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**NUTRIENT SENSING MECHANISMS**

**IN THE**

**SMALL INTESTINE:**

**Localisation of taste molecules in mice and**

**humans with and without diabetes**

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# TABLE OF CONTENTS

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STATEMENT OF ORIGINALITY AND AUTHENTICITY	x
ACKNOWLEDGEMENTS	xi
ABSTRACT	xii
KEY TO ABBREVIATIONS	xv
LIST OF TABLES	xvii
LIST OF FIGURES	xviii
PUBLICATIONS ARISING FROM THESIS	xxii
1. INTRODUCTION	
1.1 GENERAL OVERVIEW	1
1.2 NUTRIENT FEEDBACK FROM THE SMALL INTESTINE	2
1.2.1 Intestinal carbohydrate and feedback inhibition of gastric emptying	5
1.2.2 Regional specificity of intestinal carbohydrate-feedback inhibition	5
1.2.3 Transport and signalling of glucose in the small intestine	7
1.3 ADAPTATION OF NUTRIENT FEEDBACK FROM THE SMALL INTESTINE IN DIET AND DISEASE	11
1.3.1 Acute modification of intestineal function by dietary influences	11
1.3.2 Chronic modification of intestinal function by dietary influences	13
1.3.3 Adaptation of nutrient feedback in diabetes	14
Altered gastric motility in diabetes mellitus	14

Pathogenesis of upper gastrointestinal dysfunction in diabetes	15
Altered intestinal feedback in diabetes	16
1.4 PERIPHERAL GLUCOSE-SENSING MECHANISMS	17
1.4.1 Taste transduction on the tongue	18
Anatomy and innervation of lingual taste cells	18
Molecular mechanisms of taste transduction	21
1.4.2 Key molecules in sweet taste transduction	24
Sweet taste receptors T1R2 and T1R3	24
G $\alpha_{\text{gust}}$ and other transduction mediators	26
TRPM5	30
1.4.3 Taste coding in taste cells at the periphery	31
1.4.4 Neurotransmitters in taste: cell to cell communication and activation of gustatory afferents	32
1.5 EVIDENCE FOR TASTE MOLECULES IN THE GASTROINTESTINAL TRACT	34
1.6 PRIMARY CHEMOSENSORY CELL TYPES FOR CARBOHYDRATE DETECTION IN THE SMALL INTESTINE	36
1.6.1 Enterochromaffin cells	36
1.6.2 L cells	38
1.6.3 Brush cells	39
1.6.4 Enterocytes	39
1.6.5 Afferent neurons	40
1.7 RESEARCH OBJECTIVES	40
1.8 AIMS AND HYPOTHESES	42
1.8.1 Aims	42
1.8.2 Hypotheses	42

## 2. IDENTIFICATION AND LOCALISATION OF SWEET TASTE MOLECULES IN THE MOUSE SMALL INTESTINE

2.1 SUMMARY	43
2.2 INTRODUCTION	44
2.3 AIM	47
2.4 SPECIFIC HYPOTHESES	47
2.5 MATERIALS AND METHODS	47
2.5.1 Immunohistochemistry	48
2.5.1.1 Animal preparation	48
2.5.1.2 Tissue preparation and sectioning	48
2.5.1.3 Antibodies	49
2.5.1.4 Indirect immunofluorescence protocol	50
2.5.1.5 Immunohistochemical controls	50
2.5.1.6 Visualisation	51
2.5.2 Reverse-transcriptase polymerase chain reaction (RT-PCR)	51
2.5.2.1 Tissue collection	51
2.5.2.2 RNA extraction	52
2.5.2.3 Primers	53
2.5.2.4 RT-PCR protocol	57
2.5.2.5 RT-PCR controls	57
2.5.2.6 Gel electrophoresis	58
2.5.2.7 Quantification method for real time RT-PCR	59
2.5.2.8 Real time RT-PCR protocol	62
2.5.2.9 Real time RT-PCR data and statistical analysis	63
2.6 RESULTS	64

2.6.1 Immunohistochemistry	64
Validation of antibodies for taste proteins in tongue tissue	64
G $\alpha_{\text{gust}}$ expression in solitary epithelial cells of the mouse small intestine	73
G $\gamma$ 13 expression in the epithelium of mouse small intestine	75
T1R3 expression in the epithelium of mouse small intestine	77
TRPM5 expression in the epithelium of mouse small intestine	79
Immunohistochemical controls in mouse small intestine	81
Expression of taste molecule proteins in the myenteric plexus of mouse small intestine	83
2.6.2 RT-PCR	87
Expression of taste molecules in the musosa of the mouse small intestine	87
Regional expression data of taste molecules in mouse gastrointestinal tissue	90
Optimisation and verification of SYBR Green real-time RT-PCR data	96
2.7 DISCUSSION	103

### 3. PHENOTYPIC CHARACTERISATIONS OF TASTE CELLS OF THE MOUSE SMALL INTESTINE

3.1 SUMMARY	111
3.2 INTRODUCTION	112
3.3 AIMS	115
3.4 SPECIFIC HYPOTHESES	115
3.5 MATERIALS AND METHODS	115
3.5.1 Animal and tissue preparation	116
3.5.2 Single label immunohistochemistry	118
3.5.3 Double label immunohistochemistry	118
3.5.4 Lectin histochemistry	119

3.5.5 Visualisation and quantification	119
3.6 RESULTS	120
Characterisation of $G\alpha_{gust}$ immunopositive cells and their distribution in the small intestine	120
$G\alpha_{gust}$ -positive taste cells and 5-HT immunoreactivity in the small intestine	130
$G\alpha_{gust}$ -positive taste cells and GLP-1 immunoreactivity in the small intestine	134
$G\alpha_{gust}$ -positive taste cells and lectin UEA-1 label in the small intestine	137
5-HT, GLP-1 and UEA-1 expression relationships in the small intestine epithelium	139
$G\alpha_{gust}$ is expressed in different epithelial cell populations in the jejunum	139
nNOS immunoreactivity in the mouse small intestine	145
3.7 DISCUSSION	147
<b>4. EFFECTS OF GLP-1 ON MOUSE GASTROESOPHAGEAL MECHANOSENSITIVE VAGAL AFFERENTS <i>IN VITRO</i></b>	
4.1 SUMMARY	154
4.2 INTRODUCTION	155
4.3 AIM	157
4.4 SPECIFIC HYPOTHESES	158
4.5 MATERIALS AND METHODS	158
4.5.1 <i>In vitro</i> mouse gastroesophageal vagal afferent preparation	158
Characterisation of gastroesophageal vagal afferent properties	161
Application GLP-1 and assessment of effects on gastroesophageal vagal afferents	162
Data recording and analysis	162
Drugs and solutions	163
4.5.2 <i>In vitro</i> rat duodenal vagal afferent preparation	164

4.6 RESULTS	165
4.6.1 Effects of GLP-1 on mouse gastroesophageal vagal afferents	165
4.6.2 Assessment of rat duodenal vagal afferents in vitro	170
4.7 DISCUSSION	175
<b>5. EXPRESSION LEVELS OF TASTE MOLECULES IN THE MOUSE INTESTINAL MUCOSA ARE ALTERED WITH NUTRITIONAL STATE</b>	
5.1 SUMMARY	179
5.2 INTRODUCTION	180
5.3 AIMS	183
5.4 SPECIFIC HYPOTHESES	183
5.5 MATERIALS AND METHODS	183
5.5.1 Fed and fasted animals and tissue collection	184
5.5.2 RNA extraction	184
Mucosa	184
Nodose ganglia	185
5.5.3 Primers	186
5.5.4 Real time RT-PCR protocol	188
5.5.5 Data and statistical analysis	188
5.6 RESULTS	189
5.6.1 Relative expression of taste molecules in jejunal mucosa from fed and fasted mice	189
5.6.2 Relative expression of Tph-1 and Gcg in jejunal mucosa from fed and fasted mice	193
5.6.3 Relative expression of 5-HT <sub>3</sub> R and GLP-1R in nodose ganglia from fed and fasted mice	197
5.7 DISCUSSION	200

## 6. EXPRESSION OF TASTE MOLECULES IN THE UPPER GASTROINTESTINAL TRACT IN HUMANS WITH AND WITHOUT TYPE 2 DIABETES

6.1 SUMMARY	206
6.2 INTRODUCTION	207
6.3 AIMS	209
6.4 SPECIFIC HYPOTHESES	209
6.5 MATERIALS AND METHODS	209
6.5.1 Collection of human upper gastrointestinal biopsies	209
Enteroscopy biopsies in non-diabetic patients	210
Endoscopy biopsies in patients with type 2 diabetes	210
6.5.2 Absolute quantification of taste-signal molecules	211
RNA extraction	211
Primers	211
Generation of RT-PCR products as standards for target gene absolute standard curves	214
Copy number calculations for cDNA standards	215
Real time RT-PCR protocol	216
Data and statistical analysis	216
6.5.3 Immunohistochemistry	217
6.6 RESULTS	218
6.6.1 Expression of taste molecules in the human upper gastrointestinal tract	218
6.6.2 Regional specificity in expression of taste molecules in the upper gastrointestinal tract	223
6.6.3 $G\alpha_{\text{gust}}$ immunoreactivity in individual epithelial cells of the human duodenum	226
6.6.4 Expression of taste molecules in the duodenum in type 2 diabetes	231



6.7 DISCUSSION	234
<b>7. DISCUSSION</b>	
7.1 GENERAL DISCUSSION AND FUTURE EXPERIMENTS	240
7.2 CONCLUSIONS	253
 <b>APPENDIX. 5-HT IMMUNOREACTIVITY IN OTHER REGIONS OF THE GASTROINTESTINAL TRACT: ALTERATIONS IN 5-HT SIGNALLING PATHWAYS IN DISEASE</b>	
A1. INTRODUCTION	254
A2. METHODS	255
A3. RESULTS	257
A4. DISCUSSION	259
 <b>REFERENCES</b>	 261

# STATEMENT OF ORIGINALITY AND AUTHENTICITY

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Signed,

Kate Sutherland \_\_\_\_\_ Date \_\_\_\_\_.

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# ABSTRACT

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The mucosa of the small intestine is clearly able to discriminate specific chemical components of ingested meals to stimulate gastrointestinal feedback pathways and reduce further food intake. Luminal carbohydrates delay gastric emptying and initiate satiation, which are mediated by reflexes via the vagus nerve upon activation of vagal afferent endings in the mucosa. Nutrients activate these nerve fibres through intermediary epithelial cells, which release neuromediators upon transduction of luminal signals through the apical membrane. 5-hydroxytryptamine (5-HT) and glucagon-like peptide-1 (GLP-1) are released from enteroendocrine cells in response to luminal carbohydrates and both slow gastric emptying and inhibit food intake via vagal afferent pathways. The molecular mechanisms for carbohydrate detection and transduction leading to 5-HT and GLP-1 release are unknown. However molecules key to transduction of taste by receptor cells in the lingual epithelium are expressed in the gastrointestinal mucosa. The studies in this thesis aimed to investigate 1) the possibility that taste molecules expressed in the intestine form part of the carbohydrate sensing pathway that leads to 5-HT and GLP-1 release, which in turn activate mucosal vagal afferents and 2) to gauge any alterations in taste molecule expression that may relate to adaptation of carbohydrate-induced gastric motility reflexes that occurs in dietary and disease states.

Firstly these studies show key taste molecules, including sweet taste receptors T1R2 and T1R3, the G-protein gustducin (alpha-subunit  $G_{\alpha_{\text{gust}}}$ ), and the taste transduction channel TRPM5, are expressed in the mouse gastrointestinal mucosa shown by RT-PCR and were further localised to individual epithelial 'taste' cells using immunohistochemistry. Quantification of transcript levels by real time RT-PCR revealed the proximal small intestine as the preferential site of sweet taste receptor expression along the gastrointestinal tract. This finding was also confirmed in humans using gastric and intestinal mucosal biopsies obtained at enteroscopy with significantly higher transcript expression levels in the small intestine compared to stomach.

In the mouse, double label immunohistochemistry with  $G\alpha_{\text{gust}}$  antibody, as a marker of intestinal taste cells, was performed using lectin UEA-1, a marker of intestinal brush cells, and 5-HT or GLP-1 to link intestinal taste transduction to 5-HT and GLP-1 release. Results show  $G\alpha_{\text{gust}}$  is expressed within a subset of all three cell types in the small intestine but predominantly within UEA-1-expressing cells. Although  $G\alpha_{\text{gust}}$ , 5-HT and GLP-1 are largely expressed in mutually exclusive cells, within the jejunum a portion  $G\alpha_{\text{gust}}$  positive cells co-expressed 5-HT or GLP-1. This indicates a subpopulation of intestinal taste cells may be dedicated to carbohydrate-evoked gastrointestinal reflexes through 5-HT and GLP-1 mediated pathways, however, taste transduction within the small intestine appears to predominantly link to alternate mediators.

After nutrient detection at the luminal surface, activation of mucosal afferents by 5-HT released from enterochromaffin cells is well documented, however although vagal afferents express GLP-1 receptors direct activation has not been demonstrated. For this purpose the effects of GLP-1 on gastrointestinal vagal afferents were investigated through single fibre recordings in *in vitro* tissue preparations. GLP-1 had no effect on the activity of mouse gastroesophageal vagal afferents but a rat duodenal preparation proved too problematic to be able to test GLP-1 specifically on duodenal vagal afferents.

Altered gastric motility in response to carbohydrate meals due to prior dietary patterns and diabetes mellitus suggest adaptation in feedback mechanisms. Towards the second aim of this thesis taste molecule expression was quantified in fed and fasted mice by real time RT-PCR and revealed taste gene transcription is altered with the changing luminal environment, specifically transcription of taste genes was significantly decreased after feeding compared to the fasted state. Studies comparing expression in the duodenum of type 2 diabetics and non-diabetic controls show no significant difference in taste transcript levels between the two groups. However taste molecule expression was correlated to blood glucose levels

in diabetics suggesting transcription of these signal molecules is adapted to both luminal and systemic carbohydrate levels.

Findings in both the mouse and human gastrointestinal tract in terms of intestinal chemosensing are discussed.

# KEY TO ABBREVIATIONS

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5-HT	5-hydroxytryptamine
5-HT <sub>3</sub> R	5-hydroxytryptamine receptor subtype 3
bp	base pairs
BMI	body mass index
BSA	bovine serum albumin
CCK	cholesystokinin
CGRP	calcitonin gene related peptide
CNS	central nervous system
C <sub>T</sub>	threshold cycle
G $\alpha$ <sub>gust</sub>	alpha subunit of gustducin
gDNA	genomic DNA
GLP-1	glucagon-like peptide-1
GLP-1R	glucagon-like peptide-1 receptor
HbA1c	glycated hemoglobin
NO	nitric oxide
nNOS	neuronal nitric oxide synthase
NTC	no template control
PBS	phosphate buffered saline
PBST	phosphate buffered saline + Triton X-100
PCR	polymerase chain reaction
PYY	peptide YY
rRNA	ribosomal RNA
RT	reverse transcription

-RTC	no reverse transcription control
SGLT-1	sodium glucose co-transporter 1
T1R1	taste receptor family 1 member 1
T1R2	taste receptor family 1 member 2
T1R3	taste receptor family 1 member 3
$T_m$	melting temperature
TRC	taste receptor cell
TRPM5	transient receptor potential ion channel M5
UEA-1	Ulex europeaus agglutinin 1



# LIST OF TABLES

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Table 2.5.1	Primary antibody information_____	49
Table 2.5.2	Primers amplification of mouse taste molecule genes and controls in RT-PCR reactions_____	54
Table 2.6.2	Intra-assay and inter-sample variability in jejunum C <sub>T</sub> values for real time RT-PCR data analysis_____	98
Table 5.5.3	Primers for amplification of additional mouse genes in real time RT-PCR reactions_____	186
Table 6.5.1	Primers for amplification of human taste-signal molecule genes and controls in real time RT-PCR_____	212
Table 6.5.2	Primers to generate RT-PCR product containing target amplicons for use as standards_____	214

# LIST OF FIGURES

---

Figure 1.2.1	Extrinsic neural reflexes in nutrient-induced feedback control of gastric emptying_____	4
Figure 1.2.2	Hypothesised pathway activation of subepithelial vagal afferents by luminal carbohydrates_____	10
Figure 1.4.1	Anatomical arrangement of taste cells on the tongue_____	20
Figure 1.4.2	Molecular mechanisms of taste transduction_____	23
Figure 2.5.1	Approximate location of amplicon sequences in target mouse genes detected by QuantiTect Primer Assays_____	55
Figure 2.5.2	Real time PCR data acquisition and verification of product specificity_____	61
Figure 2.6.1.1	G $\gamma$ 13 immunoreactivity in taste cells of the mouse tongue_____	67
Figure 2.6.1.2	G $\alpha_{\text{gust}}$ immunoreactivity in taste cells in fungiform papillae of the mouse tongue_____	68
Figure 2.6.1.3	G $\alpha_{\text{gust}}$ immunoreactivity in taste cells of mouse foliate and circumvallate papillae_____	69
Figure 2.6.1.4	T1R3 immunoreactivity in taste cells in fungiform papillae of the mouse tongue_____	70
Figure 2.6.1.5	T1R3 immunoreactivity in taste cells in mouse foliate and circumvallate papillae_____	71
Figure 2.6.1.6	TRPM5 immunoreactivity in taste cells of mouse tongue_____	72
Figure 2.6.1.7	G $\alpha_{\text{gust}}$ immunoreactivity in solitary epithelial cells of the mouse small intestine_____	74
Figure 2.6.1.8	G $\gamma$ 13 immunoreactivity in solitary epithelial cells of the mouse small intestine_____	76
Figure 2.6.1.9	T1R3 immunoreactivity in solitary epithelial cells of the mouse small intestine_____	78
Figure 2.6.1.10	TRPM5 immunoreactivity in the mouse small intestine_____	80
Figure 2.6.1.11	Negative control sections in mouse small intestine_____	82
Figure 2.6.1.12	Taste protein immunoreactivity in the myenteric plexus of mouse small intestine_____	84
Figure 2.6.1.13	Taste protein immunoreactivity in the myenteric plexus; whole mount muscle layer_____	85
Figure 2.6.1.14	Colocalisation of G $\alpha_{\text{gust}}$ and G $\gamma$ 13 immunoreactivity with neuronal marker PGP9.5 in the myenteric plexus_____	86

Figure 2.6.2.1	Expression of taste molecules in the mucosa of the mouse small intestine detected by RT-PCR and gel electrophoresis	89
Figure 2.6.2.2	Regional expression levels of taste transcripts in mouse small intestine	93
Figure 2.6.2.3	Regional expression levels of taste transcripts along the mouse upper gastrointestinal tract	94
Figure 2.6.2.4	Relative abundance of taste molecules in tongue, antrum and small intestine	95
Figure 2.6.2.5	SYBR green fluorescence PCR amplification curves generated in Opticon Monitor software	97
Figure 2.6.2.6	Melting curve analyses for product characterisation	99
Figure 2.6.2.7	Real time PCR standard curves	101
Figure 2.6.2.8	Assessment of appropriateness of reference genes for comparisons between different gastrointestinal tissues	102
Figure 3.5.1	Regional classifications of mouse small intestine segments	117
Figure 3.6.1	Brush border membrane immunoreactivity in $G\alpha_{gust}$ -positive cells within the villous epithelium	122
Figure 3.6.2	$G\alpha_{gust}$ immunoreactive cells are frequently associated with the upper villi and villus tip	123
Figure 3.6.3	$G\alpha_{gust}$ immunoreactivity in cells located along the mid crypt-villus axis in jejunal villi	124
Figure 3.6.4	Rare $G\alpha_{gust}$ immunoreactive cells in the villus crypts in mouse small intestine	125
Figure 3.6.5	Regional specificity in frequency of $G\alpha_{gust}$ immunopositive cells in the small intestine	126
Figure 3.6.6	$G\alpha_{gust}$ immunopositive cells were most abundant in the jejunum	127
Figure 3.6.7	Characteristic appearance of $G\alpha_{gust}$ immunoreactive cells in the ileum	128
Figure 3.6.8	$G\alpha_{gust}$ immunoreactivity in individual cells of the colonic epithelium	129
Figure 3.6.9	$G\alpha_{gust}$ and 5-HT were expressed in separate cell populations in the mouse small intestine	131
Figure 3.6.10	$G\alpha_{gust}$ and 5-HT are coexpressed in a subpopulation of cells in the mouse jejunum	132
Figure 3.6.11	Coexpression of T1R3 and $G\gamma_{13}$ with 5-HT in mouse jejunum	133

Figure 3.6.12	$G\alpha_{\text{gust}}$ and GLP-1 were expressed in separate cell populations in the mouse small intestine_____	135
Figure 3.6.13	$G\alpha_{\text{gust}}$ and GLP-1 were coexpressed in a subpopulation of cells in the mouse jejunum_____	136
Figure 3.6.14	$G\alpha_{\text{gust}}$ and lectin <i>Ulex europaeus</i> agglutinin 1 (UEA-1) colabel in mouse epithelial cells_____	138
Figure 3.6.15	Lectin <i>Ulex europaeus</i> agglutinin 1 (UEA-1) binding in a subset of enterochromaffin cells in the mouse small intestine_____	141
Figure 3.6.16	Typical morphology of different $G\alpha_{\text{gust}}$ -positive cell phenotypes in the mouse jejunum_____	142
Figure 3.6.17	$G\alpha_{\text{gust}}$ expressed in different epithelial cell populations in the jejunum_____	143
Figure 3.6.18	$G\alpha_{\text{gust}}$ -positive cell populations in the mouse jejunum_____	144
Figure 3.6.19	Neuronal nitric oxide synthase (nNOS) expression in the mouse small intestine_____	146
Figure 4.5.1	Schematic diagram of the apparatus used to obtain single fibre recordings from mouse gastroesophageal vagal afferents <i>in vitro</i> _____	160
Figure 4.6.1	Responses of mouse gastroesophageal vagal afferents to mechanical stimuli_____	166
Figure 4.6.2	Response of mouse gastroesophageal vagal afferents to GLP-1_____	168
Figure 4.6.3	Effects of GLP-1 on mechanosensitivity of mouse gastroesophageal vagal afferents_____	169
Figure 4.6.4	Schematic distribution of single mechanosensitive receptive fields on the duodenum of the rat_____	173
Figure 4.6.5	Responses of rat duodenal vagal afferents to mechanical stimuli_____	174
Figure 5.5.1	Approximate location of amplicon sequences detected by QuantiTect Primer Assays in additional target mouse genes_____	187
Figure 5.6.1	Gel electrophoresis showing specific taste-signal PCR products amplified from fed and fasted mucosal RNA samples_____	191
Figure 5.6.2	Taste transcript levels in jejunal mucosa from fed and fasted mice_____	192
Figure 5.6.3	Melting curve analyses for Tph-1 and glucagon product characterisation_____	194
Figure 5.6.4	Gel electrophoresis assessment of Tph-1 and glucagon PCR products amplified from fed and fasted mucosal RNA samples_____	195

Figure 5.6.5	Tph-1 and glucagon transcript levels in jejunal mucosa from fed and fasted mice	196
Figure 5.6.6	Melting curve analyses for 5-HT <sub>3</sub> R and GLP-1R product characterisation	198
Figure 5.6.7	5-HT <sub>3</sub> R and GLP-1R transcript levels in nodose ganglia from fed and fasted mice	199
Figure 6.5.1	Approximate locations of the amplicon regions on human taste genes amplified by QuantiTect Primer Assays	213
Figure 6.6.1	Approximate locations of the amplicon regions of human taste genes amplified by QuantiTect Primer Assays	219
Figure 6.6.2	Specific expression of taste molecules in the human gastrointestinal tract shown by gel electrophoresis	220
Figure 6.6.3	Example of a standard curve generated using standards of known copy number	221
Figure 6.6.4	Expression levels of taste molecules in the human proximal intestine	222
Figure 6.6.5	Regional expression of taste molecules in the human upper gastrointestinal tract	224
Figure 6.6.6	Comparison of expression of taste molecules in the gastric and intestinal mucosa	225
Figure 6.6.7	G $\alpha_{\text{gust}}$ immunoreactive epithelial cells in the human duodenum	227
Figure 6.6.8	G $\alpha_{\text{gust}}$ and 5-HT immunoreactivity in distinct cell populations in the human duodenum	228
Figure 6.6.9	G $\alpha_{\text{gust}}$ and GLP-1 immunoreactivity in epithelial cells of the human duodenum	229
Figure 6.6.10	G $\alpha_{\text{gust}}$ and GLP-1 immunoreactivity within single epithelial cells of the human duodenum	230
Figure 6.6.11	Expression levels of taste molecules in the duodenum are not altered in type 2 diabetic patients	232
Figure 6.6.12	Taste transcript levels in the human duodenum are related to blood glucose concentration in type 2 diabetic patients	233
Figure 7.1.1	Potential carbohydrate sensing pathways in the small intestinal mucosa	246
Figure A3.1	Immunohistochemistry for 5-HT and CGRP in the mesenteric border of rat colon	258

# PUBLICATIONS ARISING FROM THESIS

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## Papers

Young RL\*, Sutherland K\*, Pezos N, Brierley SM, Horowitz M, Rayner CK, Blackshaw LA. Expression of taste receptor molecules in the upper gastrointestinal tract in humans with and without type 2 diabetes. (accepted, Gut December 2008).

Sutherland K, Young RL, Cooper NJ, Horowitz M, Blackshaw LA. Phenotypic characterization of taste cells of the mouse small intestine. *Am J Physiol Gastrointest Liver Physiol* 292: 1420-1428, 2007.

Coldwell JR, Phillis BD, Sutherland K, Howarth GS, Blackshaw LA. Increased responsiveness of rat colonic splachnic afferents to 5-HT after inflammation and recovery. *J Physiol* 579(1): 203-213, 2007.

\*these authors contributed equally to this work

## Conference Proceedings

Sutherland K, Brierley SM, Horowitz M, Rayner CK, Blackshaw LA, Young RL. Altered duodenal sweet taste receptor expression in diabetic hyperglycemia. *Digestive Diseases Week, San Diego CA, 2008.*

Sutherland K, Brierley SM, Horowitz M, Rayner CK, Blackshaw LA, Young RL. Sweet taste transduction molecules are expressed in the upper gastrointestinal tract in humans. *Digestive Diseases Week, Washington DC, 2007.*

Pezos N, Sutherland K, Brierley SM, Horowitz M, Blackshaw LA, Young RL. Vagal afferents do not directly detect intestinal glucose via a sweet-taste mechanism. *Digestive Diseases Week, Washington DC USA, 2007.*

Sutherland K, Cooper NJ, Horowitz M, Margolskee RF, Blackshaw LA, Young RL. Co-expression of taste receptor G-protein  $\alpha$ -gustducin with enteroendocrine cell markers in the mouse jejunum. *Brain Gut Interactions, Oxford UK, 2006.*

Sutherland K, Cooper NJ, Horowitz M, Margolskee RF, Blackshaw LA, Young RL. Taste receptor G-protein  $\alpha$ -gustducin does not colocalise with enteroendocrine cell markers in the mouse intestine. *Digestive diseases week, Los Angeles USA, 2006.*