

## RESEARCH GROUP: Diabetes Research Group

### Project Title:

Assessing the associated metabolic benefits of combined incretin and apelin peptide analogue therapy in obesity and diabetes.

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**Level: PhD**

### Background to the project :

Apelin is a peptide (adipokine) secreted from adipocytes which activates APJ receptors that are located in multiple tissues. Tatemoto and colleagues originally isolated the adipokine apelin from bovine stomach and discovered that it was a selective endogenous ligand for the orphan APJ receptor (Tatemoto *et al.* 1998). This APJ receptor was found in a number of tissues in rodents and man including the CNS as well as the heart and lungs. Different isoforms of apelin were found including apelin-36 and apelin-13. Apelin has been found to be multifaceted with actions on feeding behaviour, glucose utilization and insulin secretion. Recently apelin receptors have been localised mainly in pancreatic  $\beta$ -cells with some expression in  $\alpha$ -cells in man and rodents (Ringstrom *et al.* 2010). Thus apelin may well have an important role as a paracrine or autocrine messenger within pancreatic islets. We developed a range of stable apelin analogues and determined that these had significant antidiabetic actions. We found that apelin analogues stimulated insulin and GLP-1 secretion and increased cellular glucose uptake *in vitro*. Furthermore, they enhanced glucose tolerance and satiety, as well as exhibiting antidiabetic effects comparable to incretin mimetics, in acute studies using high fat fed diet-induced obese diabetic mice.

### Objectives of the research project :

New therapeutic approaches are required to combat the epidemics of obesity and diabetes. The present proposal aims to test the longer-term efficacy of apelin analogues alone and in combination with incretin mimetic therapies *in vitro* using various cell culture models, as well as in leptin deficient obese diabetic (*ob/ob* mice) and to determine their main *in vivo* mechanisms of action. Development of an assay for an apelin analogue will enable necessary pharmacokinetic studies to be undertaken.

Thus we will fully evaluate the antidiabetic potential of APJ receptor activation using the two most effective analogues developed, namely analogue A and the acylated analogue B. The intellectual property (IP) related to these peptides has been protected by a recent patent filing. Both analogues

will be tested for their effects on *in vitro* insulin secretion in BRIN-BD11 cells alone and in combination with incretin mimetics in clinical use, namely exendin-4(1-39) and liraglutide. Additional *in vitro* studies looking at the effects of single and combined use of apelin/incretin approaches on intracellular calcium, as well as cAMP second messenger responses will be investigated. A radioimmunoassay/ELISA will be developed against the most promising analogue raising polyclonal antibodies in rabbits/guinea pigs, as well as investigating the potential for an ESI MS-MS based assay for detection of apelin analogue(s) in mouse plasma samples following treatment.

For assessment *in vivo* control groups of obese hyperglycaemic (*ob/ob*) mice or heterozygous (*ob/+*) mice (n=8) will receive twice daily injections of saline. Treatment groups (*ob/ob*, n=8) will receive twice daily i.p. injections of either analogue A, analogue B, liraglutide, or the combination of analogue B with liraglutide for 42 days. We will examine functional effects of chronic administration upon food intake, body weight, body composition, energy metabolism, glucose tolerance and insulin sensitivity. Cellular actions on pancreatic islet alpha and beta cells, muscle, adipose, hypothalamus and intestinal enteroendocrine cells will be assessed. The effectiveness of treatments upon cardiovascular outcomes and lipid parameters will be evaluated. Following the development of a sensitive assay for a selected apelin analogue the *in vivo* pharmacokinetic profile of this lead analogue will be determined. Treatment effects on expression of key genes of interest in heart, hypothalamus, liver, intestine, adipose tissue and pancreatic islets will be quantified, followed by protein expression using Western blot analysis as required. Overall this work promises a better understanding of apelin's mechanisms of action and will help bring forward a new drug target for obesity related diabetes.

#### **Methods to be used :**

A wide range of modern experimental methods are required for this proposed study including HPLC purification of synthetic peptides, mass-spectrometry, *in vitro* insulin secretion studies using pancreatic beta-cell lines, cAMP second messenger assays, insulin and glucagon immunoassays, glucose assays. Additionally, animal studies will involve assessing the effects of apelin receptor agonists and/or incretin combination in a model of obesity diabetes. Furthermore, pharmacokinetic assessment of peptide biological action, peptide dose responses, glucose tolerance tests, insulin sensitivity tests, peptide desensitization studies, blood biochemistry assessments including lipid profiles, Dexa scanning, measurement of indirect calorimetry, energy expenditure and locomotor activity using Complete Laboratory Animal Monitoring System (CLAMS) metabolic chambers.

#### **Skills required of applicant :**

The applicant should ideally have good practical laboratory, computer and communication skills and show enthusiasm and commitment to work diligently on all aspects the research project to completion under the leadership of his/her supervisors. A background in biomedical sciences, pharmacology or a related subject would be desirable

#### **References :**

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