MODULATION OF MECHANOSENSITIVE GASTRO-OESOPHAGEAL VAGAL AFFERENTS BY NOVEL TARGETS

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Chapter 2:

Slattery, J.A, Page, A. J, Dorian, C.L, Brierley, S.M, Blackshaw, L.A (2006). *Potentiation of mouse vagal afferent mechanosensitivity by ionotropic and metabotropic glutamate receptors*. The Journal of Physiology. 577; 295-306

Chapter 3:

Page, A.J, Slattery, J.A, Milte, C, Laker, R, O'Donnell, T, Dorian, C.L, Brierley, S.M, Blackshaw, L, A (2007). *Ghrelin selectively reduces mechanosensitivity of upper gastrointestinal vagal afferents*. American Journal of Physiology. Gastrointestinal and Liver Physiology. 292: G1376-G1384.

Conference Proceedings

Slattery JA, Page AJ, Brierley SM, Cooper NJ, Young RL & Blackshaw LA. (2005). *The Galanin 3 Receptor- a potential vagal-specific modulator of gastric mechanosensory function*. European Society for Neurogastroenterology and Motility Meeting. Toulouse, France

Slattery JA, Page AJ, Blackshaw LA & Brierley SM. (2006). *Ionotropic Glutamate Receptor Modulation of Vagal Afferent Mechanosensitivity in Mouse*. Australian Neuroscience Society. Sydney Australia

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Slattery JA, Page AJ, Cheng E & Blackshaw LA. (2003). *Potent Inhibition and Reversal of Vagal Mechanosensitivity by Galanin*. Autonomic Neuroscience: *Basic and Clinical*. International Society of Autonomic Neurosciences (ISAN) Meeting. Calgary, Canada

Slattery JA, Page AJ, O'Donnell TA, Cooper NJ, Young RL & Blackshaw LA. (2005). *Modulation of gastro-oesophageal vagal afferents by galanin in mouse and ferret*. Visceral Pain Satellite of the World Congress on Pain. Adelaide, Australia 2005

ABBREVIATIONS

α,β-meATP; *α,β*-methylene adenosine 5'-triphosphate

AMPA; α-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

ΔCT; (Cycle threshold (CT) of GalR/iGluR/ghrelin receptor transcript - Cycle threshold (CT) of β-actin)

DRG; dorsal root ganglia

GalR; galanin receptor

GABA; γ-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, decribed 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.