How does chronic hyperglycaemia impair β-cell metabolism and thereby induce loss of both β-cell function and β-cell mass?

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Background

Diabetes is characterised by hyperglycaemia resulting from decreased pancreatic islet cell function and/or mass. We have studied the effects of hyperglycemia, in the absence of insulin resistance or dyslipidemia, using a Cre-lox mouse model that expresses an inducible activating K_{ATP} channel mutation found in patients with neonatal diabetes (ND) [4]. This enables insulin secretion to be switched off by gene induction, and immediately restored by treatment with sulphonylurea (SU) drugs (which close the open K_{ATP} channels). We found K_{ATP} channel activation specifically in β-cells of adult mice led to rapid diabetes, a dramatic reduction in insulin content, degranulation, and marked changes in β-cell metabolism. In particular, glucose no longer elevated intracellular ATP, and marked changes in expression of metabolic genes were observed, indicative of reduced oxidative phosphorylation [5]. In unpublished studies, we confirmed these mRNA changes translate into changes in protein. In addition, substantial glycogen accumulation occurred, which correlated with apoptosis and reduced β-cell mass [5]. Many (but not all) of these changes were rapidly reversed on restoration of euglycaemia by SU therapy [4,5]. In contrast to β-cells, α-cells did not accumulate glycogen or degranulate, suggesting they are protected from the deleterious effects of hyperglycemia.

Hypothesis and Aims

We hypothesise chronic hyperglycemia reduces both β-cell function and mass primarily via impairment of β-cell metabolism. Reduced oxidative metabolism (and thereby ATP production and K_{ATP} channel closure) impairs glucose-induced insulin secretion (GSIS). It leads to excessive glycogen accumulation, so triggering apoptosis and decreasing β-cell mass. Thus the aim of the project is to use a multidisciplinary approach to determine how diabetes affects β-cell metabolism. Understanding how β-cell metabolism is impaired may lead to new therapeutic targets for type 2 diabetes therapy.

Description of work to be undertaken

We will use a multidisciplinary approach to examine metabolism in β-cells/α-cells/islets from ND diabetic mice, control human/mouse islets cultured at low/high glucose and (if large cell numbers are necessary) INS1-E β-cells. We will determine how long and how high plasma glucose must rise to induce reversible changes in metabolism. Transcription factors (TFs) involved will be identified by bioinformatics of our RNAseq data, CHIPseq, and knockdown of putative TFs. Metabolomics and tracer

studies will be used to obtain data on the metabolic pathways affected. For example, we will measure (i) ATP levels (biochemically or by genetically encoded fluorescent biosensors (ii) NADH (iii) mitochondrial membrane potential (e.g. by rhodamine 123). To determine where the metabolic block in diabetes resides, we will examine GSIS and ATP in diabetic β-cells in response to substrates that enter at different steps of metabolism (e.g., leucine, methylsuccinate); and explore if high concentrations of these substrates induce changes similar to those caused by hyperglycaemia. We will examine if the enhanced basal insulin secretion of diabetic islets is due to glycogen accumulation, and if this contributes to insulin-induced hypoglycaemia in vivo. We will also determine the extent to which the changes in metabolism, gene expression etc are reversed by SU therapy.

To explore why α-cells appear to be protected from chronic hyperglycaemia, RNAseq, glucose-induced changes in cytosolic NADH/ATP, etc will be compared in α- and β-cells from control and diabetic mice.

If time permits, we will explore whether similar changes in metabolism, glycogen accumulation, autophagy and glycogen toxicity, contribute to the deleterious effects of hyperglycaemia on peripheral tissues (e.g. kidney, neurons).

Supervisor's recent relevant publications

1. 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 Communications* **7**, 13496. PMID: 27882918.

2. Brereton M, Rohm M, **Ashcroft FM** (2016). Hyperglycaemia and the pancreatic beta-cells: a crisis of identity? *Diabetes, Obesity and Metabolism* **18** (S1), 102-109.

3. Brereton MF, Iberl M, Shimomura K, Zhang Q, Adriaenssens AE, Proks P, Spiliotis II, Dace W, Mattis KK, Ramracheya A, FM, Reimann F, Clark A, Rorsman P, **Ashcroft FM** (2014) Reversible changes in pancreatic islet structure and function produced by elevated blood glucose. *Nature Communications* **5**, 4639.

4. **Ashcroft FM**, Rorsman (2013) K_{ATP} channels and islet hormone secretion: recent findings and controversies. *Nature Reviews Endocrinology* **9**, 660-669. PMID: 24042324.

5. Clark RH, McTaggart JS, Webster R, Mannikko R, Iberl M, Sim XL, Rorsman P, Glitsch M, Beeson D, **Ashcroft FM** (2010) Muscle dysfunction caused by a K_{ATP} channel mutation in neonatal diabetes is neuronal in origin. *Science* **329**, 458-461. PMID: 20595581.