

LOCALISATION AND FUNCTION OF
MECHANOSENSORY ION CHANNELS IN
COLONIC SENSORY NEURONS

Patrick Hughes

Nerve-Gut Laboratory

Department of Physiology

School of Molecular and Biomedical Sciences

University of Adelaide

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I dedicate this thesis to my wife Sarah and son Blake for their constant support and inspiration throughout

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Abstract

Irritable Bowel Syndrome (IBS) is one of the most common functional disorders of the gastrointestinal tract. Visceral hypersensitivity is the most commonly reported symptom of IBS, yet is the least adequately treated. Mechanosensitive information from the colon is relayed to the CNS by extrinsic colonic primary afferent nerves which have their cell bodies within dorsal root ganglia (DRG). This thesis aims to identify the contribution of several putatively mechanosensitive ion channels (ASIC1, 2 and 3, TRPV4 and TRPA1) toward detection of mechanical stimuli in the colon.

This involvement is assessed by both molecular and functional means. The abundance of each of these channels was assessed by comparing expression within whole DRG against that in specifically colonic DRG neurons using an *in situ* hybridization methodology developed as part of this PhD. The functional role TRPV4 and TRPA1 impart toward colonic mechanosensation was investigated by recording responses to mechanical stimuli from colonic primary afferent fibres and comparing the results from mice genetically modified to lack either TRPV4 or TRPA1 with those of their intact littermates.

The results from these studies indicate expression patterns within whole DRG do not provide accurate representation of the organ of interest, with abundances of each of the channels investigated differing between colonic DRG cells and the whole DRG. In particular ASIC3 and TRPV4 are preferentially expressed in colonic DRG neurons, unlike ASIC2 and TRPA1. Further, TRPV4 is functionally restricted to detection of noxious mechanical stimuli in the colon, while expression of TRPA1 is more widespread and

functionally less restricted. Each of these channels are each potential targets for the treatment of IBS as each affects specific aspects of colonic mechanotransduction.

Declaration of Originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Signed :

Patrick Hughes

Date

Publications arising from this thesis (bold denotes first authors)

1: **Hughes PA, Brierley SM, Young RL and Blackshaw LA.** (2007). Localization and comparative analysis of acid-sensing ion channel (ASIC1, 2, and 3) mRNA expression in mouse colonic sensory neurons within thoracolumbar dorsal root ganglia. *J. Comp. Neurol.* 500: 863-75

2: **Brierley SM, Page AJ, Hughes PA, Adam B, Liebrechts T, Cooper NJ, Holtmann G, Liedtke W, Blackshaw LA.** (2008). Selective Role for TRPV4 ion channels in visceral sensory pathways. *Gastroenterol.* 134(7):2059-69

3: **Brierley SM, Hughes PA, Page AJ, KY Kwan, CM Martin, TA O'Donnell, NJ Cooper, AM Harrington, B Adam; T Liebrechts, G Holtmann, DP Corey, G Rychkov, A Blackshaw.** (2008) TRPA1 mediates mechanotransduction in sensory neurons and is modulated by algescic stimuli. *Neuron* (in press).

Acknowledgement of Work Provided by Others

PCR was performed by Stuart Brierley in all cases, however I provided retrogradely labelled tissue for laser capture microdissection. Extracellular recordings from lumbar splanchnic colonic afferents for TRPV4 experiments (Chapter 5) were performed entirely by Stuart Brierley, and were performed by Stuart Brierley and Chris Martin for TRPA1 experiments (Chapter 6). Vagal gastroesophageal afferents recordings for TRPV4 experiments (Chapter 5) were performed entirely by Amanda Page, and were performed by Amanda Page and Tracey O'Donnell for TRPA1 experiments (Chapter 6). All colonic distension experiments were performed by Tobias Liebrechts, Birgit Adam and Gerald Holtmann. TRPV4 immunolabelling (Chapter 5) was performed by Nicole Cooper and Stuart Brierley, and TRPA1 immunolabelling (Chapter 6) was performed by Nicole Cooper, Stuart Brierley and Andrea Harrington.

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I would also like to thank Stuart Brierley for providing the time and expertise required to learn techniques including colonic electrophysiological recordings.

Finally many thanks also to my wife Sarah and my family, Peter, Marilyn and Bronwyn for their support and encouragement.