Regulation of δ-cell pancreatic secretion in health and disease

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Background

Diabetes is a trihormonal disease in which secretion from all three of the major cell types in the pancreatic islet (α-, β- and δ-cells) is dysregulated. While insulin secretion from β-cells and, more recently, glucagon secretion from α-cells has been extensively studied, our understanding of the regulation of somatostatin (Sst) secretion from δ-cells is comparatively poor. However, Sst plays a very important paracrine role in both health and disease, as it inhibits both insulin and glucagon release. Our studies suggest Sst also mediates the inhibitory effect of β-cell activation on glucagon release [1], which may explain why glucose fails to supress glucagon release in Sst KO mice. It is possible that dysregulation of Sst release in diabetes may contribute to the inappropriately elevated glucagon levels. A better understanding of the regulation of Sst release, and its paracrine roles, is therefore important.

Aims

The aim of this project is to identify the intrinsic and paracrine mechanisms regulating somatostatin release, determine how these are altered in diabetes, and define how Sst influences paracrine regulation of insulin and glucagon release in both health and disease.

Description of work to be undertaken

We will express the activating Kir6.2-V59M mutation in δ-cells by crossing floxed V59M mice [2,3] with mice expressing Cre-recombinase under the control of the Sst promoter (δSst mice). This will hyperpolarise the δ-cell, selectively switch off Sst release and thereby allow us to investigate the paracrine role of Sst on insulin and glucagon release. We can restore Sst secretion in δSst mice/islets using sulphonylureas to block the open K_{ATP} channels. δ-cells will be fluorescently labelled with RFP (triple transgenic with floxed-RFP). Using both control and δSst mice, we will examine glucose homeostasis (e.g. IPGTT), hormone secretion (insulin, glucagon; *in vivo* and in isolated islets), and islet cell electrophysiology, both at rest and in response to secretagogues. This will enhance our understanding of how Sst regulates insulin and glucagon release, and its contribution to glucose homeostasis.

δSst mice will express Kir6.2-V59M in all cells that express Sst. However, mutant K_{ATP} channels will only be present in those cells that also express the SUR1 subunit of the K_{ATP} channel. This will include certain neurones and gut cells, and provides us with an opportunity to examine the role of Sst in gut endocrine

cells (e.g using the perfused gut preparation [a]).

In separate experiments, we will inducibly express Kir6.2-V59M in β-cells, which will inhibit insulin secretion. Thus, this mouse model enables diabetes to be rapidly switched on, and euglycaemia to be subsequently restored by sulphonylurea therapy [2,3]. We will use this approach to examine the effect of diabetes on (i) Sst release (in isolated islets and perfused pancreas); and (ii) δ-cell electrophysiology, by patch-clamping δ-cells in whole islets. δ-cells will be identified by their electrophysiological fingerprint [4], Sst immunocytochemistry or by subsequent gene expression profiling of the patched cell. These studies will increase our knowledge of how δ-cell function is altered in diabetes.

The fellow will gain experience of a wide range of multidisciplinary techniques, including working with genetically modified mice (breeding programs, glucose tolerance tests, mouse surgery, etc), FACS, patch-clamping, hormone secretion assays etc.

Supervisor's recent relevant publications

1. Briant LJB, Reinbothe TM, Spiliotis I, Miranda C, Rodriguez B, **Rorsman P** (2018) δ-cells and β-cells are electrically coupled and regulate α-cell activity via somatostatin. *J Physiol* 596, 197-215
2. Brereton MF, Rohm R, Shimomura K, Holland C, Tornovsky-Babeay S, Dadon D, Iberl M, Chibalina MV, Lee S, Glaser B, Dor Y, **Rorsman P**, Clark A, **Ashcroft FM** (2016). Hyperglycaemia induces metabolic dysfunction and glycogen accumulation in pancreatic beta-cells. *Nature Commun* 7, 13496
3. Brereton MF, Iberl M, Shimomura K, Zhang Q, Adriaenssens AE, Proks P, Spiliotis II, Dace W, Mattis KK, Ramracheya R, Gribble FM, Reimann F, Clark A, **Rorsman P, Ashcroft FM** (2014). Reversible changes in pancreatic islet structure and function produced by elevated blood glucose. *Nat Commun* 5, 1-11
4. Briant LJ, Zhang Q, Vergari E, Kellard JA, Rodriguez B, **Ashcroft FM**, **Rorsman P** (2017) Functional identification of islet cell types by electrophysiological fingerprinting. *J R Soc Interface* 128
5. Braun M, Ramracheya R, Amisten S, Bengtsson M, Moritoh Y, Zhang Q, Johnson PR, **Rorsman P** (2009) Somatostatin release, electrical activity, membrane currents and exocytosis in human pancreatic delta cells. *Diabetologia* 52, 1566-78

References (in addition to those above)

1. Svendsen B, Holst JJ (2016). Regulation of gut hormone secretion. Studies using isolated perfused intestines. *Peptide* 77, 47-53