A role for the putative cannabinoid receptor GPR55 in the islets of Langerhans

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Abstract

The cannabinoid CB1 receptor is a well-known player in energy homeostasis and its specific antagonism has been used in clinical practice for the treatment of obesity. The G protein-coupled receptor GPR55 has been recently proposed as a new cannabinoid receptor and, by contrast, its pharmacology is still enigmatic and its physiological role is largely unexplored, with no reports investigating its putative role in metabolism. Thus, we aim to investigate in rats the presence, distribution and putative physiological role of GPR55 in a key metabolic tissue, the endocrine pancreas. We found high Gpr55 mRNA content in pancreatic islets and considerable protein distribution in insulin-secreting β-cells. Activation of GPR55 by the agonist O-1602 increased calcium transients (P<0.01) and insulin secretion (P<0.001) stimulated by glucose. This latter effect was blunted in Gpr55 KO mice suggesting that O-1602 is acting, at least in part, through GPR55. Indeed, acute in vivo experiments showed that GPR55 activation increases glucose tolerance (P<0.05) and plasma insulin levels (P<0.05), suggesting an in vivo physiological relevance of GPR55 systemic stimulation. Taken together, these results reveal the expression of GPR55 receptors in the endocrine pancreas as well as its function at stimulus-secretion coupling of insulin secretion, suggesting a role in glucose homeostasis. In this context, it may also represent a new target for consideration in the management of type 2 diabetes and related diseases.

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Introduction

The G protein-coupled receptor (GPCR) GPR55 has been recently proposed as a new cannabinoid receptor (Baker et al. 2006, Ryberg et al. 2007, Lauckner et al. 2008). This receptor was in silico identified from the expressed sequence tags database (Sawzdargo et al. 1999) and its low overall sequence homology with cannabinoid CB1 and CB2 receptors prevented its consideration as a putative cannabinoid receptor before. Recent pharmacological data have shown that certain cannabinoid compounds are able to activate GPR55 (Ryberg et al. 2007, Lauckner et al. 2008) and other non-cannabinoid lipid transmitters such as α-lysophosphatidylinositol (Oka et al. 2007, Yin et al. 2009). Whereas some of them were found to stimulate GTPγS binding in cells expressing GPR55, others were reported to increase intracellular calcium or stimulate RhoA activation via GPR55. Thus, it is thought that GPR55 could be enlarging the cellular repertoire of cannabinoid action. However, its pharmacology is still enigmatic and seems to change in an agonist-, tissue-, and assay-dependent manner (Ross 2009, Henstridge et al. 2010). Gpr55 mRNA is widely expressed throughout the central nervous system (CNS), including metabolically relevant areas such as the hypothalamus and also in several
peripheral tissues involved in energy homeostasis (Ryberg et al. 2007). Recent data have shown that the GPR55 receptor has a role in mechanical hyperalgesia associated with inflammatory and neuropathic pain (Staton et al. 2008) and in bone physiology (Whyte et al. 2009). In this latter study, the GPR55 agonist O-1602 inhibited mouse osteoclast formation, an effect that was not seen in osteoclasts generated from GPR55−/− bone marrow macrophages. However, at present there is no data regarding the putative role of GPR55 in metabolism or glucose homeostasis.

By contrast, the cannabinoid CB1 receptor is a well-known player in controlling energy balance (Pagotto et al. 2006). In fact, the endocannabinoid system (ECS), which comprises the cannabinoid receptors, the endogenous ligands and the enzymes responsible for synthesis and degradation of endocannabinoids, is present in all strategic tissues controlling energy balance (Cota & Woods 2005) and it has been involved in the pathogenesis of obesity (Di Marzo et al. 2009, Quarta et al. 2010). Besides its important role in the hypothalamus and brain reward system in controlling food intake, elements of the ECS are also expressed in key peripheral tissues controlling metabolism, i.e. liver, adipose tissue, muscle, and the endocrine pancreas. It is thought to have a modulatory role in several metabolic processes regulated by these tissues (Bermúdez-Silva et al. 2008, Osei-Hyiaman et al. 2008). Elucidation of the relative contribution of peripheral vs CNS circuits in the metabolic changes induced by CB1 receptor antagonists is currently under intense research and recent articles point to an important role for peripheral tissues (Nogueiras et al. 2008). In fact, because of the adverse side effects found in the clinical use ofrimonabant (a CB1 antagonist/inverse agonist capable of crossing the blood–brain barrier) for the management of obesity complications, peripheral-restricted CB1 antagonists are becoming a hot-topic in the search for new drugs against obesity-related diseases (Bermúdez-Silva et al. 2010). Indeed, some of these new kinds of drugs have recently shown promising results in animal studies (Tam et al. 2010). Interestingly, Rimonabant and AM251 (another CB1 antagonist/inverse agonist capable of crossing the blood–brain barrier) have been reported to activate GPR55, suggesting that some of the effects elicited by these drugs could be mediated by this receptor (Kapur et al. 2009, Henstridge et al. 2010). Given the important role of the ECS in the regulation of energy and glucose homeostasis, and the fact that other cannabinoid receptors are located and metabolically active in the endocrine pancreas (Bermúdez-Silva et al. 2008), we hypothesized that GPR55 might also be expressed in metabolically relevant tissues, such as the endocrine pancreas, playing a role in glucose homeostasis. In this study, we focused on the endocrine pancreas because of its preeminent role in metabolic and energy homeostasis through the secretion of insulin and glucagon.

Our findings show that GPR55 is expressed in the islets of Langerhans, both at the mRNA and protein level, specifically in insulin-secreting β-cells. Activation of GPR55 in pancreatic islets with the agonist O-1602 led to augmented intracellular calcium handling stimulated by glucose as well as increased glucose-stimulated insulin secretion (GSIS). O-1602 did potentiate GSIS in islets from Gpr55 WT mice, but this effect was blunted in islets from Gpr55 KO mice. Indeed, acute in vivo treatment with O-1602 resulted in enhanced glucose tolerance with concomitant increased plasma insulin levels. Taken together, these results reveal the expression of functional GPR55 receptors in the endocrine pancreas suggesting a role for this receptor in glucose homeostasis. In this context, GPR55 might represent a new target for consideration in the management of type 2 diabetes and related diseases.

Materials and Methods

Experimental animals

We carried out the experiments with male Wistar rats (3-month old) and GPR55 WT and KO mice (3-month old), in strict compliance with the European Communities directive 86/609/EEC regulating animal research. Rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA) and housed in groups of four in a room with controlled temperature (20 ± 2 °C) and humidity (55 ± 5%) in a 12 h light:12 h darkness cycle (lights on at 0800 h) with free access to water and standard food pellets. Food was withdrawn 12 h before the glucose tolerance test. GPR55 mice were kindly donated by Dr Ruth Ross (University of Aberdeen, Aberdeen, Scotland, UK) and individually housed in the same environmental and food conditions.

Relative gene expression measurements

Six male Wistar rats (3-month-old) were anesthetized with 2,2,2-tribromoethanol (Merck), killed by decapitation and their spleen, adipose tissue, and liver were immediately dissected out, frozen on dry ice and stored at −80 °C. Another four rats were killed in the same way and their islets of Langerhans isolated by collagenase digestion method (described below), pooled and immediately frozen on dry ice and stored at −80 °C. Real-time quantitative PCR was used to measure relative mRNA levels of Gpr55 and β-actin, the latter being used as reference gene. Total RNA was extracted from visceral adipose tissue, liver, spleen, and islets according to manufacturer’s instructions. The total RNA was cleaned and concentrated with RNeasy mini kit (Qiagen GmbH) and 1 μg of this RNA was reversed transcribed into cDNA using a transcription reverse transcriptase kit (Roche Applied Science) according to manufacturer’s instructions. Negative control included RT reactions omitting reverse transcriptase. The obtained cDNA was used as the template for real-time quantitative PCR, which was performed in an iCycler system (Bio-Rad) using the FastStart Universal SYBR Green Mastermix (Roche Diagnostics). Primers for
PCR were designed based on NCBI database sequences of rat Gpr55 mRNA (AF100789 for rat **Gpr55** and NM_031144 for rat **β-actin**) and examined for specificity with BLAST software from NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). **Gpr55** forward: 5'-GGGATA-CAGGCTTTCCACA-3', **Gpr55** reverse: 5'-AAAGGA-GACCCAGAAGACGA-3' (226 bp fragment) and **β-actin** forward: 5'-CAGGCTGTGTGTCCTCCTGTA-3', **β-actin** reverse: 5'-GCTGTGGGTCGACAGCTGTA-3' (203 bp fragment). Each reaction was run in duplicate and contained 2.5 μl cDNA template, 5 mM MgCl₂, and 0.4 μM primers in a final reaction volume of 15 μl. Cycling parameters were 95°C for 5 min to activate DNA polymerase, then 40 cycles of 95°C for 10 s to denature DNA, annealing temperature for 15 s (63-3°C for **Gpr55** and 51.4°C for **β-actin**) and a final extension step of 72°C for 15 s in which fluorescence was acquired. To assess the specificity of the PCR product, melting curve analysis and 1% agarose gel electrophoresis were performed. Melting curve analysis of our samples showed that only a single product was amplified, displaying the same melting temperature as the standards. In addition, agarose gel electrophoresis showed a unique fragment of the expected molecular size. Gene expression of **Gpr55** was normalized to the expression of the reference gene **β-actin**.

Quantification was carried out with a standard curve run at the same time as the sample with each dilution run in duplicate. The standards were a serially diluted, purified, and quantified PCR product.

**Double immunofluorescence**

Paraffin-embedded sections of rat pancreases were analyzed for the presence of GPR55 in pancreatic α (glucagon), β (insulin), and δ (somatostatin) cells by double immunofluorescence. Three adult Wistar rats (3-month-old) were deeply anesthetized with 2,2,2-tribromoethanol (300 mg/kg i.p.) and transcardially perfused with 0.1 M PBS (pH 7.4), followed by 4% paraformaldehyde in PBS at 4°C for 15–30 min. Rat pancreatic tissue was postfixed in 4% paraformaldehyde in PBS, the sections were incubated for 2 h at room temperature in a secondary anti-mouse IgG–FITC antibody (dilution 1:200; Sigma–Aldrich Quimica, cat. no. F2012) and in a secondary anti-rabbit IgG–Cy3 antibody (1:300; Jackson ImmunoResearch Laboratories, West Grove, PA, USA, cat. no. 11-166-152). Finally, the sections were washed in PBS and analyzed under epifluorescence microscopy (Olympus Europa, Hamburg, Germany). High-resolution digital microphotographs were taken with an Olympus BX41 microscope equipped with an Olympus DP70 digital camera.

Digital images were mounted and labeled using Adobe PageMaker.

**Western blot**

Liver and spleen samples were homogenized in ice-cold lysis buffer containing 50 mmol/l Tris–HCl, pH 7.5, 1 mmol/l EGTA, 1 mmol/l EDTA, 1% Triton X–100, 1 mmol/l sodium orthovanadate, 50 mmol/l sodium fluoride, 5 mmol/l sodium pyrophosphate, 0.27 mol/l sucrose, 0.1% 2-mercaptoethanol, and Complete Protease Inhibitor Cocktail (one tablet per 50 ml; Roche Diagnostics). Homogenates were centrifuged at 11 000 g for 10 min at 4°C, supernatants were removed, and aliquots were snap frozen in liquid nitrogen. Liver and spleen lysates (20 μg each lane) were subjected to SDS–PAGE on 8% polyacrylamide gels and electrotransferred on a PVDF membrane. Membranes were then blocked for 1 h in TBS–Tween (TBST: 50 mmol/l Tris–HCl, pH 7.5, 0.15 mol/l NaCl, and 0.01% Tween) containing 5% skimmed milk and probed for 16 h at 4°C in TBST, 5% skimmed milk with 1/500 dilution of the antibody (anti-GPR55, Abcam). GPR55 was detected using HRP-conjugated-coupled streptavidin (Amersham Biosciences). Detection of proteins was performed by HRP-conjugated secondary antibodies and an enhanced chemiluminescence reagent (Amersham Biosciences).

**Isolation of pancreatic islets and static secretion protocol**

Pancreatic islets from rats, WT and KO GPR55 mice, were isolated by collagenase digestion method. Briefly, pancreas was inflated with Hanks solution containing (0.8 mg/ml collagenase, 5·6 mM glucose, and 0·05% BSA, pH 7.4), removed from the rat and kept at 37°C for 10–20 min. After tissue digestion followed by three consecutive washes, the islets were collected manually under a microscope, as described previously (Rafacho et al. 2010). For static incubation, groups of five islets were first incubated for 1 h at 37°C in 1 ml Krebs-bicarbonate buffer solution of the following composition (in mM): 115 NaCl, 5 KCl, 2·56 CaCl₂, 1 MgCl₂, 24 NaHCO₃, 15 HEPES, and 5·6 glucose, 0·145 CaCl₂, 0·186 MgCl₂, and 10 HEPES, pH 7.4 and 10% FBS in HBSS.)
supplemented with 0.05% BSA and equilibrated with a mixture of 95% O₂:5% CO₂, pH 7.4. The medium was then replaced with 1 ml fresh buffer containing one of the following: 3 mM glucose, 11 mM glucose, 11 mM glucose plus 10 μM GPR55 agonist O-1602, 11 mM glucose plus 1 μM O-1602 or 11 mM glucose plus 0.1 μM O-1602, and further incubated for 1 h. O-1602, which was supplied at 10 mg/ml in a solution of methyl acetate, was purchased from Tocris Bioscience (Bristol, UK). To obtain the final concentrations of O-1602 used in the experiments, the stock was diluted from 3 × 10⁻⁴ to 3 × 10⁻⁵. At these dilutions, methyl acetate did not affect insulin secretion or calcium levels (data not shown). Equal amounts of O-1602 diluent were added at 3 and 11 mM glucose conditions without O-1602. At the end of the incubation, the samples were put at 4°C for 15 min to stop insulin secretion and stored at −20°C for subsequent measurement of insulin content by RIA using a Coat-a-count (DPC, Los Angeles, CA, USA) as described previously (Rafacho et al. 2010) or ELISA kit, according to manufacturer’s instructions (Mercodia, Uppsala, Sweden).

**Measurement of intracellular Ca²⁺ ([Ca²⁺]ᵢ)**

Measurement of [Ca²⁺]ᵢ was done according to Rafacho et al. (2010). Briefly, groups of isolated islets were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 11 mM glucose, 100 IU penicillin/ml, 100 mg streptomycin/ml, without phenol red at 37 °C in a 5% CO₂/air atmosphere for 4 h. Subsequently, they were washed twice in Krebs solution containing 5-6 mM glucose, 1% bovine albumin. They were then incubated for 2 h in the same medium with 4 μM fura-2/AM (Invitrogen) in a 5% CO₂-air atmosphere at room temperature. Islets were allowed to settle on poly-L-lysine-treated coverslips to avoid their movement during perfusion. Islets were then transferred to a thermostatically regulated open chamber (37 °C), placed on the stage of an inverted epifluorescence microscope (Zeiss, Axiovert 200), and perfused with Krebs-bicarbonate buffer without albumin at flow rate of 1.5 ml/min. The solution was continuously gassed with 95% O₂ and 5% CO₂ to maintain pH 7.4. Calcium records in whole islets of Langerhans were obtained by imaging intracellular calcium under the epifluorescence inverted microscope. Images were acquired every ~3 s with an extended Hamamatsu Digital Camera C4742-95 (Hamamatsu Photonics, Barcelona, Spain) using a dual filter wheel (Sutter Instrument Co., Novato, CA, USA) equipped with 340 and 380 nm, 10 nm bandpass filters (Omega Optics, Madrid, Spain). Data were acquired using ORCA software from Hamamatsu (Hamamatsu Photonics). Fluorescence changes are expressed as the ratio of fluorescence at 340 and 380 nm (F340/F380). Results were plotted using commercially available software (Sigmaplot, SPSS, Inc., Chicago, IL, USA). The area under the curve (AUC) of the [Ca²⁺]ᵢ oscillations was calculated for a period of 10–15 min during glucose stimulus alone (control) and for a period of 15–20 min during glucose plus GPR55 agonists stimuli application. The AUC values were obtained from the whole area under calcium curve discounting the baseline values and then normalized per minute. These values were analyzed with OriginPro7 (Northampton, MA, USA).

**Glucose tolerance test and plasma insulin levels**

Glucose tolerance test was carried out by injecting an i.p. glucose load of 2 g/kg body weight (diluted in saline) in separate groups of rats. Thirty minutes before glucose load, animals received by i.p. administration the GPR55 agonist O-1602 (0-002, 0-02, or 0-2 mg/kg) or the vehicle (2% DMSO). Tail blood samples were collected before (0 min) and 15, 30, 60, and 120 min after glucose administration. Glucose was determined using a commercial glucometer (Accu-check, Roche Diagnostic). Plasma insulin levels were measured by an ELISA method according to manufacturer’s instructions (Mercodia, Uppsala, Sweden).

**Statistical analysis**

Data are expressed as mean ± s.e.m. Comparisons were made by a two-tailed Student’s t-test. A probability level of <0.05 was considered statistically significant. Statistical analyses were performed by GraphPad Prism Software (San Diego, CA, USA).

**Results**

**Gpr55 mRNA levels in rat islets of Langerhans**

To explore the quantitative abundance of Gpr55 transcript in rat islets of Langerhans we have measured by real-time quantitative PCR the expression level of Gpr55 mRNA in several tissues. Figure 1 shows relative Gpr55 expression for metabolically relevant tissues expressed in comparison to spleen abundance. The pancreatic islets have higher expression levels than other metabolic tissues, i.e. adipose and liver. In fact, islets of Langerhans show 60% Gpr55 expression level of the spleen, a control tissue known to highly express Gpr55.

**Distribution of GPR55 protein in the endocrine pancreas**

We examined whether the high gene expression levels of Gpr55 mRNA levels in pancreatic islets were being translated into protein. For this purpose double immunofluorescence with an antibody against GPR55 and a set of antibodies against the three main hormones secreted by the islets of Langerhans was performed. Figure 2A–C clearly shows that almost all insulin-secreting β-cells expressed GPR55 protein whereas both glucagon- and somatostatin-secreting α- and δ-cells, respectively, did not express this receptor (Fig. 2D–I). The antibody specificity was confirmed in islets from...
control tissue as it is reported to express metabolically relevant tissues. Spleen was included as a positive in adipose tissue, liver, and islets of Langerhans, all

Introduction of O-1602 at any dose (0.1, 1, or 10 mM) in response to 11 mM glucose stimulation (Fig. 4 D–I). As expected, the intracellular calcium levels were elevated in (3 mM glucose) at any of the three doses assayed in rat islets.

Effects of O-1602 on GSIS

agonist O-1602 had no effect on 

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\text{[Ca}^{2+}\text{]}_i
\]

calcium pattern (Fig. 4D–I; 

Values are mean ± S.E.M. 

Figure 1 Gpr55 mRNA levels in rat tissues. Gene expression of Gpr55 in adipose tissue, liver, and islets of Langerhans, all metabolically relevant tissues. Spleen was included as a positive control tissue as it is reported to express Gpr55 at high levels. Gpr55 content has been normalized to the housekeeping gene β-actin. Values are mean ± S.E.M. 

**Effects of O-1602 on intracellular calcium handling in isolated islets**

Isolated islets of Langerhans were loaded with the calcium-sensitive probe Fura-2 and intracellular calcium was monitored after pharmacological manipulation with the GPR55 agonist O-1602 to detect possible changes in the physiological response of islets. Figure 4A and B shows that the GPR55 agonist O-1602 had no effect on 

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\text{[Ca}^{2+}\text{]}_i
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calcium at any of the three doses assayed in rat islets. As expected, the intracellular calcium levels were elevated in response to 11 mM glucose stimulation (Fig. 4D–I). Introduction of O-1602 at any dose (0.1, 1, or 10 μM) in the perfusion medium elicits a change in the oscillatory calcium pattern (Fig. 4D–I; n = 10–12 islets). Quantification of this response, by calculating the area under calcium trace both at glucose alone or glucose plus O-1602 stimuli (Fig. 4C), indicates that activation of GPR55 induced a significant increase in the intracellular calcium levels in rat islets at the three doses (n = 10–12 islets; P < 0.01).

**Effects of O-1602 on peripheral glucose tolerance**

The effect of acute systemic administration of O-1602 on glucose homeostasis has been analyzed by measuring blood glucose levels during glucose tolerance test on normal fasted rats. Figure 6A shows that O-1602 induced a significant increase in glucose tolerance, as judged by the reduced blood glucose values at 15 min (for 0.2 mg/kg O-1602, P < 0.05), 30 min (for 0.2 mg/kg O-1602, P < 0.01), and 60 min (for 0.02 and 0.2 mg/kg O-1602, P < 0.05 and P < 0.01, respectively, n = 6–8 rats). No significant effect in glycemic values was observed with 0.002 mg/kg throughout the experiment. Thus, these data suggest a dose-dependent effect of GPR55 agonist on peripheral glucose tolerance. Quantification of this response by calculating the area under the glucose curve (AUC;
showing the relative expression of Gpr55 in other tissues such as spleen, liver, and adipose fit well with the previously mRNA expression reported for this receptor (Ryberg et al. 2007). In line with the high Gpr55 mRNA expression in isolated islets, we detected significant protein distribution by double immunofluorescence that was specifically located in rat insulin-secreting β-cells. Islets from Gpr55 WT mice also displayed GPR55 protein but to a less extent than rats, probably reflecting different cellular distribution. By contrast, islets from Gpr55 KO mice showed no immunostaining. Western blot analysis revealed a band of the expected size together with two other bands probably reflecting post-translational modifications of the receptor. Importantly, the activation of GPR55 by the pharmacological agonist O-1602

Discussion

GPR55 is a novel receptor that can be activated by certain cannabinoid ligands and the bioactive lipid 1-α-lysophosphatidylinositol. The pharmacology of GPR55 is controversial perhaps due to tissue-specific differences or could depend on the agonist or functional assay employed (Ross 2009, Henstridge et al. 2010). Indeed, despite its high expression in some organs such as the adrenals, CNS, and spleen, little is known about the physiological role of this receptor. Experiments conducted in GPR55-null mice have suggested a role of GPR55 in the modulation of pain (Staton et al. 2008) whereas a more recent article suggested that it also plays a role in bone mass (Whyte et al. 2009). However, its putative role on energy homeostasis has not yet been reported. In a first attempt to address this question we have studied the functional expression of GPR55 in the endocrine pancreas, a key tissue involved in modulating energy balance mainly through the secretion of the opposing hormones insulin and glucagon.

Our mRNA expression study shows that Gpr55 is expressed in rat islets of Langerhans. Furthermore, our data Fig. 6B) revealed a significant effect at 0.2 mg/kg O-1602 concentration. To assess whether the increased glucose tolerance was concomitant to increased insulin secretion we also measured insulin in plasma after 0.2 mg/kg O-1602 injection. Figure 6C shows an increased insulin level 30 min after injection of the drug.

Figure 3 GPR55 immunohistochemistry and western blot in mice. (A and B) Distribution of GPR55 protein in pancreatic cells of WT and KO Gpr55 mice by immunofluorescence. Note there is a lack of staining in Gpr55-null mice (B). The figures are representative of several slices. Scale bars are included in each image. (C) Representative immunoblot of liver (L) and spleen (S) extracts from C57Bl/6 mice showing a band of the expected size (~37 kDa) and two other bands probably reflecting post-translational modifications of the receptor. Full colour version of this figure available via http://dx.doi.org/10.1530/JOE-11-0166.

Figure 4 Intracellular calcium recording in rat isolated islets of Langerhans. (A) Intracellular calcium recording at 3 mM glucose before and after exposure to 10 µM O-1602. (B) Note the absence of response to any of the tested doses. (C) The three doses of GPR55 agonist elicited changes in intracellular calcium oscillations at 11 mM glucose that resulted in increased area under the curve (ΔA.min⁻¹), indicating higher calcium mobilization in islets treated with the GPR55 agonist. (D and E) Intracellular calcium recording under stimulatory 11 mM glucose followed by exposure to 0.1, 1, and 10 µM O-1602 respectively. Stimuli were applied for the period indicated by the horizontal bar. A, D–I is representative of several experiments. Data from B and C are means±S.E.M. of n=10–12 islets for each condition; *P<0.01 vs 11 mM glucose alone. Unpaired Student’s t-test.
in isolated islets induced changes in glucose-induced intracellular calcium oscillations, whereas O-1602 had no effect on [Ca$^{2+}$], at 3 mM glucose. The modulation of calcium handling in response to GPR55 activation occurs similarly with all agonist concentration tested. These observations support a role for these receptors in pancreatic β-cell physiology and fit with the high mRNA and protein distribution found in these cells. Since calcium entry is a process that couples the stimulus-secretion within pancreatic β-cells we investigated the GSIS in response to GPR55 activation.

Notably, only the lower agonist concentration, 0·1 µM O-1602, was able to induce a significant increase in GSIS, compared with that of control islets (11 mM glucose alone). Thus, when properly activated, GPR55 signals may somehow potentiate the GSIS, at least in part, by increasing the calcium handling. Whether other components such as metabolic signals or protein kinases, both of which participate in insulin secretion (MacDonald et al. 2005) are involved in this process merits investigation. While the GPR55 agonist improved calcium signaling at all the concentrations tested, insulin release was only significantly affected at 0·1 µM O-1602. Thus, it is likely that O-1602, at least at the higher doses, is affecting other signals besides calcium that are also influencing β-cell exocytosis. It is likely that other parameters involved in the regulation of secretion (e.g. cAMP and ATP) may be downregulated at high concentrations of O-1602, aspects that deserve much research in the future. As has been mentioned above, GPR55 pharmacology is still enigmatic but compelling evidence highly suggests that O-1602 is a GPR55 agonist (Pertwee et al. 2010). Thus, by [35S]GTPγS binding assays, two independent research groups have reported nanomolar activity of this compound at GPR55 (Johns et al. 2007, Ryberg et al. 2007). O-1602 has been shown to promote GPR55-mediated ERK1/2 phosphorylation and RhoA activation (Whyte et al. 2009) and a calcium signal has been seen in response to O-1602 in GPR55-expressing cells (Waldeck-Weiermair et al. 2008). Indeed, this compound stimulated osteoclast polarization and resorption in vivo, with this effect being attenuated in osteoclasts from Gpr55-null mice (Whyte et al. 2009). However, there is evidence that O-1602 can activate additional targets (Johns et al. 2007, Schicho et al. 2011). To verify whether O-1602 is acting through GPR55 receptors in the islets of Langerhans, we performed in vitro GSIS in islets from Gpr55 WT and KO mice. Interestingly, in a similar way to rats, 0·1 µM O-1602 potentiated GSIS, but not higher doses. This enhancing effect of O-1602 on GSIS was totally abolished in islets from Gpr55 KO mice, suggesting that O-1602 is acting through GPR55 receptor. Taken together, these findings support that the effect of O-1602 on calcium mobilization and insulin secretion in islets is being mediated through GPR55.

Figure 5 Glucose-stimulated insulin secretion (GSIS) in isolated islets of Langerhans from rats and Gpr55 mice. Insulin release in response to basal 3 mM or stimulating 11 mM glucose plus different doses of the GPR55 agonist O-1602 (0, 0·1, 1, and 10 µM) in rats (A), Gpr55 WT mice (B), and Gpr55 KO mice (C). (A) 0·1 µM O-1602 potentiates GSIS in rat islets. Data are means ± s.e.m. of 12 wells (six wells in two different experiments from three different rats); *P<0·001 vs 3 mM glucose; 2P<0·001 vs 11 mM glucose. Unpaired Student’s t-test. (B) 0·1 µM O-1602, but not higher doses, potentiates GSIS in islets from Gpr55 WT mice. Data are means ± s.e.m. of 12–13 wells (four to six wells in three different experiments from six different mice); *P<0·05 vs 3 mM glucose; 2P<0·001 vs 11 mM glucose. Unpaired Student’s t-test. (C) None of the assayed doses of O-1602 potentiate GSIS in islets from Gpr55 KO mice. Data are means ± s.e.m. of 12 wells (six wells in two different experiments from four different mice); *P<0·001 vs 3 mM glucose. Unpaired Student’s t-test.

Figure 6 Glucose tolerance test and plasma insulin. Fasted (12 h) normo-glycemic and normo-weight rats were i.p. administered with three different GPR55 agonist O-1602 concentrations (0·002, 0·02, and 0·2 mg/kg, b.w.) 30 min before glucose load (2 g/kg, b.w.). (A) Pretreatment with the GPR55 agonist O-1602 induced a dose-dependent response with increased glucose tolerance at higher doses. (B) Quantification of this response showed that 0·2 mg/kg O-1602 significantly decreases area under glucose curve suggesting increased glucose tolerance in vivo. Data are means ± s.e.m. of 7–8 animals per group; *P<0·05 vs vehicle-treated animals. 2P<0·05 vs 0·02 mg/kg vs vehicle-treated animals. Unpaired Student’s t-test. (C) Plasma insulin levels of normo-glycemic and normo-weight rats i.p. administered with 0·2 mg/kg O-1602 30 min before glucose load (2 g/kg, b.w.). Plasma insulin was increased in O-1602-injected animals 30 min after glucose load. Data are means ± s.e.m. of 7–8 animals per group; *P<0·05 vs vehicle-treated animals.
In an attempt to evaluate the in vivo relevance of GPR55 activation, we performed a glucose tolerance test after acute i.p. injections with increasing doses of the GPR55 agonist O-1602 and we also measured plasma insulin levels. In agreement with our findings in intracellular calcium changes and insulin secretion in vitro, GPR55 activation was found to increase glucose tolerance with concomitant increase in plasma insulin levels. Our experiments indicate that glucose tolerance was dose-depending improved with O-1602. In general, it is difficult to compare in vitro and in vivo concentrations and their dose-dependence effects, because during in vivo treatment the target tissues may face different levels of the tested agents that are difficult to control. In addition, in vivo there are other tissues that can be interfering with the studied effect. In fact, the lack of changes in plasma insulin levels 15 min after glucose load, a time when there is increased glucose tolerance, suggest that O-1602 is also acting on other tissues potentiating glucose tolerance. Thus, our results suggest that in vivo O-1602 increases glucose tolerance, with part of this effect being due to enhanced insulin secretion.

Given that the CB1 antagonist AM251 has also been reported to be a GPR55 agonist (Lauckner et al. 2008), our results also suggest that the increased glucose tolerance previously reported in rats after AM251 treatment (Bermúdez-Silva et al. 2006, Bermúdez-Silva et al. 2007) could be, at least partially, mediated through GPR55. Furthermore, given that Rimonabant has also been reported as being capable of activating the GPR55 receptor (Lauckner et al. 2008), some of the beneficial effects on glucose metabolism reported after Rimonabant treatment, that has been attributed to CB1 blocking (Hildebrandt et al. 2003), could be mediated, at least partially, through GPR55 activation.

In conclusion, herein we identify a high Gpr55 mRNA content in pancreatic islets and large protein distribution in rat insulin-secreting β-cells. When activated, GPR55 increases calcium transients and insulin secretion stimulated by glucose. Accordingly, in vivo experiments have also shown that GPR55 activation increases glucose tolerance and plasma insulin levels, suggesting a role for this receptor in the acute pancreatic-mediated response to high glycemic levels. These findings may have therapeutic implications, as specific activation of GPR55 could be a new strategy to fight against hyperglycemia and the associated deleterious effects.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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