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GPRC5B a putative glutamate-receptor candidate is negative modulator of insulin secretion

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ABSTRACT

GPRC5B is an orphan receptor belonging to the group C family of G protein-coupled receptors (GPCRs). GPRC5B is abundantly expressed in both human and mouse pancreatic islets, and both GPRC5B mRNA and protein are up-regulated 2.5-fold in islets from organ donors with type 2 diabetes. Expression of Gprc5b is 50% lower in islets isolated from newborn (<3 weeks) than in adult (>36 weeks) mice. Lentiviral shRNA-mediated down-regulation of Gprc5b in intact islets from 12 to 16 week-old mice strongly (2.5-fold) increased basal (1 mmol/l) and moderately (40%) potentiated glucose (20 mmol/l) stimulated insulin secretion and also enhanced the potentiating effect of glutamate on insulin secretion. Down-regulation of Gprc5b protected murine insulin-secreting clonal MIN6 cells against cytokine-induced apoptosis. We propose that increased expression of GPRC5B contributes to the reduced insulin secretion and β-cell viability observed in type-2 diabetes. Thus, pharmacological targeting of GPRC5B might provide a novel means therapy for the treatment and prevention of type-2 diabetes.

1. Introduction

Diabetes is a multi-factorial metabolic disorder where the insulin producing pancreatic β-cell plays a central role [1,2]. The loss of adequate insulin secretion in type 2 diabetes results from a decline in β-cell secretory capacity and/or β-cell mass. Ultimately, the systemic insulin requirement exceeds the β-cells' capacity to secrete the hormone resulting glucose intolerancehomeostasis [3–5]. However, the factors culminating in β-cell dysfunction/reduced β-cell mass remain poorly defined but they are thought to involve both genetic and environmental (lifestyle) factors that culminate in altered expression of transcription factors, membrane receptors, ion channels and metabolic enzymes [2].

G protein-coupled receptors (GPCRs) constitute the largest family of transmembrane receptors in the human genome, and they are important regulators of pancreatic islet function [6]. One of the most abundant orphan receptors in human islets is GPRC5B [6], which belongs to an evolutionarily conserved subgroup of the C family of GPCRs including GPRC5A, GPRC5B, GPRC5C and GPRC5D, which are also known as retinoic acid induced genes (also known as RAIG1–4) as their expression is induced by all-trans retinoic acid [7–9]. GPRC5B displays sequence similarities with metabotropic glutamate receptors [10]. In addition to pancreatic islets, GPRC5B is also widely expressed in brain and white adipose tissue [8]. In man, a copy number variant in close proximity of the GPRC5B gene has been shown to be associated with body mass index [11], raising the interesting possibility that GPRC5B might play an important role in the regulation of human metabolism.

Due to its homology with metabotropic glutamate receptors and its high expression in the CNS, where glutamate serves as the major excitatory neurotransmitter [12], we hypothesized that GPRC5B might be involved in glutamate and/or retinoic acid-mediated receptor signaling. To investigate the function of GPRC5B, we used shRNAs delivered by lentiviral particles to selectively down-regulate the expression of Gprc5b in mouse islets and in the clonal β-cell line MIN6c4 to study its role in insulin secretion, apoptosis and proliferation.

2. Materials and methods

2.1. Chemicals

Fatty acid free bovine serum albumin (BSA) was from Boehringer Mannheim, Germany. Polyclonal rabbit anti-GPRC5B and HRP-conjugated goat anti-rabbit IgG were from Santa Cruz Biotechnologies, (CA, USA). Rabbit polyclonal anti-β-actin was from Sigma (USA). Cy2-conjugated anti-rabbit IgG and Cy5-conjugated anti-guinea pig IgG were from Jackson Immunoresearch Laboratories Inc., West Grove, PA, USA. Insulin radioimmunoassay kits were from Millipore (USA), and all other chemicals were from

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Merck AG (Darmstadt, Germany) or Sigma (USA). Gprc5b shRNA (m) (sc-62410-V) and scrambled control (sc-108080) lentiviral particles were obtained from Santa Cruz Biotechnology, Santa Cruz, CA, USA.

2.2. Isolation of human pancreatic islets

Isolated human islets from non-diabetic males and females (HbA_{1c} 4.3–6.2, BMI 20.1–30.2 kg/m^2, age 26–71 years, $n = 66$) and from diabetic males and females (HbA_{1c} 6.8–10, BMI 29.8–34.1 kg/m^2, age 30–65 years, $n = 6$) were provided by the Nordic network for clinical islet transplantation (O. Korsgren, Uppsala University, Sweden). The human islets were cultured in CMRL 1066 (ICN Biomedicals, Costa Mesa, CA) supplemented with 10 mmol/l HEPES, 2 mmol/l L-glutamine, 50 μg/ml gentamicin, 0.25 μg/ml fungizone (Gibco, BRL, Gaithersburg, MD), 20 μg/ml ciprofloxacin (Bayer Healthcare, Leverkusen, Germany) and 10 mmol/l nicotinamide at 37 °C (5% CO_2) for 1–5 days prior to the experiments. All procedures were approved by the local ethical committees at Uppsala and Lund Universities, Sweden.

2.3. mRNA expression of GPRC5B in human pancreatic islets

The expression of GPRC5B in human islets was determined using Affymetrix arrays and confirmed by quantitative real-time PCR (qPCR) using Qiagen's QuantiFast qPCR kit and QuantiTect primers (Table 1 for primers) as described elsewhere [5,6].

2.4. Animals

Female mice of the NMRI strain (B&K, Sollentuna, Sweden), weighing 25–30 g, were used for all experiments. They were housed in metabolic cages with free access to standard pellet diet (B&K) and tap water *ad libitum*. The local animal welfare committee (Lund, Sweden), approved all experimental protocols and procedures using animals.

2.5. Isolation of mouse pancreatic islets

Pancreatic islets were isolated by collagenase digestion as described elsewhere [13–15]. The islets were then handpicked under a stereomicroscope at room temperature and subjected to different experimental procedures.

2.6. Western blot

Lysates of islets (1000 islets/vial), brain, lung, heart, liver, and kidney were analyzed by SDS–PAGE, transferred to nitrocellulose, blocked for 1 h at room temperature in 5% (weight/vol) milk. The expression of mouse and human GPRC5B receptor protein relative β-actin was determined using a rabbit-raised polyclonal anti-Gprc5b antibody (1:500) and a rabbit anti-β-actin antibody (1:200) that were incubated with the membrane for 1 h at room temperature in TBST buffer with 0.01% (v/v) Tween 20. After several washes in TBST buffer, blots were probed with HRP-conjugated secondary antibodies (1:2000).

2.7. Confocal microscopy

The co-expression of Gprc5b with insulin was determined using immunohistochemistry as described elsewhere [13] using the antibodies described above.

2.8. Down-regulation of Gprc5b

Freshly isolated mouse islets were cultured with 1 ml RPMI 1670 medium for 36 h in the presence of Gprc5b shRNA lentiviral particles or scrambled control lentiviral particles according to the manufacturer's recommendations. After transfection, the islets were washed, supplied with fresh RPMI 1670 medium and allowed to recover for 12 h under cell culture conditions. The islets were then washed again and assayed for insulin secretion in the absence or presence of test agents as described elsewhere [13–15].

2.9. Cell proliferation

Clonal mouse insulin-secreting MIN6 cells were seeded at 1×10^3 cells/well into 48 well plates in DMEM Dulbecco's modified Eagle's medium (DMEM + GultaMax™-l, Gibco, USA) containing 4.5 g/l glucose supplemented with 15% fetal calf serum, 50 mg/1 streptomycin (Gibco), 75 mg/1 penicillin sulfate (Gibco) and 5 μl/ml β-mercaptoethanol (Sigma). The cells were then transfected with lentiviral particles targeting Gprc5b as described above. After transfection and a 12 h recovery period, the plates were incubated for 1–6 days at 37 °C, 5% CO_2. Cells (in individual wells) were harvested by trypsination and counted daily using a Bürcker chamber.

2.10. Cell viability measurements

After treatment with Gprc5b shRNA lentiviral particles (see above), mouse islets were dispersed into single cells using Ca^{2+} free-medium. The islet cells were then cultured with or without a cocktail of pro-apoptotic cytokines (IL-1β (100 ng/ml), TNFα (125 ng/ml), and INFγ (125 ng/ml)) for 24 h in RPMI1640 with 5 mmol/l glucose and 10% FSB supplemented with 50 μmol/l glutamate. Measurements of cell viability were performed using the MTS reagent kit according to the manufacturer's instructions (Promega).

2.11. Statistics

The results are expressed as means ± SEM for the indicated number of observations or illustrated by an observation representative of a result obtained from different experiments. Probability levels of random differences were determined by Student's *t*-test or where applicable the analysis of variance followed by Tukey–Kramers' multiple comparisons test.

3. Results

3.1. Expression of the orphan receptors GPRC5A-D in human pancreatic islets from normal and type 2-diabetic donors

Both microarray and qPCR indicate that GPRC5B is the most abundant GPRC5 group receptor expressed in human islets (Fig. 1A and B). Furthermore, qPCR and Western blot analysis demonstrated that GPRC5B was up-regulated in human pancreatic islets from diabetic donors at both mRNA (+55 ± 3%, $p < 0.001$) and protein levels (+56 ± 3%; $p < 0.01$) compared to non-diabetic islets (Fig. 1C and D).

3.2. Detection of Gprc5b protein in various mouse tissues

Gprc5b protein expression (expressed relative β-actin) in mouse pancreatic islets was compared to that in brain, lung, heart, liver and kidney. Densiometry analysis of the Western blot Gprc5b