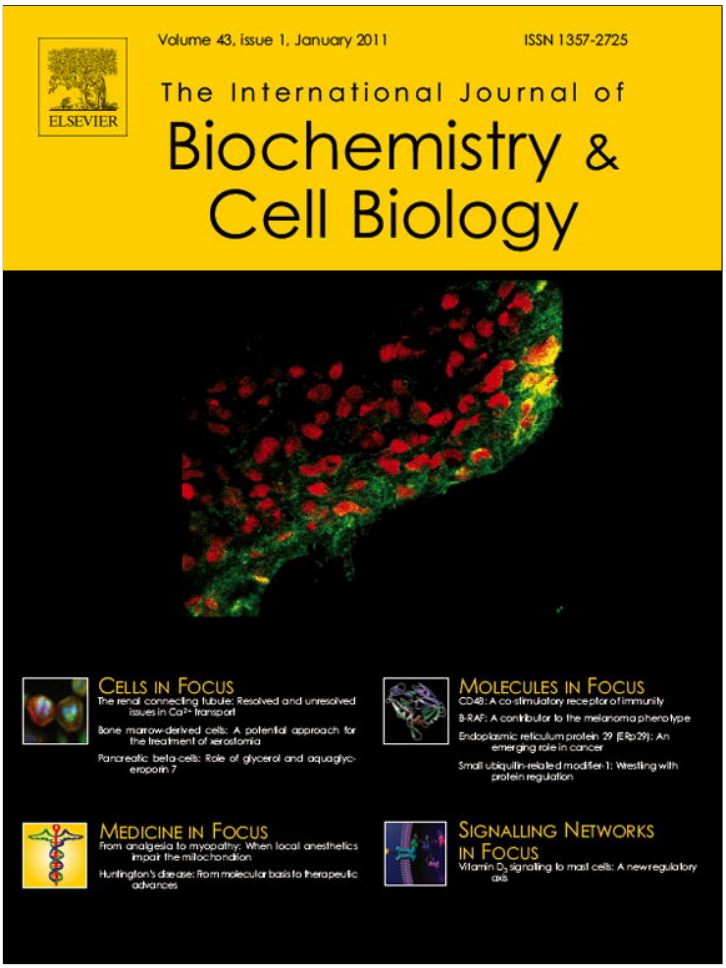

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Cells in focus

Pancreatic beta-cells: Role of glycerol and aquaglyceroporin 7

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ABSTRACT

Pancreatic β-cells originate from gut endoderm during development. Pancreatic endocrine cells represent about 10% of the mature pancreatic cells, and β-cells represent the majority of endocrine cells. β-cells secrete insulin in response to elevation of nutrient concentrations. Insulin maintains glucose homeostasis by stimulating glucose uptake into muscle and adipose tissue. Aquaglyceroporin 7, permeable to water, glycerol and urea, is expressed in pancreatic β-cells and was recently described as being involved in the control of insulin secretion.

Cell facts

- Pancreatic β-cells are endocrine cells which comprising ∼1–2% of the total cell mass of the pancreas, and ∼65–85% of the Langerhans islets cells.
- Pancreatic β-cells synthesize, store in secretory granules and secrete insulin in response to rising concentration of circulating glucose.
- Aquaglyceroporin 7, a water and glycerol permeable protein channel, is expressed by pancreatic β-cells.

1. Introduction

Paul Langerhans, during his MD thesis, made a famous histological discovery: the pancreatic islets, groups of small polygonal cells with an almost perfect homogeneous content (Langerhans, 1869). It was not until 1893 that Edouard Laguesse postulated that these cells might produce an endocrine secretion. This secretion was later supposed to regulate sugar in the bloodstream. In 1922, insulin was isolated from islets of Langerhans by Frederick Banting and John Macleod (Bliss, 1982).

Pancreatic β-cells derive from gut endoderm during development. In mature pancreas, endocrine and exocrine cells account respectively for 10% and 90% of total cells. The function of pancreatic β-cells is to release insulin in response to increase nutrients such as glucose, amino acids, and fatty acids. Subsequent insulin release then stimulates glucose uptake principally in muscle and adipose tissue, to maintain glucose homeostasis. Recently, aquaglyceroporin 7 (AQP7), permeable to water as well as glycerol and urea, was shown to be expressed in pancreatic β-cells. Consequently, the role of AQP7 in the insulin secretion process was investigated. Diabetes is characterized by abnormal regulation of blood glucose levels. Type 1 and type 2 diabetes result respectively, from the loss or the impaired function of pancreatic β-cells. Due to the high prevalence of diabetes worldwide, understanding the control of insulin secretion and pancreas development is crucial to develop new therapeutic approaches.

2. Cell origin and plasticity

During vertebrate development, three germline layers emerge after gastrulation: the ectoderm, mesoderm and endoderm. A series of coordinated signalling events and regulatory transcriptional cascades play crucial roles in pancreas patterning, originating from the gut endoderm and progressing to the adult organ (Gittes, 2009; Puri and Hebrok, 2010). By E15.5, fate specification of all differentiated cells types forming the mouse adult pancreas has been undergone (Jørgensen et al., 2007). During the formation of the complex three-dimensional branched structure of the pancreas, endocrine precursors delaminate from the epithelium and clusters, while cells remaining within the epithelium adopt exocrine fates (Puri and Hebrok, 2010). Mature pancreas consists of 90% of exocrine cells and 10% of endocrine cells. Exocrine cells comprise acinar cells, synthesizing and secreting digestive enzymes, and ductal cells, releasing mostly fluid (McManaman et al., 2006). Endocrine cells are a compound structure termed islet of Langerhans. Islets of

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Langerhans are made of a majority of insulin-producing β-cells surrounded by glucagon-producing α-cells, somatostatin-producing δ-cells and pancreatic polypeptide-producing PP cells.

After birth, the rate of β-cell mass expansion, governed by the rates of new β-cells formation and β-cells loss, slows down. A better understanding of the mechanisms involved in β-cell expansion has contributed to the identification of a whole set of distinct signaling pathways and molecules that can be used as potential target to allow *in vitro* and *in vivo* proliferation of β-cells (Puri and Hebrok, 2010; Dhawan et al., 2007). Generation of β-cells can be achieved by exocrine-to-endocrine transdifferentiation (Baeyens and Bouwens, 2008), differentiation of stem and progenitors cells (Dhawan et al., 2007; Puri and Hebrok, 2010).

Both insulin and insulin like growth factor (IGF) signalling pathways through insulin receptor substrate proteins (IRS) play important roles for proper maintenance and functioning of beta cells (Kubota et al., 2000). The latter is essential for the transmission of intracellular signalling from the insulin receptor to downstream pathways and transcriptional regulation.

Recently, it has been demonstrated that glucose effect on beta-cells growth and survival requires activation of insulin receptors (Assmann et al., 2009). Likewise, IRS1 and IRS2 seem to play critical roles in beta cells mass, as supported by IRS1 or IRS2 knockout mice (Burks and White, 2001).

3. Functions

During postprandial periods, β-cell insulin secretion is stimulated by elevated plasma glucose, free fatty acids (Nolan et al., 2006) and amino acids concentrations (Sener and Malaisse, 2002). Insulin secretion is required to maintain glucose homeostasis by promoting glucose uptake principally by muscle and adipose tissue.

In current models of stimulus-secretion (i.e. insulin) coupling, β-cells are featured as electrically excitable fuel sensors under hormonal and neural control. Stimulus-secretion coupling models emphasize the role of mitochondrial metabolism, ATP-sensitive potassium channels (K_{ATP} channels), calcium influx and increase in cytosolic calcium concentration (Henquin and Meissner, 1984). In addition to this triggering pathway, an amplifying pathway, increasing triggering calcium stimulated exocytosis, is also involved in insulin secretion (Henquin, 2009).

Unlike epithelial cells, β-cells are not likely to be exposed to important changes in extracellular osmolarity. However, alterations in intracellular osmolarity and cell volume are likely to occur in response to transport and intracellular metabolism of substrate and metabolites. It has been shown that β-cell volume increases in response to an increase in glucose concentration (Miley et al., 1997). Importantly, cell volume was shown to profoundly influence the activity of β-cells. Indeed, exposure of β-cells to hypotonic extracellular solutions induce cell swelling, activating volume-regulated anion channel (VRAC) and cell membrane depolarization, leading to activation of voltage-sensitive calcium channels, allowing calcium entry, and thereby insulin exocytosis (Best et al., 1996; Drews et al., 2010).

Independently of the origin of β-cell swelling, e.g. exposure to hypertonic extracellular medium or hypertonic intracellular medium, aquaporins are likely to play a role in cell swelling. Aquaporins are membrane water channels of about 28 kDa possessing six transmembrane domains and two repeating Asn-Pro-Ala (NPA) sequences motifs, present in the first intracellular and third extracellular loop, involved in the formation of the water pore (Agre, 2004). The thirteen mammalian aquaporins cloned so far can be classified into three subfamilies according to their permeability: (1) aquaporins: exclusively permeable to water (AQP1, AQP2, AQP4, AQP5, AQP6, AQP8); (2) aquaglyceroporins: permeable to water as well as small solutes such as glycerol and urea (AQP3, AQP7, AQP9, AQP10); and (3) aquaporins with unusual NPA motifs and permeability remaining controversial (AQP11, AQP12) (Agre, 2004). Aquaglyceroporin AQP7 is expressed in mouse (Matsumura et al., 2007) and rat (Best et al., 2009) β-cells and pancreatic β-cell line BRIN-BD11 (Delporte et al., 2009) (Fig. 1). Pancreatic β-cells from AQP7 knockout mice display reduced size, mass, insulin content, and increased rates of basal and glucose-stimulated insulin secretion (Matsumura et al., 2007). Furthermore, β-cells from AQP7 knockout mice also showed increased glycerol and triglyceride contents, increased glycerol kinase activity and decreased forskolin-induced glycerol release through cAMP lipolysis stimulation (Matsumura et al., 2007). Based on the analysis of AQP7 knockout mice, the role of AQP7 in pancreatic β-cells insulin secretion is summarized in Fig. 2. Isoosmotic addition of glycerol to the extracellular medium of normal rat β-cells induced in β-cell the sequential swelling, VRAC activation, membrane depolarization, electrical activity and concomitant insulin release (Best et al., 2009) (Fig. 3). The effects of glycerol, in contrast to urea, persist throughout exposure to osmolytes. Moreover, glycerol activates β-cells even when added hyperosmotically in the extracellular medium. These data suggest that β-cell activation by glycerol is the results of its transport into the cells as well as an additional action concomitant to glycerol metabolism (Best et al., 2009). In BRIN-BD11 cells, glycerol uptake occurs rapidly and is higher when cells are submitted to hypotonic extracellular medium or to medium deprived of 50 mM NaCl but enriched with 100 mM urea, compared to isoosmotic medium. Insulin output was concomitantly higher in BRIN-BD11 cells exposed to hypotonic extracellular medium or to medium deprived of 50 mM NaCl but enriched with 100 mM urea or 100 mM glycerol, compared to isoosmotic medium. Furthermore, an inhibitor of VRAC, 5-nitro-2-(3-phenylpropylamino)benzoate, suppress insulin output induced by medium deprived of 50 mM NaCl but enriched with 100 mM urea or 100 mM glycerol. These data confirm that under extracel-

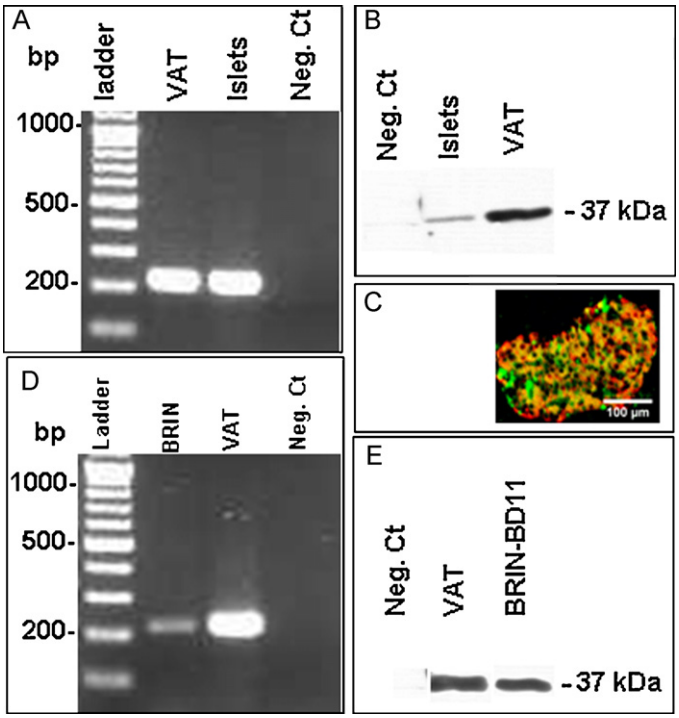

Fig. 1. AQP7 expression in rat pancreatic islets and BRIN-BD11 cells. (A and D) RT-PCR detection of AQP7 mRNA. (B and E) AQP7 detection of expression by Western blot. (C) Double fluorescent immunolabelling of AQP7 expression in rat pancreatic β-cells. AQP7 labelling, in green, overlaps with that of insulin, in red.

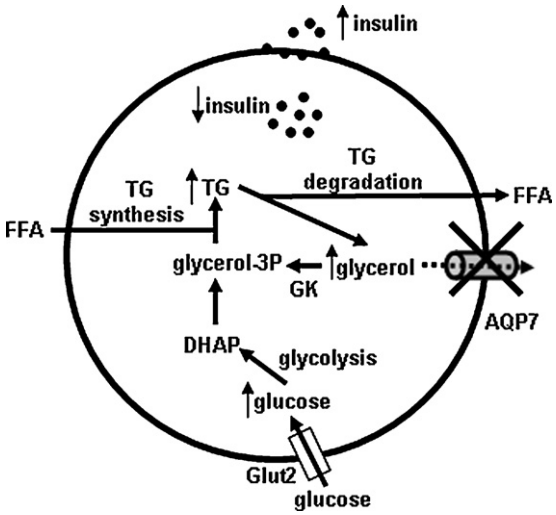

Fig. 2. Role of AQP7 in pancreatic β-cells based on the analysis of AQP7 knockout mice. AQP7 knockout mice display increased glucose entry, increased intracellular glycerol and triglycerides (TG) concentrations, decreased intracellular insulin concentrations, increased insulin secretion. Increased intracellular glycerol concentrations induce increased glycerol kinase (GK) activity. Glycerol kinase promotes re-esterification of glycerol and accelerates TG accumulation. Increased glucose capitation contributes to increased glucose metabolization by glycolysis.

lular isotonicity, both glycerol and urea rapidly enter BRIN-BD11 cells, inducing cell swelling, subsequent VRAC activation, membrane depolarization, calcium entry, increase cytosolic calcium concentration and insulin secretion (Delporte et al., 2009). The role of glycerol and AQP7 in insulin secretion corroborates with the observations made in AQP7 knockout mice (see above; Matsumura et al., 2007). Under pathophysiological conditions, rise in local intracellular glycerol concentration may result from either intensive starvation concomitantly to glycerol entry, insulin increase that downregulates AQP7 expression (Kishida et al., 2001; Kondo et al., 2002), or genetic alteration of AQP7 gene leading to non-functional or decreased AQP7 expression (see below). Furthermore, rise in local intracellular glycerol/glycerol metabolites concentrations may occur during insulin resistance leading to increased blood glucose. Indeed, glucose-stimulated insulin secretion has been shown to correlate with β-cell lipolysis (Mulder et al., 2004; Winzell et al., 2006). Lipolysis will increase both intracellular glycerol and fatty acids, the latter being necessary to support insulin secretion (Yaney and Corkey, 2003). Glucose oxidation will accelerate production of glycerol-3P. These events will lead to an increase in glycerol/glycerol metabolites and may therefore induce the observed *in vitro* glycerol effect (Best et al., 2009). Furthermore, the downregulation of AQP7 expression by insulin (Kishida et al., 2001; Kondo et al., 2002) will be sustained and mimic thereby the mouse AQP7 knockout situation. Clarification of timing and metabolic steps/metabolite(s) involved in these processes will require further studies. For example, conditional knockout of AQP7 in pancreatic β-cell should provide an additional tool to determine the role of AQP7 and glycerol in the process of insulin secretion under physiological conditions.

4. Associated pathologies: β-cells and diabetes

Diabetes results from either loss (type 1) or impaired functions (type 2) of which pancreatic β-cells are involved. As a consequence, abnormal regulation of blood glucose levels and significant complications occur. According to the World Health Organization, the prevalence of diabetes reached about 180 millions of people worldwide in 2000, and is expected to double by 2030. Approaches aiming at improving β-cell replacement therapy have focused on finding the most suitable progenitor cell capable of generating functional β-cells in large number: β-cells themselves, embryonic or adult stem cells, or other cell types that can undergo reprogramming (such as exocrine-endocrine transdifferentiation) (Puri and Hebrok, 2010; Baeyens and Bouwens, 2008; Dhawan et al., 2007).

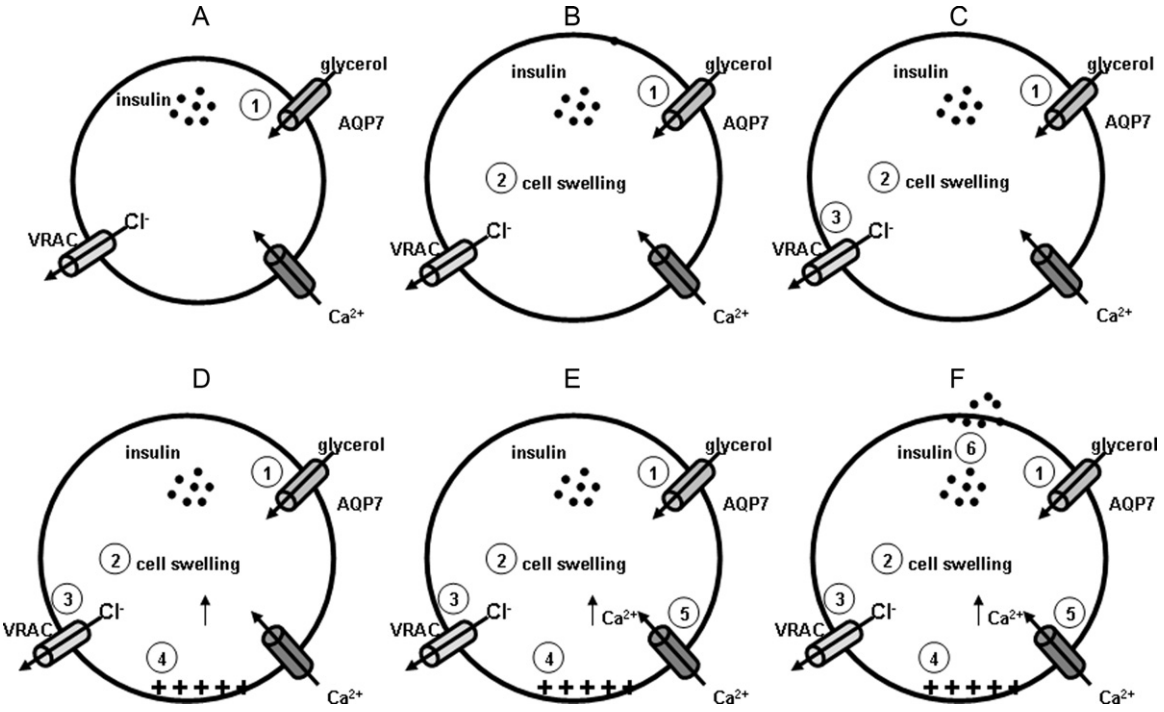

Fig. 3. Sequential events leading to insulin exocytosis in response to glycerol entry via AQP7. (A) Glycerol enters β-cells via AQP7 (1). (B) Glycerol entry causes cell swelling (2). (C) Cell swelling activates VRAC channels with concomitant Cl^- exit (3). (D) Cl^- exit induces membrane depolarization (4). (E) Membrane depolarization stimulates voltage-sensitive calcium channel and calcium entry into the cell (5). (F) Increased intracellular calcium concentration induces insulin exocytosis (6).

Pancreatic beta cells are sensitive to attack by reactive oxygen or nitrogen species (ROS or RNS) and contribute to gluco- and lipotoxicity in the development of diabetes. Furthermore, isolations of islets for transplantation undergo detrimental oxidative stress. Consequently, the addition of a redox modulation strategies could be a beneficial clinical approach for islets preservation for islets transplantation (Sklavos et al., 2010).

AQP7, controlling glycerol transport, plays a key role in insulin secretion. Two AQP7 knockout mice display hyperinsulinemia (Matsumura et al., 2007; Hibuse et al., 2005, accompanied (Hibuse et al., 2005) or not accompanied (Matsumura et al., 2007) by hyperglycemia. In a third AQP7 knockout mice model, insulin and glucose concentration have not been determined (Hara-Chikuma et al., 2005). Finally, a fourth AQP7 knockout mice model displays normal glycaemia, while insulinemia has not been determined (Skowronski et al., 2006). The existence of different AQP7 knockout mice phenotypes may be attributed to the different genetic backgrounds of the mice. In Japanese subjects, the frequency of missense mutations (R12C, V59L, G264V) and silent mutations (A103A, G250G) of AQP7 were not associated with the phenotype for diabetes or obesity, despite the absence of glycerol transport in a subject with the G264V mutation (Kondo et al., 2002). More recently, it was shown that a single-nucleotide polymorphism in the human AQP7 promoter region (A-953G) resulted in a decreased C/EBP-β DNA binding and AQP7 expression in adipose tissue from obese individuals (Prudente et al., 2007). Furthermore, this polymorphism is associated with increased risk of type 2 diabetes in women (Prudente et al., 2007). Concerning the possible existence of a relationship between the level of AQP7 expression and obesity and type 2 diabetes, data remain controversial. Indeed, decreased AQP7 expression in adipose tissue was observed in severe obesity (Ceperuelo-Mallafre et al., 2007), while no relationship was found between AQP7 expression in adipose tissue and the presence of glucose abnormalities in morbid obesity (Miranda et al., 2009; Catalan et al., 2008). No modification (Ceperuelo-Mallafre et al., 2007), or an increase in AQP7 expression was observed in adipose tissue from patients with type 2 diabetes (Miranda et al., 2010). Different pathological conditions involve different sets of perturbed metabolic pathways and therefore the implication of a single gene product cannot necessarily have the same impact in all settings. Therefore, it remains difficult to assess if the AQP7 polymorphism (A-953G), AQP7 mutations, and/or AQP7 expression level contribute to obesity and type 2 diabetes. Further studies will hopefully validate or invalidate the hypothesis. Moreover, AQP7 regulation could become an attractive drug-target pathway for therapeutic obesity and type 2 diabetes strategies.

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