Obesity Induced Dysfunction of Gastric Vagal Afferent Signalling

Stephen James Kentish

Discipline of Medicine

School of Medicine

The University of Adelaide

July 2013

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 to Stephen James Kentish 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.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below*) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Stephen James Kentish

BSc. (Biomed) (Honours, First Class)

July 11th 2013

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to sincerely thank my primary supervisor Associate Professor Amanda Page for the tireless supervision she has provided me over the past three years. Without the support she provided I doubt that I would have been able to complete this thesis. I am truly thankful for her guidance and support in allowing me to develop research ideas and implement them. I am furthermore grateful for the opportunities provided to me to attend a number of local and international scientific meetings.

I am also grateful to my co-supervisor, Professor Ashley Blackshaw. Whilst for the majority of my candidature he was not in the country; the advice and guidance he provided both remotely and in person were well appreciated.

I would also like to offer my thanks to a Tracey O'Donnell for her contributions in caring for the obese mice and Gary Wittert for his excellent advice at various stages of my candidature as well as acting as a pseudo supervisor. I would also like to thank every member of the Nerve Gut Laboratory for their support. My time in this lab has made me realise just how specialised the work is and how much effort is put in. I wish every member of the lab every success imaginable.

On a more personal note I wish to thank my parents for seeing me through the initial stages of my education and encourage me to take my desire to learn as far as I have (Pete I think I may have beaten your PhD completion record by just a tad).

I would also like to give very special endless heartfelt thanks to my partner of 6 years, Alice. She who has had to endure the mountains of paper, the ridiculous hours, the stress that shaves years off your life and all with a smile on her face and a kind word to be said.

“Far and away the best prize that life offers is the chance to work hard at work worth doing.”- Thomas Jefferson

CONFERENCE PROCEEDINGS

Kentish S.J., O'Donnell T., Wittert G., Page A.J. (2012) Obesity induced suppression of gastric satiety signals are only partially reversed by dietary change. Presented at NGM 2012, Bologna, Italy.

Kentish S.J., O'Donnell T., Wittert G., Page A.J. (2012) Obesity induced suppression of gastric satiety signals are not reversed by dietary change. Presented at SSIB 2012, Zurich, Switzerland.

Kentish S.J., Wittert G., Blackshaw L.A., Page A.J. (2012) A chronic high fat diet alters the homologous and heterologous control of satiety peptide receptor expression. Presented at DDW 2012, San Diego, United States of America.

Kentish S.J., O'Donnell T.A., Wittert G., Blackshaw L.A., Page A.J. (2011) High fat diet feeding switches the second messenger system activated by leptin on vagal afferents. Presented at ANZOS 2011, Adelaide, Australia.

Kentish S., O'Donnell T., Wittert G., Blackshaw L.A. & Page A. (2011) The "leptin switch" mechanism in gastric vagal afferents. Presented at Digestive Disease Week 2011, Chicago, United States of America.

Kentish S., O'Donnell T., Li H., Wittert G., Blackshaw L.A. & Page A. (2011) Obesity switches the effect of leptin on vagal afferent mechanosensitivity. Presented at Digestive Disease Week 2011, Chicago, United States of America .

Kentish S., T., Wittert G., Blackshaw L.A. & Page A. (2010) Increased Ghrelin Inhibition of Vagal Afferents in an Obese Mouse Model. Presented at Digestive Disease Week 2010, New Orleans, United States of America.

ABBREVIATIONS

ACh; Acetylcholine

AgRP; Agouti-related peptide

α-MSH; α-Melanocyte-stimulating hormone

ANOVA; Analysis of variance

AP; Area postrema

ARC; Arcuate nucleus

AT; Adaptive thermogenesis

BBB; Blood brain barrier

BKCa; Large conductance calcium activated potassium channel

CART; Cocaine- and amphetamine-regulated transcript

CB1; Cannabinoid receptor 1

CCK; Cholecystokinin

CCK1R; CCK receptor 1

ChAT; Choline acetyltransferase

CNS; Central nervous system

CT; Cycle threshold

db/db; Leptin receptor knockout mouse

DMH; Dorsal medial hypothalamus

DMV; Dorsal motor nucleus of the vagus

DRG; Dorsal root ganglion

ENS; Enteric nervous system

GABA; γ-Amino butyric acid

GHS-R; Growth hormone secretagogue receptor

GPCRs; G-protein coupled receptors

GPR7; G-Protein coupled receptor 7 (endogenous receptor for neuropeptide W)

IGLE; Intraganglionic laminar endings

IL-; Interleukin

IMA; Intramuscular array

IP; Intraperitoneal

IRS; Insulin receptor substrate

JAK; Janus kinase

LDL; low density lipoprotein

LepR; Leptin receptor

LH; Lateral hypothalamus

MCR; Melanocortin receptor

mRNA; Messenger RNA

NANC; Non-adrenergic non-cholinergic

NPW; Neuropeptide W

NPY; Neuropeptide Y

NTS; Nucleus tractus solitarii

Ob/Ob; Leptin knockout mouse

PDE; Phosphodiesterase

PI3K; Phosphatidylinositide 3-kinases

PLC; Phospholipase C

POMC; Pro-opiomelanocortin

PYY; Peptide YY

PVN; Paraventricular nucleus

QRT-PCR; Quantative reverse transcription polymerase chain reaction

RMR; Resting metabolic rate

RNA; Ribonucleic acid

RT; Reverse transcription

RYGB; Roux-en-Y gastric bypass

5-HT; 5-hydroxytryptamine

SEM; Standard error of the mean

SGLT1; Sodium-glucose transporter 1

SOCS; Suppressor of cytokine signalling

STAT; Signal transducer and activator of transcription

TRPC; Transient receptor potential: Canocial subtype

TRPV; Transient receptor potential: Vannilloid subtype

UCP; Uncoupling protein

VTA; Ventral tegmental area

ABSTRACT

Background: The stomach has the ability to respond to chemical and mechanical stimuli to mediate satiety through vagal pathways. Within the stomach specialised endocrine and epithelial cells synthesise and secrete leptin and ghrelin, which influence food intake through vagal afferent pathways. However, it remains to be determined if mechanosensitive gastric vagal afferent signalling is disrupted in obesity and whether this may play a role in the overconsumption of energy required for the maintenance of diet-induced obesity. Furthermore, whether leptin can modulate mechanically sensitive gastric vagal afferents and whether any ability of leptin and ghrelin to modulate mechanically sensitive endings is altered in obesity has not been conclusively determined.

Aims: To determine in lean mice and in high fat diet induced obese mice:

1) The effect of gastric peptides ghrelin and leptin on gastric vagal afferent mechanosensitivity.

2) The effect of gastric peptides on the expression of their own and other peptide receptors.

3) The reversibility of diet-induced obesity.

Methods: Lean and diet-induced obese mice were created by feeding 8 week old female C57BL/6 mice a standard chow diet (N=4-20; 7% energy from fat) or a high-fat diet (N=4-20; 60% of energy from fat) respectively. An *in vitro* gastro-oesophageal vagal flat sheet preparation was utilised to determine the

mechanosensitivity of vagal afferent endings and the effect of leptin, ghrelin and diet-induced obesity on this mechanosensitivity. Messenger RNA (mRNA) content in nodose ganglia was measured by QRT-PCR. Specific gastric vagal afferent cell bodies were identified by retrograde labelling and this technique was combined with QRT-PCR to determine mRNA content in specific gastric cell bodies. Anterograde tracing by injection of tracer into the nodose ganglia allowed visualisation of the distribution of gastric vagal afferents in relation to leptin and ghrelin positive cells. Nodose ganglia were cultured overnight in medium containing leptin, ghrelin or neuropeptide W (NPW) followed by QRT-PCR to determine any homologous or heterologous receptor expression regulation.

Results: Diet-induced obesity caused a reduction in the mechanosensitivity of gastric tension receptors. Furthermore, it increased the inhibitory effect of ghrelin on gastric vagal afferent mechanosensitivity and resulted in a switch in the effect of leptin from potentiating to inhibitory. The gut peptides leptin, ghrelin and NPW modified the mRNA content of their own and each other's receptors in a manner that was dependent on dietary group. Placing obese mice back on a chow diet resulted in an initial weight loss but subsequent increased food consumption and weight gain. The decrease in mechanosensitivity caused by the high fat diet was not reversible by placing diet-induced obese mice back on a chow diet and the effects of leptin were only partially reversed.

Conclusions: Vagal afferent function is altered in diet-induced obesity to the extent that both the baseline response and the effects of leptin and ghrelin may act to facilitate increased food intake. Given the lack of reversibility of changes observed in diet-induced obesity this suggests that gastric vagal afferents may play a role in the maintenance of obesity and may act to oppose weight loss.