT from enteric nerves using Immunohistochemistry and mass spectrometry	126
4.5. Discussion	127
4.5.1. By what mechanism could 5-HT antagonists cause temporary or sustained inhibition of peristaltic contractions in preparations depleted of endogenous 5-HT?	128
4.5.2. Why do the same 5-HT3 and 5-HT4 antagonists have different effects on peristaltic contractions evoked in the same preparation of distal colon?	129
4.6. Conclusion	131
FIGURES: CHAPTER 4	133
Figure 1. Experimental set up	134
Figure 2. Transient effect of combined antagonism of 5-HT3 and 5-HT4 receptors in guinea pig distal colon <i>ex vivo</i>	136
Figure 3. Proportional breakdown of the effect of 5-HT3 and 5-HT4 antagonists on guinea pig distal colon <i>ex vivo</i>	138
Figure 4. Effect of 5-HT3 and 5-HT4 antagonists on force of peristaltic contractions	140

Figure 5. Effect on frequency of contractions in the presence of 5-HT3 and 5-HT4 receptor antagonists	142
Figure 6. Recovery of peristalsis in the presence of 5-HT3 and 5HT4 receptor antagonists	144
Figure 7. Effect of 5-HT3 and 5-HT4 receptor antagonists in guinea pig distal colon with mucosa resected	146
Figure 8. Proportional breakdown of the effect of 5-HT3 and 5-HT4 receptor antagonists	148
Table 1. Efficacy of reserpine induced neuronal 5-HT depletion as measured by Mass Spectrometry	150
CHAPTER 5: IDENTIFICATION OF NEUROGENIC PERISTALSIS IN THE <i>EX VIVO</i> HUMAN SMALL INTESTINE	153
5.1. Abstract	154
5.2. Introduction:	155
5.3. Methods:	157
5.3.1. Ethics approval	157
5.3.2. <i>Ex vivo</i> recordings	157
5.3.3. <i>In vivo</i> recordings	162
5.3.4. Measurements and statistics:	162
5.4. Results:	164
5.4.1. Presence of an underlying phasic contractile activity in the human terminal ileum	164
5.4.2. Propagating events	165
5.4.3. Presence of enteric nervous system modulating the underlying phasic contractile activity	166
5.4.4. <i>In vivo</i> water perfused recordings	167
5.4.5. Example of luminal distension that modifies the directionality of motor activity in isolated human small bowel	167
5.4.6. Video imaging of contractile activity	168
5.5. Discussion	168
5.5.1. Confirmation of the role of the enteric nervous system in the generation of motor activity in the isolated human small intestine	168
5.5.2. Role of combining multiple simultaneous recording techniques	169
5.5.3. Increased frequency of phasic contractile activity <i>ex vivo</i> compared to <i>in vivo</i>	171
5.5.4. Are <i>ex vivo</i> experiments representative of <i>in vivo</i> physiological conditions?	172
5.6. Conclusion	174
FIGURES: CHAPTER 5	175
Figure 1. Cross sectional cartoon of experimental set up	176
Figure 2. Photograph of experimental technique	178
Figure 3. View from high-resolution video imaging for generation of spatio-temporal mapping	180

Figure 4. Characteristics of contractions generated by both force transducer and fibre-optic catheter recordings	182
Figure 5. Presence of propagating activity in the <i>ex vivo</i> human terminal ileum recorded using 3 separate recording techniques	184
Figure 6. Characteristics of propagating contractions recorded from isolated human terminal ileum	186
Figure 7. Effect of lidocaine on propagating activity	188
Figure 8. Presence of an underlying phasic contractile activity unmasked in the presence of lidocaine	190
Figure 9. Presence of a dominant underlying phasic contractile frequency as seen using Fast Fourier transformation	192
Figure 10. Effect of luminal distension on human terminal ileum <i>ex vivo</i>	194
Figure 11. <i>In vivo</i> recording allows for comparison of parameters seen <i>ex vivo</i>	196
Figure 12. Presence of segmental contractile activity seen <i>in vivo</i> in the small bowel of anaesthetised human patients	198
CHAPTER 6: COMPARISON OF <i>IN VIVO</i> WITH <i>EX VIVO</i> RECORDINGS OF THE HUMAN COLON IN SLOW TRANSIT STATES REVEALS INSIGHT INTO MECHANISMS OF PERISTALSIS AND DISEASE PATHOPHYSIOLOGY	201
6.1. Abstract	202
6.2. Introduction	203
6.3. Methods	205
6.3.1. Overview	205
6.3.2. Patient selection	206
6.3.3. Profiles for control patients	207
6.3.4. Profiles for STC patients	208
6.3.5. Colonic manometry	209
6.3.6. Colonoscopic placement of the fiber-optic catheter	210
6.3.7. <i>Ex vivo</i> recording of colonic motility	210
6.3.8. Data Analysis	211
6.4. Results	212
6.4.1. Table 1: Anterograde propagating sequences	213
6.4.2. Table 2: Retrograde propagating sequences	214
6.4.3. Non-propagating activity	215
6.5. Discussion:	215
FIGURES: CHAPTER 6	219
Figure 1. Experimental protocol	220
Table 1. STC patient profiles	222
Figure 2. Evidence for STC and exclusion of other conditions in patient 1	224

Figure 3. Evidence for STC and exclusion of other conditions in patient 2	226
Figure 4. Presence of contractile activity in the control specimens of human colon <i>ex vivo</i>	228
Figure 5. Presence of contractile activity in the human STC colon <i>ex vivo</i>	230
Figure 6. Presence of an underlying phasic contractile activity in the <i>ex vivo</i> colon	232
CHAPTER 7: DISCUSSION	234
REFERENCES	247

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

Acknowledgements

This thesis in whole was carried out under the supervision of Associate Professor Nick Spencer, Professor David Wattchow, Associate Professor Phil Dinning and Professor Simon Brookes. It was with their guidance, support and friendship that this thesis has come to its completion.

This thesis would not have been possible if not for my primary supervisor and dear friend Dr Nick Spencer. These three years of expert guidance, stimulating discussions and ongoing encouragement have been invaluable.

In addition to patient guidance, encouragement and useful critiques of the research work in this thesis, I am also grateful to Professor Wattchow for his role in my clinical education in general surgery. I have learnt significantly from this apprenticeship in surgery, both academically and technically.

I am indebted to Dr Phil Dinning for his expertise and support in realising our human experiments, and his amazing abilities to bridge the gap between the world of basic science and clinical medicine.

Special thanks to Professor Simon Brookes for his constructive advice and piercing insights into our experiments.

It was a privilege to have learnt from Professor Marcello Costa, a true giant in the field of Neurogastroenterology.

Melinda Kyloh, Kelsi Dodds- 2 special people whom I am forever grateful for their help, support, and never-ending entertainment over the years. They are true experts in their fields, and experts in the science of happiness. Lee Travis, Merel Kuizenga and Sarah Nicholas, for their help in the experimental processes and friendship.

Dr Dayan De Fontgalland- who started this whole crazy idea of devoting 3 years out of clinical training to have the best time of my life.

My family. To which I am forever grateful.

And lastly, Kailin Teh for her unending support.

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, Kylloh, 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; Kylloh, 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; Zekas, 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; Kylloh, 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; Kylloh, 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

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)