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GPR55-dependent stimulation of insulin secretion from isolated mouse and human islets of Langerhans

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Abstract

Aims: The novel cannabinoid receptor GPR55 is expressed by rodent islets and it has been implicated in β -cell function in response to a range of ligands. This study evaluated the effects of GPR55 ligands on intracellular calcium ([Ca²⁺]_i) levels and insulin secretion from islets isolated from GPR55 knockout (GPR55^{-/-}) mice, age-matched wildtype (WT) mice, and human pancreas.

Materials and methods: GPR55 expression was determined by western blotting and fluorescent immunohistochemistry. Changes in $[Ca^{2+}]_i$ were measured by Fura-2 microfluorimetry. Dynamic insulin secretion was quantified by radioimmunoassay following perifusion of isolated islets. RhoA activity was monitored using a Rho binding domain pull down assay.

Results: Western blotting indicated that MIN6 β -cells, mouse and human islets express GPR55 and its localisation on human β -cells was demonstrated by fluorescent immunohistochemistry. The

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pharmacological GPR55 agonist O-1602 (10 μ M) significantly stimulated [Ca²⁺]_i and insulin secretion from WT mouse islets and these stimulatory effects were abolished in islets isolated from GPR55^{-/-} mice. In contrast, while the putative endogenous GPR55 agonist lysophosphatidylinositol (LPI, 5 μ M) and the GPR55 antagonist cannabidiol (CBD, 1 μ M) also elevated [Ca²⁺]_i and insulin secretion, these effects were sustained in islets from GPR55^{-/-} mice. Stimulatory effects of O-1602 on [Ca²⁺]_i and insulin secretion were also observed in experiments using human islets, but O-1602 did not activate RhoA in MIN6 β-cells.

Conclusions: Our results therefore suggest that GPR55 plays an important role in the regulation of mouse and human islet physiology, but LPI and CBD exert stimulatory effects on islet function by a GPR55-independent pathway(s).

Introduction

GPR55 is a seven transmembrane spanning G-protein coupled receptor (GPCR) whose activation is regulated by endogenous and pharmacological cannabinoid ligands, and it has been proposed as a novel cannabinoid receptor (1). However, its low sequence homology with the classic CB1 and CB2 cannabinoid receptors (2) and its activation by the non-cannabinoid endogenous phospholipid L-α-lysophosphatidylinositol (LPI) (3-10) means that its identity as a cannabinoid receptor is questionable. In addition, identifying the physiological roles of GPR55 has been hampered by the complicated pharmacology of GPR55 ligands. Thus, the CB1 receptor antagonist rimonabant acts as a GPR55 agonist as does AM251, a rimonabant analogue (1, 9). O-1602 is a synthetic compound derived from the native phytocannabinoid, cannabidiol (CBD), which was developed as a selective GPR55 agonist with little binding affinity for CB1 and CB2 (1, 5, 11). However, O-1602 may exert GPR55-independent effects to stimulate food intake (12, 13), and it increases calcium and activates MAP kinase in a cell line overexpressing the GPCR GPR18 (14). CBD is reported to act as a GPR55 antagonist (1, 4, 10, 15), but it may also possess GPR55 agonist activity since it provided protection against acute pancreatitis in mice to an extent similar to that observed using its synthetic canalogue O-1602 (16). To further confound interpretation of studies using CBD,

there are several reports that it can modulate cellular responses in a GPR55-independent manner (17-20). Similarly, LPI also exhibits GPR55-independent effects in endothelial cells (21-23), suggesting alternative mechanisms for transducing LPI-mediated signalling.

GPR55 may be involved in the regulation of islet β -cell function. GPR55 mRNA has been detected in β -cell lines and in rodent and human islets (24-26), and GPR55 protein expression in rodent islets is localised primarily to β -cells. It has long been known that LPI generated in islets from phosphatidylinositol hydrolysis stimulates insulin release (27), although its mode of action in β -cells has not been established, and two studies using pharmacological GPR55 agonists reported the stimulation of insulin release from rodent islets and BRIN-BD11 insulinoma cells (25, 26). However, the potential lack of specificity of GPR55 agonists means that such studies cannot be considered as conclusive. We have therefore used islets isolated from a transgenic mouse model with homozygous deletion of the GPR55 gene (GPR55^{/-} mice) to investigate the specificity for GPR55 of LPI, 0-1602 and CBD, and we have studied the effects of GPR55 activation on human islet function.

Materials and methods

Materials. Culture media and supplements, cell dissociation solution, collagenase type XI, Fura-2, LPI and glucagon antibody: Sigma-Aldrich (Dorset, UK). ECL western blotting reagents and Hyperfilm: GE Healthcare Life Sciences (Little Chalfont, UK). GPR55 antibody for Western blotting: Cayman (Cambridge, UK); Tissue culture flasks, Petri dishes, cover slips, 10% polyacrylamide gels, NuPAGE[®] sample buffer and transfer buffer, GPR55 antibody for immunohistochemistry and anti-rabbit secondary antibody: Thermo Fisher Scientific (Paisley, UK). Insulin antibody: DAKO (Cambridge, UK), somatostatin antibody Abcam (Cambridge, UK). Rainbow molecular weight markers and polyvinylidene fluoride (PVDF) membrane: Millipore (Watford, UK). RhoA Activation Assay Kit[™]: Cytoskeleton Inc. (Denver, USA). CBD and O-1602: Tocris Biosciences (Bristol, UK). **Experimental animals.** GPR55^{-/-} mice were generated by homologous recombination, with a loxPflanked Neo cassette replacing a single GPR55 coding exon and backcrossed for 11 generations towards C57BL/6. Mice heterozygous for the deletion were inter-crossed to produce homozygous C57BL/6 GPR55^{-/-} offspring (28), a colony of which was maintained at King's College London, with food and water supplied *ad libitum*. Wild type (WT) C57BL/6 mice were purchased from Harlan (Bicester, UK). We did not detect any significant changes in CB1 and CB2 mRNA expression following GPR55 deletion (cerebral cortex, CB1 expression relative to β -actin; WT: 1.12±0.05; GPR55^{-/-}: 1.10±0.14; CB2 expression relative to β -actin; WT: 0.11±0.03; GPR55^{-/-}: 0.07±0.01; n=4; p>0.2).

Islets and MIN6 cells. Islets were isolated from 8-12 week old male GPR55^{-/-} mice and from agematched WT C57BL/6 mice by collagenase digestion (29), and human islets were isolated from nondiabetic pancreas donors at King's College Hospital Islet Transplantation Unit with appropriate ethical approval (30). Islets were maintained overnight at 37°C in culture medium supplemented with 5.6mM glucose, 10% FCS, 2mM glutamine and penicillin-streptomycin (100 U/ml, 0.1 mg/ml), and MIN6 β -cells were maintained as for islets, but in culture medium supplemented with 25mM glucose.

SDS-PAGE/Western blotting and immunohistochemistry. Protein-matched extracts from MIN6 βcells, and mouse and human islets were fractionated on 10% polyacrylamide gels, transferred to PVDF membranes and GPR55 expression was detected by Western blotting (1:160 dilution of rabbit anti-GPR55 antibody and 1:7,500 dilution of a HRP-linked anti-rabbit secondary antibody). Mouse and human pancreas sections were incubated overnight at 4°C with a rabbit anti-GPR55 antibody (1:10 dilution) in combination with antibodies directed against insulin, glucagon or somatostatin (at 1:100, 1:50 and 1:25 dilutions, respectively). Immunoreactive GPR55 was detected by incubation with an AlexaFluor488-conjugated secondary antibody (1:150) and islet hormones were detected with AlexaFluor594-conjugated secondary antibodies (all at 1:150). **Single cell calcium microfluorimetry.** Mouse and human islets were dispersed using cell dissociation solution, and groups of 100,000 islet cells or 100,000 MIN6 β -cells were seeded onto glass coverslips. Cells were maintained in culture overnight then loaded with 5µM Fura-2 AM and perifused (1 ml/min) on a heated stage with a physiological salt solution (31) in the absence or presence of pharmacological agents. Real-time changes in $[Ca^{2+}]_i$ were determined by illuminating cells alternately at 340nm and 380nm, with the emitted light being filtered at 510nm.

Dynamic insulin secretion. Isolated mouse or human islets were perifused (37°C, 0.5 ml/min) with a physiological salt solution (31) in the absence or presence of O-1602, LPI or CBD. Samples were collected at 2 min intervals and secreted insulin was measured by radioimmunoassay (32).

RhoA activation assay. Serum-starved MIN6 β -cells were treated for 10 minutes with a salt solution containing 2mM or 20mM glucose, in the presence or absence of 10 μ M O-1602 and activity of RhoA was determined using a kit in which the Rho binding domain (RBD) of the Rho effector protein rhotekin binds specifically to GTP-bound RhoA. In brief, 50 μ g of rhotekin-RBD affinity beads was added to protein-matched MIN6 cell samples, the mixtures were incubated (4°C, 1 hour) to allow active RhoA binding, then beads were pelleted and samples were fractionated by SDS-PAGE. Total and active RhoA was detected by Western blotting (1:500 dilution of anti-RhoA primary antibody; 1:5,000 dilution of anti-mouse secondary antibody).

Statistical analyses. Numerical data are expressed as means±SEM and statistical comparisons were made by Student's t tests. Values of P<0.05 were considered statistically significant.

Results

Expression of GPR55 by MIN6 β -cells and mouse and human islets

GPR55 is a 319 amino acid protein with a predicted molecular weight of 37kDa (2). An immunoreactive protein of 37kDa was detected in Western blots of MIN6 β-cells, human islets and islets from wildtype mice, but, as expected, not in islets isolated from GPR55^{-/-} mice (Figure 1A). Proteins of 33kDa and 50kDa were also detected in MIN6 β-cells and islets from WT mice but not in those isolated from GPR55^{-/-} mice, nor in human islet extracts. Fluorescence immunohistochemistry indicated that GPR55 was not detected in pancreases from GPR55^{-/-} mice, as expected, while it was present in islets of WT mice (Figure 1B). Dual immunostaining with GPR55 and islet hormone antibodies in mouse and human pancreas sections indicated that GPR55 was highly expressed by β-cells of both species (Figure 1C-1F). It was also present in a small proportion of mouse islet α-cells (Figure 1E) and in the majority of human islet α-cells (Figure 1D, 1F), but it was absent or at very low levels in δ-cells of both species (Figure 1C-1F). The immunohistochemical staining also indicated that GPR55 was preferentially expressed in the endocrine pancreas, with only faint immunoreactivity in the exocrine pancreas in mice and humans.

O-1602, but not LPI and CBD, increases β -cell [Ca²⁺]_i via activation of GPR55

To identify the roles of the putative GPR55 ligands O-1602, LPI and CBD in β -cells, and to examine the requirement of GPR55 for their functional effects, $[Ca^{2+}]_i$ was measured by single cell microfluorimetry in islets isolated from WT and GPR55^{-/-} mice. Previous studies have identified β -cell responses in dispersed islet populations through elevated $[Ca^{2+}]_i$ levels in response to stimulatory concentrations of glucose and to the K_{ATP} channel blocker tolbutamide (33-35), so in our experiments dispersed islet cells were classified as β -cells if they responded to 20mM glucose and to 100µM tolbutamide. Figure 2A shows that 10µM O-1602 caused a rapid and reversible increase in $[Ca^{2+}]_i$ in WT islet β -cells at 2mM glucose, and these cells then responded to 20mM glucose with a similar amplitude of calcium increase (peak response, 340:380 ratio: 0.821 vs 0.815). Subsequent exposure to carbachol (500µM) and tolbutamide (100µM) also resulted in reversible elevations in $[Ca^{2+}]_i$. O-1602 also stimulated elevations in $[Ca^{2+}]_i$ at 20mM glucose in islet β -cells from WT mice (Figure 2B)

and these β -cells were able to reversibly respond to carbachol and tolbutamide after removal of O-1602. Analysis of responses by individual cells indicated that the stimulatory effect of O-1602 at 2mM glucose was observed in 48±2% of β -cells, and 49±7% of β -cells responded to O-1602 at 20mM glucose (Figure 2E). The individual [Ca²⁺], traces indicated that the stimulatory effects of O-1602 at both basal and stimulatory glucose levels were no longer observed in β -cells from GPR55^{-/-} mice (Figure 2C and 2D), and analysis of all cells demonstrated an absence of effect of O-1602 in the majority of β -cells, as expected (Figure 2E). The putative GPR55 endogenous ligand LPI (5µM) also induced reversible increases in [Ca²⁺], at both basal and stimulatory glucose concentrations in β -cells from WT mice (Figure 2F and 2G). In contrast to the loss of O-1602-induced elevations in [Ca²⁺], in β -cells from GPR55^{-/-} mice (Figure 2C and 2D), the effects of LPI to increase [Ca²⁺], at both basal and stimulatory glucose levels were sustained in β -cells from GPR55^{-/-} mice (Figure 2J) indicated that similar proportions of β -cells from WT and GPR55^{-/-} mice responded to LPI at 2mM glucose (WT: 56±3%, GPR55^{-/-}: 62±1%) and 20mM glucose (WT: 53±4%, GPR55^{-/-}: 58±20%), indicating that it increases [Ca²⁺], in β -cells by a GPR55-independent mechanism.

The effects of CBD on $[Ca^{2+}]_i$ in β -cells from WT and GPR55^{-/-} mice were also examined in these experiments. Surprisingly, in contrast to the proposed antagonist effect of this cannabinoid on GPR55, CBD (1µM) induced reversible elevations in $[Ca^{2+}]_i$ in β -cells at both 2mM and 20mM glucose (Figure 2K and 2L). These stimulatory effects of CBD were not mediated via interaction with GPR55 since they were still observed in β -cells from GPR55^{-/-} mice (Figure 2M and 2N), with no decline in the total number of β -cells responding at either 2mM glucose (WT: 45±9%, GPR55^{-/-}: 44±11%) or 20mM glucose (WT: 38±8%, GPR55^{-/-}: 39±15%; Figure 2O).

O-1602 increases $[Ca^{2+}]_i$ in MIN6 and human β -cells

Since GPR55 is also expressed by MIN6 β -cells and human islets (Figure 1), the effects of the GPR55-selective agonist O-1602 on calcium handling in MIN6 cells and dispersed human islet cells

was also investigated. It can be seen from Figure 3A that 10μ M O-1602 induced a robust, reversible increase in $[Ca^{2+}]_i$ in MIN6 cells and subsequent exposure to 100μ M tolbutamide also promoted reversible elevation in $[Ca^{2+}]_i$, demonstrating the viability of MIN6 cells following treatment with O-1602. Similar stimulatory effects of O-1602 were observed in calcium microfluorimetry experiments in dispersed human β -cells (Figure 3B). Thus, 10μ M O-1602 stimulated elevations in $[Ca^{2+}]_i$ at 2mM glucose in dispersed human β -cells and these β -cells were able to reversibly respond to tolbutamide after removal of O-1602. Stimulatory effects of O-1602 on $[Ca^{2+}]_i$ at 2mM glucose were observed in $42\pm3\%$ of the 19 human β -cells analysed.

O-1602 does not activate RhoA in MIN6 β -cells

To determine whether GPR55 activation is also coupled to activation of RhoA, a key Rho small GTPase activated by GPR55 in other cells (1, 6, 15, 36), MIN6 cells were exposed to buffers supplemented with 2 or 20mM glucose in the absence or presence of 10µM O-1602, and RhoA activity was measured. As shown in Figure 4, exposure of MIN6 cells to 20mM glucose for 10 minutes induced RhoA activation, which was detected by immunoblotting after a pull down assay, but 10µM O-1602 failed to elicit RhoA activation at either 2mM or 20mM glucose.

O-1602, but not LPI and CBD, increases insulin secretion via activation of GPR55

The effects of O-1602, LPI and CBD on dynamic insulin secretion were examined in perifusion experiments using islets isolated from WT and GPR55^{-/-} mice. There was no significant difference in glucose-stimulated insulin secretion in islets from GPR55^{-/-} and WT mice (insulin secretion in response to 20mM glucose, AUC; WT: 126±37; GPR55^{-/-}: 120±21; n=4, p>0.2). In WT islets the GPR55 agonist, O-1602, stimulated an approximately 3-fold sustained increase in insulin release at 2mM glucose that was reversible upon removal of the agonist. Islets were then perifused with a buffer supplemented with 20mM glucose, which induced a rapid, biphasic increase in insulin release and exposure to O-1602 potentiated the plateau phase of this secretory response (Figure 5A, solid line).

In parallel experiments performed using islets isolated from GPR55^{-/-} mice O-1602 did not have any effect on insulin secretion, either at 2mM or 20mM glucose (Figure 5A, dotted line), confirming that the stimulatory effects of O-1602 on insulin secretion are mediated through activation of GPR55. Perifusions were also used to investigate the effects of LPI on dynamic insulin secretion, using islets isolated from WT and GPR55^{-/-} mice. In several separate experiments with islets from WT mice it was observed that 5µM LPI only produced a transient, non-significant elevation of insulin release at 2mM glucose, but it promoted a sustained potentiation of glucose-induced insulin secretion (Figure 5B, solid line). This potentiation of insulin release by LPI was independent of GPR55 activation since similar stimulatory profiles of LPI-induced insulin secretion were observed in islets obtained from both WT and GPR55^{-/-} mice (Figure 5B, dotted line). The effect of CBD on insulin secretion and the requirement for GPR55 in this process was also investigated in perifusions using islets isolated from WT and GPR55^{-/-} mice. Data presented in Figure 5C demonstrated that CBD does not have an antagonistic effect on insulin secretion, which is in agreement with its effect on $[Ca^{2+}]_i$ observed in our calcium microfluorimetry experiments. Instead, in islets obtained from both WT and GPR55^{-/-} mice, 1µM CBD induced a small increase in insulin secretion at 2mM glucose and a transient elevation in glucose-stimulated insulin secretion. Thus, although glucose-induced insulin secretion from GPR55^{-/-} islets peaked at a lower level than that from WT islets in the experiments shown in Figure 5C, 1µM CBD was able to potentiate glucose induced insulin secretion from both WT and GPR55^{-/-} islets with similar magnitudes.

O-1602 stimulates insulin secretion from isolated human islets

The insulinotropic effect of the GPR55-dependent agonist O-1602 was further investigated in perifusions with human islets. It can be seen from Figure 5D that 10μ M O-1602 induced a significant elevation in insulin secretion at 2mM glucose that was readily reversible upon removal of the agonist, and the islets then responded to a subsequent exposure to 20mM glucose. 10μ M O-1602

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also potentiated glucose-induced insulin secretion from human islets, stimulating a transient elevation above the plateau phase after exposure to 20mM glucose (Figure 5E).

Discussion

The endocannabinoid system (ECS) plays an important role in fuel homeostasis via effects on the central nervous system and on peripheral tissues involved in metabolic regulation (24, 37, 38). It is now acknowledged that some cannabinoids that were previously thought to act exclusively through CB1 and CB2 receptors may exhibit activities on other receptors such as GPR55 (1, 39), and this has informed studies on cannabinoid signalling in islets. For example, we have reported previously that antagonism of the CB1 receptor by AM251 does not inhibit insulin release from human islets in response to the CB1 agonist ACEA, and AM251 alone exerts reversible, stimulatory effects on insulin secretion (40). Since AM251 is a potent agonist for GPR55 (9, 41), its direct stimulation of insulin secretion may be a consequence of activation of GPR55.

We have previously detected GPR55 mRNA in MIN6 β -cells and mouse and human islets (24), and have now confirmed that it is translated into a 37kDa protein in these cells. Two additional immunoreactive proteins of 33kDa and 50kDa were detected in MIN6 β -cells and islets from WT mice, but absent in islets from GPR55^{-/-} mice. GPR55 undergoes degradation (42) and glycosylation (36), so the lower and higher molecular weight proteins most likely represent a GPR55 degradation product and a glycosylated form of GPR55, respectively. There appears to be species-specific processing of GPR55, since only the native 37kDa protein was identified in human islets. Fluorescence immunostaining indicated that GPR55 is localised to β -cells in mouse and human islets, consistent with two previous rodent studies where GPR55 was reported to be expressed in the BRIN-BD11 cell line and mouse islet β -cells (26) and rat islet β -cells (25). We have also shown here that GPR55 is highly expressed by human islet α -cells, whereas very few mouse α -cells express this receptor, suggesting that it may play a more important role in regulating α -cell function in man than Accepted Article

in mouse. Further studies are required to identify its role in the regulation of glucagon secretion from human islets.

GPR55 couples to G α q and/or G α 13, both of which have been associated with elevations in $[Ca^{2+}]_i$ following activation of PLC and IP₃ generation (1, 4, 6). GPR55 also activates Rho small GTPases via Rho-associated protein kinase (ROCK), which in turn increases $[Ca^{2+}]_i$ both via PLC-mediated IP₃ synthesis, and through actin cytoskeleton remodelling (4). O-1602 elevates $[Ca^{2+}]_i$ in human endothelial cells (5) and our data demonstrate that it also increases $[Ca^{2+}]_i$ in mouse islet cells, consistent with earlier reports in BRIN-BD11 cells (26) and rat islets (25). The lack of effect of O-1602 on RhoA activity in our studies indicates that, at least in β -cells, GPR55 signalling downstream of O-1602 binding to elevate $[Ca^{2+}]_i$ is not through the G $_{\alpha 13}$ pathway and is most likely via G $_{\alpha q}$ activation. Signalling cascades downstream of GPR55 may therefore be cell type-specific since GPR55-mediated RhoA activation has previously been reported in HEK293 and PC12 cells (1, 6, 36). However, two of these earlier studies used LPI as a GPR55 agonist (6, 36), which we have shown here not to function via GPR55 in islets, and cells in which GPR55 had been overexpressed were used in two of the previous reports (1, 6).

It is intriguing that only approximately 50% of β -cells responded to O-1602 treatment in our experiments. This most likely reflects functional heterogeneity of islet β -cells, which has been observed previously in terms of β -cell [Ca²⁺]_i responses (43-45). Similar proportions of mouse islet β -cells also showed elevations in [Ca²⁺]_i in response to LPI, and, surprisingly, to the phytocannabinoid CBD, which is proposed to act as a GPR55 antagonist. The use of islets isolated from GPR55^{-/-} mice indicated that only the O-1602-induced increases in [Ca²⁺]_i were via GPR55-dependent signalling, while similar proportions of islet cells from both WT and GPR55^{-/-} mice responded to LPI and CBD, indicating that these responses were not via GPR55. These findings are in line with previous studies in other cells demonstrating that LPI increased [Ca²⁺]_i independently of GPR55 activation (21-23).

Consistent with its effects on [Ca²⁺]_i, O-1602 reversibly stimulated insulin secretion from mouse and human islets. The use of islets from GPR55^{-/-} mice indicated that GPR55 was required for

O-1602-induced insulin secretion, demonstrating that this cannabidiol analogue is a selective GPR55 agonist in β -cells. In accordance with these data, O-1602 improves glucose tolerance in rats, an effect that has been attributed to its stimulation of insulin secretion via GPR55 activation (25, 26), and GPR55 deletion in mice is associated with insulin resistance (46). However, although our data also support LPI being an effective insulin secretagogue, its capacity to stimulate insulin release does not require interaction with GPR55 since it was equally effective in GPR55^{-/-} islets. LPI can activate non-selective cation channels, Ca²⁺-activated K⁺ channels and L-type voltage-gated calcium channels (21-23, 47), all of which are expressed by islet β -cells and play regulatory roles in β -cell electrophysiology and insulin secretion (48-50). In addition, LPI has been reported to inhibit Na⁺/K⁺ ATPase to alter $[Ca^{2+}]_i$ independently of GPR55 (21) and it may exert its effects via this mechanism in islets. We also found that CBD has insulinotropic effects in mouse islets despite it being considered as a potent inhibitor of GPR55 in HEK293 cells overexpressing GPR55 (1, 4, 15), and being able to inhibit O-1602- and LPI-induced responses in osteoclasts (15). It has been proposed that certain cannabinoid ligands bind to an orthosteric binding site on GPR55 and act as agonists when applied alone, but bind to an allosteric site and inhibit agonist-induced activation in a non-competitive manner when used in tandem (51). However, our demonstration that CBD increased both $[Ca^{2+}]_i$ and insulin secretion following deletion of GPR55 indicates that it transduces its effects by a signalling pathway(s) other than through interaction with GPR55. In particular, CBD may act as an agonist at TRPV2 cation channels (17), PPAR γ (52) and 5-HT_{1A} receptors (18), and activation of TRPV2 (53) and PPARγ (54) are known to stimulate insulin secretion. Deletion of GPR55 did not significantly affect glucose-induced insulin secretion, so the differences in response to these ligands were not a consequence of alterations in glucose responsiveness of the islets.

In summary, this study has demonstrated that GPR55 is expressed by islet β -cells and it plays a positive role in regulating $[Ca^{2+}]_i$ and insulin secretion in response to the pharmacological agonist O-1602. The putative GPR55 antagonist CBD and endogenous ligand LPI induced similar effects to O-1602 on calcium handling and insulin secretion, but, unlike O-1602, they act independently of GPR55

activation in islets. The precise signalling pathways downstream of GPR55 activation in islets remain to be defined but $G_{\alpha 12/13}$ is not implicated as O-1602 did not stimulate β -cell RhoA activity. Our identification of O-1602 as a selective GPR55 agonist in β -cells highlights a therapeutic potential for O-1602-related compounds in type 2 diabetes, especially given the non-psychoactive effects of O-1602, which are most likely a consequence of its lack of activity at the abundant CB1 receptors in the central nervous system. However, our observations that O-1602 can stimulate insulin secretion in the absence of a glucose stimulus suggest that its use in vivo may be associated with hypoglycaemia, as may occur with sulphonylureas, and further investigation of this is necessary in future studies.

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Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement

The study was designed by BL, SS and SJP. Data were collected and analysed by BL, SS, IRM, AP, PMJ and SJP. DB provided the GPR55 null mice and GCH provided the isolated human islets of Langerhans. The article was drafted by BL, SS, SJP and PMJ. All authors revised the article critically for important intellectual content. All authors gave their final approval of the current version to be published.

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References

1. Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol. 2007;152(7):1092-101.

2. Baker D, Pryce G, Davies WL, Hiley CR. In silico patent searching reveals a new cannabinoid receptor. Trends Pharmacol Sci. 2006;27(1):1-4.

3. Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T. Identification of GPR55 as a lysophosphatidylinositol receptor. Biochem Biophys Res Commun. 2007;362(4):928-34.

4. Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. Proc Natl Acad Sci USA. 2008;105(7):2699-704.

5. Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M, et al. Integrin clustering enables anandamide-induced Ca²⁺ signaling in endothelial cells via GPR55 by protection against CB1-receptor-triggered repression. J Cell Sci. 2008;121(Pt 10):1704-17.

6. Henstridge CM, Balenga NA, Ford LA, Ross RA, Waldhoer M, Irving AJ. The GPR55 ligand Lalpha-lysophosphatidylinositol promotes RhoA-dependent Ca²⁺ signaling and NFAT activation. FASEB J. 2009;23(1):183-93.

7. Oka S, Toshida T, Maruyama K, Nakajima K, Yamashita A, Sugiura T. 2-Arachidonoyl-snglycero-3-phosphoinositol: a possible natural ligand for GPR55. J Biochem. 2009;145(1):13-20.

8. Kargl J, Brown AJ, Andersen L, Dorn G, Schicho R, Waldhoer M, et al. A selective antagonist reveals a potential role of G protein-coupled receptor 55 in platelet and endothelial cell function. J Pharmacol Exp Ther. 2013;346(1):54-66.

9. Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS, et al. Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. J Biol Chem. 2009;284(43):29817-27.

10. Sylantyev S, Jensen TP, Ross RA, Rusakov DA. Cannabinoid- and lysophosphatidylinositolsensitive receptor GPR55 boosts neurotransmitter release at central synapses. Proc Natl Acad Sci USA. 2013;110(13):5193-8. 11. Johns DG, Behm DJ, Walker DJ, Ao Z, Shapland EM, Daniels DA, et al. The novel endocannabinoid receptor GPR55 is activated by atypical cannabinoids but does not mediate their vasodilator effects. Br J Pharmacol. 2007;152(5):825-31.

12. Diaz-Arteaga A, Vazquez MJ, Vazquez-Martinez R, Pulido MR, Suarez J, Velasquez DA, et al. The atypical cannabinoid O-1602 stimulates food intake and adiposity in rats. Diabetes, Obesity & Metabolism. 2012;14(3):234-43.

Schicho R, Bashashati M, Bawa M, McHugh D, Saur D, Hu HM, et al. The atypical cannabinoid
O-1602 protects against experimental colitis and inhibits neutrophil recruitment. Inflamm Bowel Dis.
2011;17(8):1651-64.

14. Console-Bram L, Brailoiu E, Brailoiu GC, Sharir H, Abood ME. Activation of GPR18 by cannabinoid compounds: a tale of biased agonism. Br J Pharmacol. 2014;171(16):3908-17.

15. Whyte LS, Ryberg E, Sims NA, Ridge SA, Mackie K, Greasley PJ, et al. The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass in vivo. Proc Natl Acad Sci USA. 2009;106(38):16511-6.

16. Li K, Feng JY, Li YY, Yuece B, Lin XH, Yu LY, et al. Anti-inflammatory role of cannabidiol and O-1602 in cerulein-induced acute pancreatitis in mice. Pancreas. 2013;42(1):123-9.

17. Qin N, Neeper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM. TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. J Neurosci. 2008;28(24):6231-8.

18. Campos AC, Guimaraes FS. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology. 2008;199(2):223-30.

19. De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini PC, Orlando P, et al. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. J Pharmacol Exp Ther. 2008;325(3):1007-15.

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20. O'Sullivan SE, Sun Y, Bennett AJ, Randall MD, Kendall DA. Time-dependent vascular actions of cannabidiol in the rat aorta. Eur J Pharmacol. 2009;612(1-3):61-8.

21. Bondarenko A, Waldeck-Weiermair M, Naghdi S, Poteser M, Malli R, Graier WF. GPR55dependent and -independent ion signalling in response to lysophosphatidylinositol in endothelial cells. Br J Pharmacol. 2010;161(2):308-20.

22. Bondarenko AI, Malli R, Graier WF. The GPR55 agonist lysophosphatidylinositol acts as an intracellular messenger and bidirectionally modulates Ca²⁺-activated large-conductance K⁺ channels in endothelial cells. Pflugers Arch. 2011;461(1):177-89.

23. Bondarenko AI, Malli R, Graier WF. The GPR55 agonist lysophosphatidylinositol directly activates intermediate-conductance Ca2+-activated K+ channels. Pflugers Arch. 2011;462(2):245-55.

24. Li C, Jones PM, Persaud SJ. Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. Pharmacology & Therapeutics. 2011;129(3):307-20.

25. Romero-Zerbo SY, Rafacho A, Diaz-Arteaga A, Suarez J, Quesada I, Imbernon M, et al. A role for the putative cannabinoid receptor GPR55 in the islets of Langerhans. J Endocrinol. 2011;211(2):177-85.

26. McKillop AM, Moran BM, Abdel-Wahab YH, Flatt PR. Evaluation of the insulin releasing and antihyperglycaemic activities of GPR55 lipid agonists using clonal beta-cells, isolated pancreatic islets and mice. Br J Pharmacol. 2013;170(5):978-90.

27. Metz SA. Lysophosphatidylinositol, but not lysophosphatidic acid, stimulates insulin release. A possible role for phospholipase A₂ but not de novo synthesis of lysophospholipid in pancreatic islet function. Biochem Biophys Res Commun. 1986;138(2):720-7.

28. Sisay S, Pryce G, Jackson SJ, Tanner C, Ross RA, Michael GJ, et al. Genetic background can result in a marked or minimal effect of gene knockout (GPR55 and CB2 receptor) in experimental autoimmune encephalomyelitis models of multiple sclerosis. PloS one. 2013;8(10):e76907.

29. Papadimitriou A, King AJ, Jones PM, Persaud SJ. Anti-apoptotic effects of arachidonic acid and prostaglandin E_2 in pancreatic beta-cells. Cell. Physiol. Biochem. 2007;20(5):607-16.

30. Huang GC, Zhao M, Jones P, Persaud S, Ramracheya R, Lobner K, et al. The development of new density gradient media for purifying human islets and islet-quality assessments. Transplantation. 2004;77(1):143-5.

31. Gey GO, Gey MK. The Maintenance of Human Normal Cells and Tumor Cells in Continuous Culture: I. Preliminary Report: Cultivation of Mesoblastic Tumors and Normal Tissue and Notes on Methods of Cultivation. Am J Cancer. 1936;27(1):45-76.

32. Jones PM, Salmon DM, Howell SL. Protein phosphorylation in electrically permeabilized islets of Langerhans. Effects of Ca²⁺, cyclic AMP, a phorbol ester and noradrenaline. Biochem J. 1988;254(2):397-403.

33. Gustavsson N, Larsson-Nyren G, Lindstrom P. Cell specificity of the cytoplasmic Ca²⁺ response to tolbutamide is impaired in beta-cells from hyperglycemic mice. J Endocrinol. 2006;190(2):461-70.

34. Berts A, Ball A, Dryselius G, Gylfe E, Hellman B. Glucose stimulation of somatostatinproducing islet cells involves oscillatory Ca²⁺ signaling. Endocrinology. 1996;137(2):693-7.

35. Quesada I, Nadal A, Soria B. Different effects of tolbutamide and diazoxide in alpha, beta-, and delta-cells within intact islets of Langerhans. Diabetes. 1999;48(12):2390-7.

36. Obara Y, Ueno S, Yanagihata Y, Nakahata N. Lysophosphatidylinositol causes neurite retraction via GPR55, G13 and RhoA in PC12 cells. PLoS One. 2011;6(8):e24284.

37. Liu B, Song S, Jones PM, Persaud SJ. GPR55: from orphan to metabolic regulator? Pharmacology & Therapeutics. 2015;145:35-42.

38. Simcocks AC, O'Keefe L, Jenkin KA, Mathai ML, Hryciw DH, McAinch AJ. A potential role for GPR55 in the regulation of energy homeostasis. Drug Discovery Today. 2014;19(8):1145-51.

39. Brown AJ. Novel cannabinoid receptors. Br J Pharmacol. 2007;152(5):567-75.

40. Li C, Bowe JE, Huang GC, Amiel SA, Jones PM, Persaud SJ. Cannabinoid receptor agonists and antagonists stimulate insulin secretion from isolated human islets of Langerhans. Diabetes, Obesity & Metabolism. 2011;13(10):903-10.

41. Yin H, Chu A, Li W, Wang B, Shelton F, Otero F, et al. Lipid G protein-coupled receptor ligand identification using beta-arrestin PathHunter assay. J Biol Chem. 2009;284(18):12328-38.

42. Kargl J, Balenga NA, Platzer W, Martini L, Whistler JL, Waldhoer M. The GPCR-associated sorting protein 1 regulates ligand-induced down-regulation of GPR55. Br J Pharmacol. 2012;165(8):2611-9.

43. Squires PE, Persaud SJ, Hauge-Evans AC, Gray E, Ratcliff H, Jones PM. Co-ordinated Ca²⁺signalling within pancreatic islets: does beta-cell entrainment require a secreted messenger. Cell Calcium. 2002;31(5):209-19.

44. Squires PE, Hauge-Evans AC, Persaud SJ, Jones PM. Synchronization of Ca²⁺-signals within insulin-secreting pseudoislets: effects of gap-junctional uncouplers. Cell Calcium. 2000;27(5):287-96.

45. Salomon D, Meda P. Heterogeneity and contact-dependent regulation of hormone secretion by individual B cells. Experimental Cell Research. 1986;162(2):507-20.

46. Meadows A, Lee JH, Wu CS, Wei Q, Pradhan G, Yafi M, et al. Deletion of G-protein-coupled receptor 55 promotes obesity by reducing physical activity. Int J Obesity. 2016;40(3):417-24.

47. Ben-Zeev G, Telias M, Nussinovitch I. Lysophospholipids modulate voltage-gated calcium channel currents in pituitary cells; effects of lipid stress. Cell Calcium. 2010;47(6):514-24.

48. Leech CA, Habener JF. A role for Ca²⁺⁻sensitive nonselective cation channels in regulating the membrane potential of pancreatic beta-cells. Diabetes. 1998;47(7):1066-73.

49. Ferrer J, Wasson J, Salkoff L, Permutt MA. Cloning of human pancreatic islet large conductance Ca2+-activated K+ channel (hSlo) cDNAs: Evidence for high levels of expression in pancreatic islets and identification of a flanking genetic marker. Diabetologia. 1996;39(8):891-8.

50. Braun M, Rantracheya R, Bengtsson M, Zhang Q, Karanauskaite J, Partridge C, et al. Voltagegated ion channels in human pancreatic beta-cells: Electrophysiological characterization and role in insulin secretion. Diabetes. 2008;57(6):1618-28.

51. Anavi-Goffer S, Baillie G, Irving AJ, Gertsch J, Greig IR, Pertwee RG, et al. Modulation of Lalpha-lysophosphatidylinositol/GPR55 mitogen-activated protein kinase (MAPK) signaling by cannabinoids. J Biol Chem. 2012;287(1):91-104.

52. O'Sullivan SE, Sun Y, Bennett AJ, Randall MD, Kendall DA. Time-dependent vascular actions of cannabidiol in the rat aorta. Eur J Pharmacol. 2009;612(1-3):61-8.

53. Hisanaga E, Nagasawa M, Ueki K, Kulkarni RN, Mori M, Kojima I. Regulation of calciumpermeable TRPV2 channel by insulin in pancreatic beta-cells. Diabetes. 2009;58(1):174-84.

54. Masuda K, Okamoto Y, Tsuura Y, Kato S, Miura T, Tsuda K, et al. Effects of Troglitazone (CS-045) on insulin secretion in isolated rat pancreatic islets and HIT cells: an insulinotropic mechanism distinct from glibenclamide. Diabetologia. 1995;38(1):24-30.

Figure legends

Panel A: Western blotting indicates that GPR55 is expressed by MIN6 β -cells, mouse and human islets.

Panel B: Fluorescence immunohistochemistry demonstrates that GPR55 (green) is expressed by islets in pancreases from WT mice, while it is absent in islets of GPR55^{-/-} mice.

Panel C: Fluorescence immunohistochemistry in individual mouse pancreas sections demonstrates that GPR55 (green) is co-localised with insulin (red) but not with glucagon or somatostatin (both in red).

Panel D: Fluorescence immunohistochemistry in individual human pancreas sections demonstrates that GPR55 (green) is co-localised with insulin and glucagon (both in red) but not somatostatin (red). Panels E & F: Quantification of GPR55-expressing islet cells in multiple sections from mouse (E) and human (F) pancreases reveals that GPR55 is expressed by β -cells of both species; it is present in a small proportion of mouse islet α -cells and in the majority of human α -cells, but it was absent or at very low levels in δ -cells of both species.

Figure 2. Effects of O-1602, LPI and CBD on $[Ca^{2+}]_i$ in WT and GPR55^{-/-} mouse islet β -cells.

Panels A-D, F-I, K-N: Single microfluorimetry traces indicating that 10μ M O-1602 increased $[Ca^{2+}]_i$ at 2mM glucose (A) and 20mM glucose (B) in β -cells from WT mice but not GPR55^{-/-} mice (C,D). 5 μ M LPI increased $[Ca^{2+}]_i$ in β -cells from WT (F,H) and GPR55^{-/-} mice (G,I). 1μ M CBD increased $[Ca^{2+}]_i$ in β -cells from WT (K,M) and GPR55^{-/-} mice (L,N).

Panels E, J, O: Analysis of the percentage of β -cells responding to O-1602, LPI and CBD with changes in $[Ca^{2+}]_i$. Data are presented as mean+SEM, n=16-51 cells from 2-3 separate experiments per genotype. **p<0.01.

Figure 3. Effect of O-1602 on $[Ca^{2+}]_i$ in MIN6 and human β -cells.

10 μ M O-1602 increased [Ca²⁺]_i at 2mM glucose in MIN6 β -cells (A) and human islet β -cells (B). Results for MIN6 cells are mean±SEM representative of at least 5 different experiments and for human islet β -cells are shown as a single trace representative of at least 19 cells from 2 experiments.

Figure 4. O-1602 does not activate RhoA in MIN6 β -cells.

Immunoblot (A) and fold change in RhoA activity (B) illustrated that 20mM glucose induced RhoA activation in MIN6 cells, but 10μ M O-1602 was without effect at either 2mM or 20mM glucose. Data in panel B are presented as mean+SEM, n=3.

Figure 5. Effects of O-1602, LPI and CBD on insulin secretion from isolated mouse and human islets.

10μM O-1602 stimulated basal and glucose-induced insulin secretion from islets from WT mice but not from GPR55^{-/-} mice (A; AUC; WT vs GPR55^{-/-}, O-1602 at 2mM glucose: 209.8±31.3 vs 53.5±14.4; O-1602 at 20mM glucose: 605.0±68.4 vs 268.6±94.6; p<0.05). Stimulation of insulin secretion by 1μM LPI (B; AUC; WT vs GPR55^{-/-}, at 2mM glucose: 35.1±8.8 vs 27.9±8.2; at 20mM glucose: 206.4±23.5 vs 213.3±107.2; p>0.2) and 5μM CBD (C; AUC; WT vs GPR55^{-/-} at 2mM glucose: 82.9±26.9 vs 80.4±20.5; at 20mM glucose: 586.9±112.6 vs 374.9±64.7; p>0.2) were observed in islets from WT and GPR55^{-/-} mice. 10μM O-1602 increased insulin secretion from human islets at 2mM glucose (D; AUC; basal: 7.7±0.2; O-1602: 30.5±6.3; p<0.05) and 20mM glucose (E; AUC: plateau phase of glucose-stimulated insulin secretion: 60.5±8.1; O-1602: 94.5±5.0, p<0.05). Results for mouse islets are means±SEM, n=3, representative of 3 experiments for each agonist. Results for human islets are means±SEM, n=3 for a single islet donor, representative of 3 experiments using islets from different

donors.

MIN6 WT GPR55^{-/-} Human β-cells 30kDa → 37kDa → 33kDa →



В



D

С







Figure 1. Expression of GPR55 by islets and $\beta\text{-cells}$

Ε

F



2mM glucose 20m

20mM glucose



2mM glucose 20mM glu

20mM glucose



Figure 2. Effects of O-1602, LPI and CBD on $[Ca^{2+}]_i$ in islet β -cells from WT and GPR55^{-/-} mice.



В



Figure 3. Effect of O-1602 on $\left[\text{Ca}^{2+}\right]_i$ in MIN6 and human $\beta\text{-cells}$



Figure 4. O-1602 does not promote RhoA activation in MIN6 β -cells.





Figure 5. Effects of O-1602, LPI and CBD on insulin secretion from isolated mouse and human islets.