NUTRIENT SENSING MECHANISMS IN THE SMALL INTESTINE:

Localisation of taste molecules in mice and humans with and without diabetes

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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 (alpha-subunit $G\alpha_{gust}$), 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 $G\alpha_{gust}$ 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 $G\alpha_{gust}$ is expressed within a subset of all three cell types in the small intestine but predominantly within UEA-1-expressing cells. Although $G\alpha_{gust}$, 5-HT and GLP-1 are largely expressed in mutually exclusive cells, within the jejunum a portion $G\alpha_{gust}$ 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 ABBRIEVIATIONS

5-HT	5-hydroxytryptamine
$5\text{-HT}_3\text{R}$	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_T	threshold cycle
$G\alpha_{gust}$	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

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PUBLICATIONS ARISING FROM THESIS

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.

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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 α-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 α-gustducin does not colocalise with enteroendocrine cell markers in the mouse intestine. Digestive diseases week, Los Angeles USA, 2006.