This work contains no material which has been accepted for the award of any other
degree or diploma in any university or tertiary institution, and to the best of my
knowledge, contains no material previously published or written by another person
except where due reference has been made in the text,
I give consent to this copy of my thesis, when deposited in the university library, to
be made available for loan and photocopying, subject to provisions of the Copyright
Act 1968.

James Arthur Slattery
B. Sci (Biomed) (Hons, First Class), MBBS

December 2010
# TABLE OF CONTENTS

## ACKNOWLEDGEMENTS
- Publications arising from this thesis
- Conference Proceedings

## ABBREVIATIONS

## SUMMARY

## INTRODUCTION

1. Anatomy of Innervation of the Gastro-intestinal tract
   1.1 Vagus Nerve
      - Figure 1. Vagal afferent and efferent pathway.
   1.1 a) Intra Ganglionic Laminar Endings (IGLE)
   1.1 b) Intramuscular Arrays
   1.1 c) Mucosal Afferents
   1.2 Spinal Afferents

2. Functional properties of visceral afferent endings
   2.1 Vagal Afferents
      - 2.1 a) Mucosal Receptors
      - 2.1 b) Tension Receptors
      - 2.1 c) Tension Mucosal (TM) Receptors
   2.2 Spinal Afferents
      - 2.2 a) Mucosal Receptors
      - 2.2 b) Muscular Receptors
      - Lumbar Splanchnic Nerve:
      - Pelvic Nerve:
      - 2.2 c) Serosal and Mesenteric

3. Pharmacology of Visceral Afferents
   3.1 Excitatory Receptors
      - 3.1 a) Adenosine triphosphate (ATP)
      - 3.1 b) Bradykinin
      - 3.1 c) Cholecystokinin (CCK)
      - 3.1 d) Ionotropic Glutamate Receptors (iGluRs)
      - 3.1 d i) NMDA receptors:
      - 3.1 d ii) AMPA receptors:
      - 3.1 d iii) Kainate Receptors:
      - 3.1 e) Metabotropic glutamate receptors (mGluR)
      - 3.1 f) Prostaglandin Receptors:
      - 3.1 g) 5-Hydroxytryptamine (5-HT):
      - 3.1 h) Vanilloid Receptors: transient receptor potential channels:

   3.2 Inhibitory Receptors
      - 3.2 a) γ-Amino butyric acid (GABA):
      - 3.2 b) Group II and III mGluR:
      - 3.2 c) Galanin:
      - 3.2 d) Ghrelin:
3.2 e) Opioids:

4. Molecular Mechanisms of Mechanotransduction

4.1 Mechanosensory Ion Channels

Acid Sensing Ion Channels (ASIC):

Transient Receptor Potential (TRP) Channels:

TRPV1:

TRPV4:

TRPA1:

5. Clinical Aspects of Gastro-oesophageal Reflux Disease (GORD)

5.1 Epidemiology of GORD

5.2 Pathophysiology: The lower oesophageal sphincter (LOS) and reflux

5.3 Neural pathway of transient LOS relaxations (TLOSR)

5.4 Current treatments for GORD

5.5 Pharmacology of TLOSR pathways

AIMS

CHAPTER 1 : Identification of Receptors Responsible for Neuromodulation of Mouse Gastro-oesophageal Vagal Afferents by Galanin

SUMMARY

INTRODUCTION

MATERIALS AND METHODS

Generation of GalR1-/- Mutant Mice

Nodose Ganglia Dissection and RNA extraction for RT-PCR and Quantitative RT-PCR

Determination of galanin receptor transcript expression and relative galanin receptor transcript expression using Quantitative RT-PCR

In vitro mouse gastro-oesophageal afferent preparation

Characterisation of gastro-oesophageal vagal afferent properties

Effects of Galanin on mechanosensitivity of vagal afferents

Effect of a GalR3 antagonist on the inhibitory effects of Galanin

Effects of AR-M961 on mechanical sensitivity of GalR1 -/- vagal afferents

Data Recording and Analysis

Drugs

RESULTS

Expression of Galanin receptors in mouse nodose ganglion

Quantitative RT-PCR

Electrophysiology

Effect of galanin on mechanosensitivity of gastro-oesophageal vagal afferents

Effect of a GalR3 antagonist on the inhibitory effect of galanin

Effect of a GalR1/2 agonist on the mechanosensitivity of gastro-oesophageal vagal afferents

DISCUSSION

Sources of endogenous galanin

Galanin receptors on vagal afferents

Role of GalR1

Role of GalR2

Role for GalR3

CONCLUSION
CHAPTER 2: Potentiation of Vagal Afferent Mechano sensitivity by Ionotropic and Metabotropic Glutamate Receptors

SUMMARY

INTRODUCTION

MATERIALS AND METHODS

RESULTS

DISCUSSION

CHAPTER 3: Ghrelin Selectively Reduces Mechano sensitivity of Upper Gastrointestinal Vagal Afferents

SUMMARY

INTRODUCTION

MATERIALS AND METHODS

RESULTS

DISCUSSION

Table 1

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodose Ganglia Dissection and RNA extraction for RT-PCR and Quantitative RT-PCR</td>
<td>173</td>
</tr>
<tr>
<td>Determination of ghrelin and ghrelin receptor transcript expression in Nodose Ganglia using RT-PCR</td>
<td>174</td>
</tr>
<tr>
<td>Determination of Relative ghrelin and ghrelin receptor transcript expression using Quantitative RT-PCR</td>
<td>175</td>
</tr>
<tr>
<td>RESULTS</td>
<td>177</td>
</tr>
<tr>
<td>RT PCR localisation of ghrelin and ghrelin receptor transcripts</td>
<td>177</td>
</tr>
<tr>
<td>Quantitative RT-PCR comparing relative transcript expression</td>
<td>177</td>
</tr>
<tr>
<td>Electrophysiology</td>
<td>178</td>
</tr>
<tr>
<td>Effect of ghrelin on the mechanosensitivity of gastro-oesophageal vagal afferents</td>
<td>178</td>
</tr>
<tr>
<td>Mouse</td>
<td>178</td>
</tr>
<tr>
<td>Effect of specific ghrelin receptor antagonist D-Lys-3]-GHRP-6</td>
<td>179</td>
</tr>
<tr>
<td>Ferret</td>
<td>179</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>180</td>
</tr>
<tr>
<td>Figure 1.</td>
<td>187</td>
</tr>
<tr>
<td>Figure 2.</td>
<td>189</td>
</tr>
<tr>
<td>Figure 3.</td>
<td>191</td>
</tr>
<tr>
<td>Figure 4.</td>
<td>193</td>
</tr>
<tr>
<td>Table 1.</td>
<td>195</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>196</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>203</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I would firstly like to acknowledge my two supervisors Dr. Amanda Page and Professor L. Ashley Blackshaw for the supervision they have given me during the time of this thesis. Whilst encouraging creative thought and resourcefulness, they have been an unending source of support and ideas. I am truly thankful for their tireless work and every opportunity they have provided. They have both always led by example and I cannot thank them enough for the wonderful opportunities to learn new skills, develop knowledge and to travel and present work at international conferences.

I would also like to thank members of the nerve gut lab for their support and comic relief. It is a hub of innervation and I thoroughly enjoy seeing the hard work culminate into the success it so deserves. Special thanks to Dr. Stuart Brierley who was heavily involved in introducing the RT-PCR technique to the lab and was just as involved in ironing out many methodical problems I had with the procedure.

Several people need to be recognised for their contribution to work included in chapter 3. Tracey O’Donnell, Caitlin Wilte, Rheanna Laker and my supervisor, Dr. Amanda Page all contributed to electrophysiological mouse and ferret studies using ghrelin and ghrelin receptor analogues included in this work. Although I completed the bulk of the work, the contribution to study numbers was invaluable and much appreciated. Dr Amanda Page needs to be recognised for her contribution of MTEP data presented in Chapter 2 which is also greatly appreciated.
I would like to thank the University of Adelaide for the opportunity to undertake this process and for the scholarship I received during my time spent in the laboratory. I would also like to acknowledge the work of Associate Professor Mike Nordstrom, who always made himself available at short notice and was always helpful.

Finally I would like to thank my Mum, Dad and three brothers Chris, Charles and Henry, I am extremely grateful for everything they have done for me. Most of all I would like to thank my own family, my wonderful wife, France, and my two main men, Lachlan and Alexander, who have enabled me to persevere with all my endeavours and are a continual source of love and support.
Publications arising from this thesis

Chapter 1:

Chapter 2:

Chapter 3:

Conference Proceedings


ABBREVIATIONS

$\alpha,\beta$-meATP; $\alpha,\beta$-methylene adenosine 5’-triphosphate

AMPA; $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AP-5, D-(-)-2-amino-5-phosphonopentanoic acid

ASIC; Acid Sensing Ion Channels

BK; Bradykinin

C; carboxyl terminus

CCK; Cholecystokinin

CNS; central nervous system

CRD; colorectal distension

CT; Cycle threshold

DEG/ENaC; Degenerin/Epithelial Na$^+$ Channel

$\Delta$CT; (Cycle threshold (CT) of GalR/iGluR/ghrelin receptor transcript - Cycle threshold (CT) of $\beta$-actin)

DRG; dorsal root ganglia

GalR; galanin receptor

GABA; $\gamma$-Amino butyric acid

Glu-IR; Glutamate immunoreactivity

IMG; inferior mesenteric ganglion

IGLEs; intraganglionic laminar endings

IMAs; intramuscular arrays

iGluR; ionotropic glutamate receptors

LSN; lumbar splanchnic nerve

mGlur; metabotropic glutamate receptor
MTEP, 3-[(2-Methyl-1, 3-thiazol-4-yl)ethynyl]-pyridine

N; amino terminus

NBQX, 2,3-dioxo-6-nitro-1, 2, 3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide

NO; Nitric oxide

-/-; null mutant

NMDA; N-methyl-D-aspartate

PN; sacral pelvic nerve

PCR; polymerase chain reaction

QRT-PCR; Quantitative reverse transcription polymerase chain reaction

RA; rapidly adapting mechanoreceptor

rIGLEs; rectal intraganglionic laminar endings

RNA; ribonucleic acid

RT; reverse transcription

5-HT; serotonin

spikes / sec; spikes per second

SD; standard deviation

TM; transmembrane domain

TLOSRs; transient lower oesophageal relaxations

TRP; transient receptor potential

TRPV1; transient receptor potential vanilloid receptor 1

VIP; Vasoactive intestinal peptide

ANOVA; analysis of variance

+/-; wild-type
SUMMARY

Modulation of signals from peripheral vagal afferent mechanoreceptors to the central nervous system has been identified as the most accessible target for control of neuronal pathways and reflexes central to gastrointestinal disorders such as GORD, disordered food intake and functional dyspepsia.

There are numerous candidates for modulation of vagal afferent signals from the gastrointestinal tract to the CNS, all of which may represent novel targets for therapeutic treatment of gastrointestinal disorders. These candidates include excitatory ionotropic receptors as well as inhibitory and excitatory (metabotropic) G-protein coupled receptors. Four were chosen for study in this thesis. These are:

1) Galanin receptors, which may be excitatory or inhibitory GPCRs depending on their subtype

2) Excitatory ionotropic glutamate receptors, and their relative contribution compared with excitatory metabotropic glutamate receptors.

3) Ghrelin receptors, which may have excitatory or inhibitory actions on nerves elsewhere.

Aims

Determine the roles of four groups of identified receptors in modulation of mechanosensitivity of peripheral gastro-oesophageal mechanoreceptors and to identify endogenous ligands and receptors in vagal cell bodies to complement their known location in stomach.
Methods:

Novel in vitro mouse and ferret vagal gastro-oesophageal preparations have been previously reported. Accurate quantification of mechanical responses was performed according to the primary stimulus for the type of afferent. Mechanical sensitivity of primary afferents was established by mechanical stimulation of the preparation via circumferential tension (0.5-7g) or mucosal stroking with von Frey hairs (10-1000mg). Afferent responses to mechanical stimulus were tested in the presence of selective agonists and antagonists of galanin, ionotropic and metabotropic glutamate as well as ghrelin receptors. In additional studies, the effects of galanin and selective receptor agonists and antagonist on GalR1 wild type (+/+), and null mutant (-/-) mice were determined.

Results:

Two types of vagal afferent mechanoreceptors were identified in the mouse model, described as tension and mucosal sensitive afferents. An additional sub-type, tension-mucosal was identified in the ferret oesophagus.

1) Galanin induced potent inhibition of mechanosensitivity of both types of mouse afferent, an effect mimicked by a GalR1/2 agonist but was absent in null mutant GalR1 (-/-) mice. A GalR1/2 agonist demonstrated minor potentiation of mechanosensitivity in null mutant GalR1 (-/-) mice. There was no significant effect of GalR3 selective ligands observed however.

2) Selective iGluR receptor agonists AMPA and NMDA dose dependently potentiated responses of vagal afferents to mechanical sensitivity, an effect reversed by both selective and non-selective antagonists, whilst the mGluR5 antagonist MTEP concentration dependently inhibited mechanosensitivity.
Efficacy of agonists and antagonists for the various receptor sub-types differed between mucosal and tension receptors. No role for Kainate receptors was observed in this study.

3) In a mouse model ghrelin significantly reduced the response of tension sensitive afferents to circumferential tension, an effect reversed by a selective receptor antagonist. This effect was not observed in mouse mucosal receptors. In the ferret model, ghrelin significantly reduced the response of mucosal and tension mucosal receptors to mucosal stroking however did not affect responses to circumferential tension.

Conclusions:
The current study highlights the complex interaction between excitatory and inhibitory receptors, located on peripheral vagal afferent terminals, that serve to modulate afferent signalling to the CNS and thus allows precise control over gut reflex and secretory function. This study further adds to an expanding list of modulators of peripheral vagal afferent mechanoreceptors, providing additional possible novel therapeutic candidates for treatment of upper gastro-intestinal dysfunction.