2009

The role of hypothalamic neuropeptides and BDNF in obesity and the effects of dietary intervention

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THE ROLE OF HYPOTHALAMIC NEUROPEPTIDES AND BDNF IN OBESITY AND THE EFFECTS OF DIETARY INTERVENTION

A thesis submitted in fulfilment of the

requirements for the award of the degree

Doctor of Philosophy

from

SCHOOL OF HEALTH SCIENCES

UNIVERSITY OF WOLLONGONG

by

Yinghua Yu

2009

CERTIFICATION

I, Yinghua Yu, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Health Sciences, University of Wollongong, is entirely my own work unless otherwise referenced or acknowledged. This manuscript has not been submitted for qualification at any other academic institution.

Yinghua Yu

2009

ACKNOWLEDGMENTS

I would like to thank the following people for their support and guidance throughout my research project.

To my supervisors Professor Xu-Feng Huang and Dr Chao Deng, I would like to thank you for your consistent encouragement, guidance and support. In particular, thanks to Xu-Feng for your support in the preparation of my published papers and thesis. Your support and encouragement was greatly appreciated and will never be forgotten. Thank you for your time and patience on improving my academic writing.

I would like thank Mr Tim South and Dr Dennis Wang for their help and advice in the preparation of manuscripts. To Professor Len Storlien and Ms Teresa Marie du Bois, thank you for the careful editorial reading my manuscripts.

Thank you to Dr Kelly Newell, Ms Teresa Marie du Bois and Dr Mandy Reid for the enthusiastic editorial reading of my thesis. Thank you to Ms Teresa Marie du Bois, Ms Sara Grafenauer, Dr Kelly Newell and Mr Tim South for the critical proofreading of my final thesis.

I would also like to thank Ms Mei Han, Ms Gita Rahardjo and Ms Yingxu Liu for helping me to learn and carry out experimental techniques in the lab.

Thanks to Dr Pam Davy from the School of Mathematics and Applied Statistics, University of Wollongong, for suggestions regarding the statistical analyses.

Thank you to Dr Tracy Maddocks for your guidance and assistance in the preparation of the ingestive behaviour study in the animal house.

To my family, thank you for your continual support and understanding throughout this period of study. I give special thanks to my parents for encouraging me and believing that I can achieve this great outcome in my life. Special thanks also to my husband, Hongtao, who provided tremendous support not only in life but also academically. Thank you for your expertly designed program for the ingestive behaviour study and for helping me overcome the obstacles in this project. Thanks to my son, Zhubao, who brought me both fun and trouble during my study.

Finally to our talented team of researchers, thank you for friendly and effective assistance when I needed it during this project.

PUBLICATIONS

The following publications and presentations have arisen directly from the work conducted for this thesis.

Publications in Refereed Journals

Yu, Y., South, T., Wang, Q. and Huang, X.F., (2008) Differential expression of hypothalamic CART mRNA in response to body weight change following different dietary interventions. *Neurochemistry International*. 52:1422-1430.

Yu, Y., South, T.and Huang, X.F., (2009) Inter-meal interval is increased in mice fed a high whey, as opposed to soy and gluten, protein diets. Appetite. 52 (2):372-379.

Yu, Y., Wang, Q. and Huang, X.F., (2009) Energy-restricted pair-feeding normalizes low levels of brain-derived neurotrophic factor/tyrosine kinase B mRNA expression in the hippocampus, but not ventromedial hypothalamic nucleus, in diet-induced obese mice. Neuroscience 160 (2), 295-306.

Yu, Y., Deng, C. and Huang, X.F., Obese reversal by a chronic energy restricted diet leaves an increased Arc NPY/AgRP, but no alteration in POMC/CART, mRNA expression in diet-induced obese mice. *American Journal of Physiology – Regulatory*. Submitted (4 November, 2008).

Publications in Conference Proceedings

Yu, Y., South, T. and Huang, X.F., (2007) The role of CART in obesity development and dietary intervention. *7th World Congress of Neuroscience*, IBRO, Melbourne, Australia, pp. 284.

Yu, Y., Li, Y. and Huang, X.F., (2006) Arc neuropeptides mRNA expression in response to diet macronutrients and obesity reversal in diet-induced obese and resistant mice. *Proceeding of 4th Federation of Asian-Oceanian Neuroscience Societies (FAONS) Congress*, Hong Kong, pp. 122.

Yu, Y., South, T., and Huang X.F., (2006) AdipoR1 mRNA expression levels are increased in the VMH of mice prone to diet-induced obesity. *Proceeding of the 26th ANS Annual Meeting*, Sydney, Australia, pp. 140.

Additional Publications

The following publications have arisen from other projects I have been involved in throughout my doctoral study.

Huang, X.F., ***Yu, Y.,*** Li, Y., Tim, S., Deng, C., Wang, Q., (2008) Ventromedial Hypothalamic NPY Y2 Receptor in the Maintenance of Body Weight in Diet-Induced Obesity in Mice. *Neurochemical Research*. 33(9):1881-1888.

Huang, X.F., Zavitsanou, K., Huang, X., ***Yu, Y.,*** Wang, H., Chen, F., Lawrence, A. J., Deng, C., (2006) Dopamine transporter and D2 receptor binding densities in mice prone or resistant to chronic high fat diet-induced obesity. *Behavior Brain Research*. 175(2):415-419.

Huang, X.F., ***Yu, Y.,*** Zavitsanou, K., Han, M. and Storlien, L., (2005) Differential expression of dopamine D2 and D4 receptor and tyrosine hydroxylase mRNA in mice prone, or resistant, to chronic high-fat diet-induced obesity. *Molecular Brain Research*. 135:150-161.

ABSTRACT

Obesity is a serious metabolic disorder that has reached epidemic proportions and has produced a heavy financial burden on health care systems worldwide. Obesity is usually induced by excessive energy intake and is highly resistant to treatment by lifestyle intervention. Using a mouse model, this study investigated the role of hypothalamic neuropeptides and neurotrophic factors in diet-induced obesity and obesity reversal by dietary interventions. Furthermore, this study tested the satiating capacity of various protein sources (whey, soy and gluten) through dietary intervention by analysing the meal pattern behaviour of mice.

In the first two chapters, the mRNA expression of hypothalamic cocaine- and amphetamine- regulated transcript (CART), brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine kinase B (TrkB) was examined by *in situ* hybridisation in diet-induced obese (DIO) and resistant (DR) mice following dietary intervention. CART mRNA expression was increased in the arcuate hypothalamic nucleus (Arc) and the paraventricular nucleus (PVN), and was decreased in the dorsomedial hypothalamic nucleus (DM) and lateral hypothalamic area (LH) of DIO mice compared to DR mice. These results demonstrated for the first time that two groups of CART neurons in the hypothalamus are differentially expressed in DIO mice. BDNF/TrkB mRNA expression was decreased in the hippocampus in DIO mice, suggesting a weakened inhibitory control of food intake. In the ventromedial hypothalamic nucleus (VMH), BDNF mRNA expression was lowered in DIO mice even after obesity reversal compared to DR mice. The low level of BDNF expression in the VMH may indicate an intrinsic nature of obese mice which makes them susceptible to overconsumption of a high-fat diet. Furthermore, energy restricted pair-feeding eliminated the differences between

DIO and DR mice in both body weight and mRNA expression of hypothalamic CART and hippocampal BDNF/TrkB, which suggests that CART and BDNF/TrkB expression are related to body weight changes.

The aim of the third chapter was to examine the expression of Arc orexigenic and anorexigenic neuropeptides in response to weight loss after chronic energy intake restriction. DIO and DR mice were placed on an energy restricted diet or continued on their high-fat diet *ad libitum.* An additional group was fed a low-fat diet throughout the entire study as controls. The results showed that chronic energy restriction corrected the obesity status and decreased plasma leptin in the DIO mice. Chronic energy restriction increased the expression of hypothalamic orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP), however, it had no effect on the expression of Arc proopiomelanocortin (POMC) and CART mRNA. These results suggest that orexigenic NPY and AgRP (but not anorexigenic CART and POMC) may contribute to the re-establishment of a body weight set-point after body weight loss.

Following on from the above three chapters which investigated the role of hypothalamic neuropeptides and neurotrophic factors in food intake, the fourth chapter investigated the satiating capacities of single or combined whey, soy and gluten protein diets through analysing the meal pattern behaviour of mice. It was found that the whey protein diet potently prolonged intermeal interval and diminished spontaneous meal frequency. This increase in intermeal interval, suggestive of postprandial and postabsorptive satiety effects, is mainly responsible for the inhibition of total energy intake after a whey protein diet. Combinations of whey and gluten caused a lower energy intake, longer inter-meal interval and lower meal number compared to the other paired combinations. Therefore, a combination of whey and gluten may be a better

formula to provide a high satiety effect and suppress energy intake for antiobesity purposes.

In conclusion, this thesis reveals that dietary intervention has a pronounced impact on the gene expression of hypothalamic neuropeptides and neurotrophic factors. High expression of orexigenic neuropeptides NPY/AgRP and low expression of BDNF/TrkB after weight loss may contribute to the recurrence of obesity. Combining dietary protein manipulations for maximising satiety with inhibition of the orexigenic neuropeptides and stimulation of BDNF/TrkB might be critical for potential treatment of obesity and maintenance of weight loss in human obese individuals.

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LIST OF ABBREVIATIONS

3V	Third ventricle
ACTH	Adrenocorticotropic hormone
AgRP	Agouti-related protein
Arc	Arcuate hypothalamic nucleus
Arc-M	Middle part of arcuate nucleus
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
α-MSH	α-melanocyte stimulating hormone
β-MSH	β-melanocyte stimulating hormone
γ-MSH	γ-melanocyte stimulating hormone
CART	Cocaine- and amphetamine-regulated transcript
DIO	High-fat diet-induced obese
DIO-H	DIO mice on high-fat diet ad libitum
DIO-L	DIO mice on low-fat diet
DIO-P	DIO mice on energy restricted pair-feeding diet
DM	Dorsomedial hypothalamic nucleus
DM-C	Caudal part of dorsomedial hypothalamic nucleus
DM-R	Rostral part of dorsomedial hypothalamic nucleus
DR	High-fat diet-resistant
DR-H	DR mice on high-fat diet ad libitum
DR-L	DR mice on low-fat diet
DR-P	DR mice on energy restricted pair-feeding diet
GMP	Glycomacropeptide
HF	High-fat diet
LF	Low-fat diet
LH	Lateral hypothalamic area
MC3R	Melanocortin receptor 3
MC4R	Melanocortin receptor 4
MCH	Melanin-concentrating hormone
NPY	Neuropeptide Y
NT3	Neurotrophins 3
NT4/5	Neurotrophins 4/5
POMC	Proopiomelanocortin
PYY	Peptide YY
PVN	Paraventricular nucleus
TrkB	Tyrosine kinase B
VMH	Ventromedial hypothalamic nucleus
VTA	ventral tegmental area