British Society for Paediatric Endocrinology & Diabetes Research Award Report

Quantification of oxytocin (OXT) and arginine-vasopressin (AVP) concentrations in patients with hypothalamic tumours and septo-optic dysplasia

Background

Hypothalamic obesity (HyOb) is a syndrome of morbid, inexorable obesity first described more than 100 years ago(1). Despite multiple attempts using a variety of interventions from dietary and lifestyle modification(2), medical therapy(3-5) and surgery(6), no single treatment modality has been shown to have long-term efficacy, or reports have been limited to small case series with insufficient long-term follow-up data. Failure of these treatments has been hampered by our incomplete understanding of the pathophysiology of HyOb, with the pathways underlying the imbalances between anorexigens (leptin, insulin, α-MSH) and orexigens (ghrelin, neuropeptide Y, adiponectin) only beginning to be elucidated(7). Management of HyOb is complicated further by its association with hyperphagia, sleep and temperature dysregulation and behavioural disorders which often lie on the autistic spectrum; all features of the "hypothalamic syndrome".

Additionally, the wider role of the neuropeptides oxytocin (OXT) and arginine-vasopressin (AVP) beyond parturition, lactation and sodium and water balance respectively in metabolism, social interaction and thermoregulation has yet to be completely understood(8-14). More specifically, plasma OXT concentrations have been shown to be lower in Prader-Willi syndrome and autistic patients(15, 16), and intranasal OXT trials have demonstrated some effect on reducing food intake in healthy adults(10, 14) and improving food-related behaviours in Prader-Willi syndrome(17). However, current assays for both OXT and AVP vary significantly in their validity and reliability, depending on the method of plasma purification to remove interfering substances, the method of pre-analysis sample preparation, and the sensitivity and specificity of the assay to detect these small nonapeptides which are present in low concentrations in human plasma(18).

This project therefore aimed to optimise the plasma purification process for plasma OXT and AVP, determine the reliability and validity of commercially available immunoassays, and then translate these findings into clinical use to characterise the plasma physiology of these hormones in subcohorts with and without congenital (septo-optic dysplasia) and acquired (hypothalamic tumours) hypothalamic disorders who were obese (BMI ≥ +2 SDS) and lean (BMI < 2 SDS), as well as to correlate these with their degree of hyperphagia and other clinical features.

Results

A total of 122 patients with and without hypothalamic disorders have been recruited as part of a multi-way case-control study design (HyOb 52, hypothalamic lean (HyLean) 28, common obesity 24, healthy lean 18), and their clinical features with regards to their auxology, eating behaviours (using the Dykens Hyperphagia Questionnaire Score(19)), their Endocrine Morbidity Score (EMS)(20), and the presence of autism, learning difficulties, sleep disturbances and temperature dysregulation have been characterised. An assay for OXT has been optimised and

validated and now been translated for use on our patient samples to determine the pathophysiology of OXT in HyOb, particularly in comparison to common obesity, alongside other appetite-regulating hormones including insulin, leptin, α-MSH, ghrelin, AgRP, and BDNF, some of which have been performed in collaboration with our colleagues at the University of Cambridge. Additionally, development and validation of an AVP assay has been abandoned in favour of utilising measurements of plasma copeptin (a much more stable, stoichiometric co-secreted marker) in collaboration with Newcastle-upon-Tyne Hospitals NHS Foundation Trust.

This study has led to the following presentations and abstract publications (full publications are in progress):

- *Presentations*
    - Gan HW, Lesson C, Aitkenhead H, Spoudeas HA, Martinez-Barbera JP, Dattani MT. *Oxytocin deficiency is associated with hyperphagia and weight gain in hypothalamic and common obesity: a first-in-humans, proof-of-concept study.* (Poster, 55th European Society for Paediatric Endocrinology (ESPE) Meeting 2016, Paris, France)
    - Gan HW, Leeson C, Aitkenhead H, Spoudeas HA, Martinez-Barbera JP, Dattani MT. *Oxytocin deficiency is associated with hyperphagia and weight gain in hypothalamic and common obesity: preliminary data from a first-in-humans proof-of-concept study.* (Poster, 98th Endocrine Society Annual Meeting (ENDO 2016), Boston, USA)
    - Gan HW, Leeson C, Aitkenhead H, Spoudeas HA, Dattani M. *Hypothalamic obesity, hyperphagia and hyperinsulinaemia: time for a paradigm shift in assumptions?* (Poster, 54th ESPE Meeting 2015, Barcelona, Spain)
- *Abstracts*
    - Gan HW, Leeson C, Aitkenhead H, Spoudeas H, Martinez-Barbera JP, Dattani M. *Oxytocin deficiency is associated with hyperphagia and weight gain in hypothalamic and common obesity: a first-in-humans proof-of-concept study.* Horm Res Paediatr 2016; 86(S1):421(21).
    - Gan HW, Leeson C, Aitkenhead H, Spoudeas HA, Dattani M. *Hypothalamic obesity, hyperphagia and hyperinsulinaemia: time for a paradigm shift in assumptions?* Horm Res Paediatr 2015: 84(S1):211(22).

Benefit to applicant

BSPED funding has supported the translation of the OXT assays that have been optimised in-house by Dr. Hoong-Wei Gan into use for the clinical portion of the study, i.e. the elucidation of the pathophysiology of HyOb, as well as the purchasing of various other immunoassays for the other appetite-regulating hormones of interest. Through this PhD project, Dr. Hoong-Wei Gan has also obtained transferable skills in assay development which will be useful for his future clinical and academic career.

Benefit to department/ institution

This project has been generously supported by patients and their families through their participation, particularly those who were recruited as controls. Preliminary results have been fed back to the local Septo-optic Dysplasia Patient Support Group, with many expressing interest regarding the possibility of taking part in any future interventional trials. Apart from extending the research portfolio of the department, it has also benefited indirectly as many other clinically needed endocrine tests were performed concurrently during the admissions for research testing; and this resulted in smoother coordination of the admissions process in many cases.

Benefit to endocrinology

This project has expanded our understanding of HyOb and common obesity, leading to the understanding that there is significant overlap in the disrupted appetite-regulating pathways in both cases. Future more targeted therapies for this currently untreatable disorder may be planned, depending on the final results of this study which is currently in analysis.

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21. 55th Annual Meeting of the European Society for Paediatric Endocrinology (ESPE), Paris, France, September 10-12, 2016: Abstracts. Horm Res Paediatr. 2016;86 Suppl 1:1-556.
22. 54th Annual Meeting of the European Society for Paediatric Endocrinology (ESPE). Barcelona, Spain, October 1-3, 2015: Abstracts. Horm Res Paediatr. 2015;84 Suppl 1:1-622.