Building a diabetes knowledge bank

Human physiology expert **Professor Marcia Hiriart** is focusing her latest efforts on improving understanding about Type 2 diabetes, insulin secretion in pancreatic cells and metabolic syndrome

Could you describe your main duties as Director of the Cellular Physiology Institute at the National Autonomous University of Mexico (UNAM)?

I coordinate the work of 54 head of group researchers (Professors), five Associated researchers, 84 Academic Technicians, students (nearly 350) and employees that attend the laboratories, and others that support secretarial work, etc., in order to facilitate the development of science. Our Institute is one of the best in UNAM and the country, so this is a major responsibility.

What are the primary aims of your research?

I want to understand the regulation of insulin secretion in different developmental stages of pancreatic beta cells and their physiopathological responses in metabolic syndrome and Type 2 diabetes mellitus. In my lab, we study the mechanisms that couple glucose stimulation and insulin secretion. We are mainly interested in the behaviour of ionic channels and the glucose transporter GLUT2 in single beta cells of rats. Also, we are interested in the modulation of these structures by nerve growth factor (NGF), different cytokines and lipids.

In another research direction, we have developed a model of metabolic syndrome in rats, by giving them 20 per cent sucrose in drinking water. After two months of treatment, rats are obese, and present high levels of triglycerides in plasma, high insulin secretion and insulin resistance, and mild hypertension. All these factors are characteristic of metabolic syndrome in humans. We are studying the changes in beta cells due to this excess sucrose in the diet.

Could you outline some of the symptoms and causes of metabolic syndrome? Why do these factors sometimes lead to Type 2 diabetes?

Metabolic syndrome is a group of signs that increases the risk of developing cardiovascular disease, Type 2 diabetes and some cancer types. To define the syndrome, the individual must present at least three of the following: central obesity, dyslipidaemia (such as high levels of triglycerides and cholesterol), hypertension, hyperinsulinaemia and insulin resistance.

It is not known exactly why this condition may lead to Type 2 diabetes. However, it may be that inflammation mediators (for example, cytokines and growth factors) secreted by the adipose tissue stimulate beta cells to secrete large amounts of insulin. With time, beta cells become exhausted and no longer secrete enough insulin to maintain glucose at normal levels, thus diabetes develops.

What is the role of NGF in insulin secretion and how did you elucidate this link?

First, we discovered that beta cells develop neurite-like processes when cultured in the presence of NGF. This was exciting because beta cells are not neurons. Moreover, this is not normally seen in pancreatic islets. We also observed that NGF increased insulin secretion, as beta cells have NGF receptors (13, 14, 21). I investigated these effects for a couple of years, and asked if beta cells could be normally exposed to NGF. Initially, I thought that NGF could come from the exocrine tissue, which surrounds the islets, but we discovered that beta cells themselves produced and secreted NGF (17). We then discovered that autocrine regulation of insulin secretion (24) could be happening. In addition, we identified that human beta cells also produce NGF (28).

What methods did you use to study NGF and insulin secretion further and what insights did you gain into foetal development?

To measure insulin secretion from single beta cells, we used an insulin reverse haemolytic plaque assay. To measure NGF secretion by beta cells, we used the enzyme-linked immunosorbent assay (ELISA) technique alongside immunofluorescence to identify both markers. I knew from the literature that the high-affinity NGF receptor was necessary for the correct development of islets. If foetal beta cells were producing the neurotrophic factor, then it could be an autocrine/paracrine regulator of development.

Do you have a clear plan for your research in the next few years?

I am interested in the reversibility of the effects of metabolic syndrome in beta cells, and the timing of this process. We are looking forward to interpreting microarrays comparing immature and mature beta cells, and trying to characterise the behaviour of the principal genes where we find differences. We will also develop some studies in humans, trying to understand some of the changes observed in metabolic syndrome in rats.

In addition to further studying the adipose tissue of rats under different developmental stages, with and without metabolic syndrome, and looking at the effects on beta cell physiology, we will focus on other channels in beta cells.

New insights into metabolic syndrome

Researchers from the Cellular Physiology Institute at the **National Autonomous University of Mexico** are making important contributions to the field of diabetes through their work on metabolic syndrome and beta cells under different conditions

THERE ARE NEARLY 350 million people worldwide with diabetes and, with global levels of Type 2 diabetes rapidly increasing, there has been much scientific attention recently placed on unravelling the mechanisms behind this disease. The World Health Organization (WHO) believes that the emerging global diabetes pandemic can be traced back to rapid increases in overweight, obesity and physical inactivity.

Professor Marcia Hiriart, an internationally acclaimed physiology researcher in this field and Director of the Cellular Physiology Institute at the National Autonomous University of Mexico (UNAM), is spearheading work to improve understanding of insulin secretion in pancreatic cells and metabolic syndrome. This syndrome can be defined as a group of signs that increases the risk of developing Type 2 diabetes and other serious conditions. Whilst metabolic syndrome development is accelerated by genetic susceptibility, there are some environmental factors known to hasten its onset, including a nutrient-rich diet and low physical activity.

THE ROLE OF A HIGH SUCROSE DIET

Along with a number of contributors from the University, Hiriart's laboratory has been investigating the mechanisms that lead to hyperinsulinaemia. This step was seen as being essential to help explore the ways in which beta cells fail in a patient with Type 2 diabetes. In this study, they looked at how giving adult male Wistar rats a chronically high sucrose diet over a period of several months induces metabolic syndrome. To do this, the researchers analysed the effect of the internal environment of rats with metabolic syndrome and how such a diet impacted on their pancreatic beta cells.

After two months, the rats demonstrated the kind of signs the team was looking for: "After this exposure time, the rats displayed central obesity, hyperinsulinaemia and insulin resistance, and their systolic blood pressure and triglyceride plasma levels increased," Hiriart expounds. After six months of treatment, symptoms got worse and the rats also became hyperglycaemic. These are classic symptoms of the onset of metabolic syndrome and show that environment – in this case, a high sucrose diet – is one of the key drivers triggering the development of the condition.

> Hiriart's research emphasises that, regardless of genetic background, a high sucrose diet leads to the development of metabolic syndrome

Prior to this work, Hiriart's laboratory explored the role that nerve growth factor (NGF) plays in the differentiation and function of pancreatic beta cells; given NGF is known to induce morphological and physiological changes in pancreatic beta cells. They were able to show that pancreatic beta cells in adult rats were actually producing and secreting this growth factor. In addition, they highlighted the different impacts of NGF and cyclic adenosine monophosphate (cAMP) on the release of insulin. "Taken together, our findings suggest endocrine and autocrine roles for pancreatic beta-cell NGF which, in turn, could be related to the pathogenesis of Type 2 diabetes where serum NGF levels are diminished," adds Hiriart. This was the first time that a laboratory demonstrated that pancreatic beta cells synthesise and secrete NGF. Hiriart considers that this particular project has been one of her most important contributions to this field of medical research.

LINKAGES WITH BETA CELLS

Under Hiriart's direction, the group has recently been awarded a grant to analyse genetic and functional regulation of pancreatic beta cells in the development and physiopathology of metabolic syndrome. Hiriart points out that it is essential to first be cognisant of the normal behaviour of beta cells and understand how they become deregulated and exhausted when the external medium is too rich in nutrients: "Pancreatic beta cells are the only cell type that secrete insulin and are essential for nutrient homeostasis," she outlines.

INTELLIGENCE

ANALYSIS OF GENETIC AND FUNCTIONAL REGULATION OF PANCREATIC BETA CELLS IN DEVELOPMENT AND PHYSIOPATHOLOGY OF METABOLIC SYNDROME

OBJECTIVES

To understand the regulation of insulin secretion in different developmental stages of pancreatic beta cells and their physiopathological responses in metabolic syndrome and Type 2 diabetes mellitus.

KEY COLLABORATORS

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PROFESSOR MARCIA HIRIART URDANIVIA is Director of the Cellular Physiology Institute, UNAM in Mexico, first elected in 2009 and now re-elected for a further four years (2013-17). Hiriart completed an MD at the UNAM School of Medicine (1975-80), an MSc in Physiology and Biophysics at the Center for Advanced Studies, Instituto Politécnico Nacional (CINVESTAV, IPN; 1980-83) and a PhD also at CINVESTAV, IPN (1988). She conducted her thesis research in the School of Medicine, University of Pennsylvania, USA, where she also undertook postdoctoral training.

"During development, rat beta cells are immature, meaning they secrete discrete amounts of insulin and are not sensitive to changes in extracellular glucose concentrations". It is for this reason that rats make a very useful model to learn more about the stages of physiologic insulin resistance, which in this case is present around the weaning period. Hiriart is utilising this in her work: "We want to compare the gene expression and physiology of these cells to adult mature cells and cells under metabolic syndrome".

The main objective of the study is to look at changes in the transcriptome of pure rat beta cells in different rat subjects. The investigation is based on their previously developed metabolic syndrome model. In this latest project, they used young rats, normal adult rats and adult rats with metabolic syndrome for comparison. "We correlated this result with the functional expression of molecules that are important in stimulus-secretion coupling in cells, like ionic channels and glucose transporter GLUT2," reveals Hiriart. They then analysed the expression and secretion of NGF by beta cells in all the different animal models. "We were hopeful that this effort would enable us to garner some new understanding about how relevant this molecule is in the maturing process of cells, and were pleased with the results."

INSIGHTS INTO METABOLIC SYNDROME

Their findings over a number of years are certainly insightful. The team has been able to demonstrate that the changes they have seen in ionic channels could partially explain the increase in insulin secretion in rats with metabolic syndrome. "However, some beta cells showed smaller calcium currents or no current at all. These cells may represent a beta cell subpopulation as it becomes exhausted by the long-term high sucrose diet," Hiriart muses.

Taken together, Hiriart's research emphasises that, regardless of genetic background, a high sucrose diet leads to the development of metabolic syndrome – but there is still much to learn. Along with expert contributors, she has recently been involved in reviewing the existing body of evidence about phosphorylation of transient receptor potential (TRP) channels in beta cells as well as discussing the most current expert opinions about insulin secretion. Her laboratory has also been using the metabolic syndrome model to show that the condition induces electrical remodelling of the sinus node and produces arrhythmias, or problems with heart rates or rhythms.

COLLABORATE TO INNOVATE

Hiriart has been collaborating closely with a number of graduate students through this project, and to lesser extent postdoctoral fellows. Through this mutually beneficial partnership, she has been able to harness molecular biology techniques that the students have learned in their courses. Hiriart's group has also been building close relationships with different researchers and professors from the Institute, as well as from other places in the University, such as UNAM's School of Medicine, and the Autonomous University of Puebla (UAP). All of these groups offer important expertise to support the studies.

Aiming to disseminate her work broadly through the community, Hiriart has been sharing the knowledge garnered through years of research in the global arena by attending important international meetings and conferences. She has presented her findings and discussed their implications in regard to the management and treatment of diabetes. Hiriart has also prepared a number of papers which have been published in respected journals, such as *Current Diabetes Reviews* and *PLoS ONE*. Such activities show that Hiriart will continue to offer her enthusiasm and expertise in order to further add to the knowledge base around diabetes and the disease's relationship with both metabolic syndrome and beta cells.