NUTRIENT SENSING MECHANISMS
IN THE
SMALL INTESTINE:
Localisation of taste molecules in mice and
humans with and without diabetes

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A thesis submitted in fulfilment of the Degree of Doctor of Philosophy

Discipline of Physiology
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STATEMENT OF ORIGINALITY AND AUTHENTICITY

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Signed,

Kate Sutherland ___________________________ Date ___________.
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ABSTRACT

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 (\( \text{G}_\text{\alpha_{gust}} \)), 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 Gαgust 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 Gαgust is expressed within a subset of all three cell types in the small intestine but predominantly within UEA-1-expressing cells. Although Gαgust, 5-HT and GLP-1 are largely expressed in mutually exclusive cells, within the jejunum a portion Gαgust 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.
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<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>5-HT₃R</td>
<td>5-hydroxytryptamine receptor subtype 3</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CCK</td>
<td>choleystokinin</td>
</tr>
<tr>
<td>CGRP</td>
<td>calcitonin gene related peptide</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>Cₜ</td>
<td>threshold cycle</td>
</tr>
<tr>
<td>Gα₉ust</td>
<td>alpha subunit of gustducin</td>
</tr>
<tr>
<td>gDNA</td>
<td>genomic DNA</td>
</tr>
<tr>
<td>GLP-1</td>
<td>glucagon-like peptide-1</td>
</tr>
<tr>
<td>GLP-1R</td>
<td>glucagon-like peptide-1 receptor</td>
</tr>
<tr>
<td>HbA1c</td>
<td>glycated hemoglobin</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>nNOS</td>
<td>neuronal nitric oxide synthase</td>
</tr>
<tr>
<td>NTC</td>
<td>no template control</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PBST</td>
<td>phosphate buffered saline + Triton X-100</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PYY</td>
<td>peptide YY</td>
</tr>
<tr>
<td>rRNA</td>
<td>ribosomal RNA</td>
</tr>
<tr>
<td>RT</td>
<td>reverse transcription</td>
</tr>
<tr>
<td>Abbreviation</td>
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<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>-RTC</td>
<td>no reverse transcription control</td>
</tr>
<tr>
<td>SGLT-1</td>
<td>sodium glucose co-transporter 1</td>
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<tr>
<td>T1R1</td>
<td>taste receptor family 1 member 1</td>
</tr>
<tr>
<td>T1R2</td>
<td>taste receptor family 1 member 2</td>
</tr>
<tr>
<td>T1R3</td>
<td>taste receptor family 1 member 3</td>
</tr>
<tr>
<td>$T_m$</td>
<td>melting temperature</td>
</tr>
<tr>
<td>TRC</td>
<td>taste receptor cell</td>
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<tr>
<td>TRPM5</td>
<td>transient receptor potential ion channel M5</td>
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<tr>
<td>UEA-1</td>
<td>Ulex europeaus agglutinin 1</td>
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PUBLICATIONS ARISING FROM THESIS

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*these authors contributed equally to this work

Conference Proceedings


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