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Differential expression of glucagon-like peptide-2 (GLP-2) is involved in pancreatic islet cell adaptations to stress and beta-cell survival

Short title: Glucagon like peptide-2 and beta-cells

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Highlights

- GLP-2 had no effect on glucose disposal or insulin secretion
- However, GLP-2 had positive effects on beta-cell apoptosis and proliferation
- Insulin deficiency and resistance altered islet localisation of GLP-2
- GLP-2 is involved in adaptations to pancreatic islet cell stress encountered in diabetes

Abstract

Recent studies have confirmed that locally released proglucagon derived gene products, other than glucagon, have a major influence on pancreatic endocrine function. We assessed the
impact of glucagon-like peptide-2 (GLP-2) on beta-cell secretory function, proliferation and apoptosis, as well as glucose tolerance, feeding behaviour and islet adaptions to chemically-induced insulin deficiency and resistance. The GLP-2 receptor was evidenced on cultured rodent and human beta-cells, rodent alpha-cells and isolated mouse islets. GLP-2 had no effect on insulin secretion from beta-cells, or isolated mouse islets. *In vivo*, GLP-2 administration significantly (*P*<0.05 to *P*<0.01) decreased food intake in mice. Conversely, GLP-2 had no discernible effects on glucose disposal or insulin secretion. As expected, streptozotocin treatment decreased and hydrocortisone increased beta-cell mass in mice. GLP-2 was visualised in mouse islets and intestinal L-cells. Islet GLP-2 co-localisation with glucagon was significantly decreased (*P*<0.01) by both streptozotocin and hydrocortisone. In contrast, both interventions increased (*P*<0.05) co-localisation of GLP-2 with somatostatin. Interestingly, GLP-2 positive cells were reduced (*P*<0.05) in the intestines of streptozotocin, but not hydrocortisone, treated mice. Further *in vitro* investigations revealed that GLP-2 protected rodent and human 1.1B4 beta-cells against streptozotocin induced DNA damage. Furthermore, GLP-2 augmented (*P*<0.05) BRIN BD11 beta-cell proliferation, but was less efficacious in 1.1B4 cells. These data highlight the involvement of GLP-2 receptor signalling in the adaptations to pancreatic islet cell stress.

*Keywords:* Beta-cell, alpha-cell, islets, proglucagon, GLP-2, GLP-2 receptor, insulin secretion, diabetes, proliferation, apoptosis

**Introduction**
Glucagon-like peptide-2 (GLP-2) is a 33 amino-acid peptide hormone secreted from intestinal L-cells in response to feeding that exerts a number of well characterised metabolic actions. These include direct trophic effects on the intestine that are linked to increased proliferation and inhibition of apoptosis of crypt cells [14], stimulation of nutrient digestion and absorption [33] and inhibition of acid secretion [42]. However, recently GLP-2 has been shown to play a role in the control of glucose homeostasis, insulin sensitivity [34] and lipid metabolism [7], which is presumed to relate to the observed effects of GLP-2 to stimulate glucagon release from pancreatic alpha-cells [28]. Thus, intestinal L-cells and pancreatic alpha-cells both express the proglucagon gene, from which GLP-2 is potentially derived [32].

The conventional view is that the proglucagon gene is processed in a tissue-specific manner, yielding GLP-1, GLP-2 and related molecules in the gut through the action of the enzyme prohormone convertase 1/3 (PC1/3), with glucagon being released in the islets by PC2 processing [18]. However, more recent evidence reveals that alpha-cells can express PC1/3 [16,29,31,41]. Consistent with this, GLP-2 protein and receptor expression have been evidenced in rodent and human alpha-cells [1,10]. Moreover, alpha-cell expression of the related PC1/3 proglucagon gene product, glucagon-like peptide-1 (GLP-1), has also been confirmed in rodent and human alpha-cells [25,29]. Indeed, studies in isolated islets reveal alpha-cells secrete GLP-1 in response to arginine stimulation [25]. Furthermore, paracrine effects of alpha-cell derived GLP-1 are now heavily implicated in pancreatic beta-cell compensatory responses to insulin resistance and cellular stress [40]. In agreement, alpha-cell expression of GLP-1 has been shown to be enhanced under situations of beta-cell loss and stress [11,17,29].

Although there is clear proof for islet alpha-cell GLP-1 production and related beneficial autocrine and paracrine effects [41], there is only partial evidence for a role of GLP-2 in the regulation of islet function. Thus, in terms of the limited information available, GLP-
2 receptor knockout mice present with normal glucose homeostasis and islet architecture [4]. However, GLP-2 receptor knockout in severely diabetic ob/ob mice leads to increased alpha-, and reduced beta-cell masses, resulting in a deterioration of glucose tolerance, implying that GLP-2 modulates islet adaptations to metabolic stress [4]. Finally, in high fat fed diabetic mice administration of the GLP-2 receptor inhibitor, GLP-2(3-33), impairs glucose tolerance and insulin sensitivity, whereas the long-acting GLP-2 agonist, (Gly2)GLP-2, improves glucose tolerance and increases beta-cell mass [6]. Therefore, based on these recently described islet actions of GLP-2, it can be hypothesised that GLP-2 may have an important impact upon beta-cell function and survival, particularly in the adaptive responses to islet cell stress.

Therefore, in the present study we have evaluated the effects and potential mechanisms of GLP-2 on the modulation of insulin secretion in vivo and in vitro from cultured clonal rat beta-cells and human 1.1B4 cells, as well as isolated mouse islets. To determine the impact of GLP-2 in islet adaptations to beta-cell loss and insulin resistance, relative changes in islet cell GLP-2 gene expression, and co-localisation with alpha- and delta-cells, was assessed in mice following streptozotocin and hydrocortisone treatment. Finally, effects of GLP-2 on beta-cell proliferation and apoptosis was studies in rodent beta-cells and human 1.1B4 cells. Our data support the concept that GLP-2 plays a role in the beta-cell survival and function.

Materials and methods

In vitro studies

Messenger RNA (3 μg) for GLP-2 and glucose-dependent insulinotropic polypeptide (GIP) receptor expression analyses was extracted and isolated from studied murine islets and cell lines, and real time RT-PCR amplification conditions applied, as described previously [22].
Amplification conditions were set at 95°C for initial and final denaturation, 58°C for primer annealing and 72°C for extension for 40 cycles, followed by a melting curve analysis, with temperature range set at 60°C to 90°C. The Ct values for GLP-2 in BRIN BD11, 1.1B4 and αTC1.9 cells, as well as mouse islets, was 30.5, 31.5, 24.6 and 30.8, respectively. All expression data was normalised to expression of the housekeeping gene β-actin.

GLP-2 and GLP-1 peptides were obtained (>95% purity) from Genscript (USA) and GL-Biochem Limited (China), respectively, and further characterised in-house using HPLC and mass spectrometry before experimentation, as described previously [19]. Effects of test peptides (10⁻⁸ – 10⁻⁶ M) on insulin secretion from clonal rodent BRIN-BD11 beta-cells (ECACC 10033003) [26] and human 1.1B4 cells (ECACC 10012801) [27] following 20 minute incubations, or isolated mouse islets following a 60 min incubation period, was also determined as described in detail elsewhere [22]. In addition, effects of GLP-2 (10⁻⁶ M) on GLP-1 (10⁻⁸ M) induced insulin secretion from BRIN BD11 cells were also examined. Insulin concentrations were measured by radioimmunoassay [15]. Mechanistic studies in beta-cells, including assessment of membrane potential and [Ca²⁺], were conducted in BRIN BD11 cells as documented previously from our laboratory [22].

**Animals**

Animal studies were conducted using adult male C57BL/6 or NIH Swiss mice (12-14 weeks of age, Harlan Ltd, UK). All mice were housed individually in air conditioned room at 22±2°C with 12 hours light and dark cycle and *ad libitum* access to drinking water and standard rodent diet (10% fat, 30% protein and 60% carbohydrate; Trouw Nutrition, Northwich, UK). All experiments were carried out in accordance with the UK Animal Scientific Procedures Act 1986.
Acute in vivo effects

Effects of GLP-1 and GLP-2 (both at 25 nmol/kg; i.p.) on food intake, glucose homeostasis and glucose-induced insulin secretion were evaluated in overnight (18 h) fasted NIH Swiss mice as detailed before [22]. This dose was chosen on the basis of our previous detailed in vivo studies using pro-glucagon derived peptides [19,29,30,39]. Blood glucose and plasma insulin concentrations were determined by standard procedures as detailed in our previous work [22]. Food intake was measured at 30 min intervals, up to and including 180 mins after, i.p administration of GLP-1 or GLP-2 (both at 25 nmol/kg; i.p.) in 18 h fasted NIH Swiss mice.

GLP-2 islet and intestinal expression, as well as islet localisation in streptozotocin and hydrocortisone treated diabetic mice

Insulin-deficient and insulin-resistant diabetes were induced in C57BL/6 mice by multiple low dose streptozotocin (50 mg/kg body weight, i.p.) or daily hydrocortisone injections (70 mg/kg body weight, i.p.), as described previously [22]. After 10 days, pancreatic and small intestinal tissues were excised and fixed in 4% paraformaldehyde (PFA) for 48 h at 4°C. Tissues were processed and sectioned as detailed within our previous work [22]. Primary and secondary antibodies are listed in Table 1 and Table 2, respectively. Specificity of antibodies was previously confirmed by blocking experiments using the respective native peptides (10^{-3} M), and no cross reactivity was observed (see supplementary Figure 1). Slides were mounted, and immunofluorescent islet sections viewed and photographed, as previously described within our laboratory [22]. All procedures were conducted in a blinded manner. Approximately 100 islets were analysed per group. All parameters were determined using the ‘closed polygon’ tool in Olympus CellF analysis software.
**In vitro beta-cell proliferation and apoptosis**

We used BRIN BD11 beta-cells and human 1.1B4 cells to further probe GLP-2 mediated beta-cell effects in terms of proliferation (16 h incubation) and protection against streptozotocin induced (5 mM, 2 h incubation) apoptosis. Studies were carried out in the presence of GLP-2, or GLP-1 as positive control (both at 10^{-6} M), in an essentially similar manner to those described previously by our laboratory [22]. The choice of peptide doses was based on our previous beta-cell proliferation and apoptosis studies [22]. As such, Ki-67 immunostaining was used to assess proliferation with comet assay performed to determine % tail DNA and olive tail moment [22].

**Statistical analysis**

Statistical analyses were performed using GraphPad PRISM software (Version 5.0). Values are expressed as mean±S.E.M. Comparative analyses between groups were carried out using a One-way ANOVA with Bonferroni post hoc test or student’s unpaired t-test, as appropriate. The difference between groups was considered significant if $P<0.05$.

**Results**

*Expression of GLP-2 receptor by rodent alpha- and beta-cells, human beta-cells and isolated mouse islets*

Expression of the GLP-2 receptor was confirmed on rodent alpha- and beta-cells, isolated mouse islets, as well as cultured human 1.1B4 cells (Fig. 1A-D). GLP-2 receptor expression was lower in both BRIN BD11 ($P<0.05$) and 1.1B4 ($P<0.01$) beta-cells when compared to relative expression of the well characterised GIP receptor (Fig. 1A,B). However, GLP-2
receptor mRNA levels were comparable to the GIP receptor in isolated mouse islets and alpha TC1.9 cells (Fig. 1C,D).

**Effects of GLP-2 on insulin release from rodent and human beta-cells, as well as isolated mouse islets**

KCl (30 mM), alanine (10 mM) and GLP-1 (10^{-8} \text{ M} \text{ and } 10^{-6} \text{ M}) significantly (P<0.01 to P<0.001) amplified insulin secretion from BRIN BD11 beta-cells at both 5.6 and 16.7 mM glucose (Fig. 2A,B). In contrast, GLP-2 (10^{-8} - 10^{-6} \text{ M}) had no significant effect on basal (5.6 mM) or glucose-stimulated (16.7 mM) insulin release from BRIN BD11 cells (Fig. 2A,B). Similar observations were made in human 1.1B4 cells (Fig. 2C) and isolated mouse islets (Fig. 2D), where GLP-2 had no significant effect on augmentation of insulin release. In keeping with this, GLP-2 (10^{-6} \text{ M}) had no effect on GLP-1 (10^{-8} \text{ M}) induced insulin secretion (Fig. 2E). In agreement with lack of insulinotropic actions of GLP-2, the peptide (10^{-6} \text{ M}) did not alter membrane potential or [Ca^{2+}], in BRIN BD11 cells (Fig. 3A-D), whereas KCl (10 mM) and alanine (10 mM) significantly (P<0.001) increased cell membrane potential and [Ca^{2+}], respectively, at 5.6 mM glucose (Fig. 3A-D).

**Effects of GLP-2 on glucose tolerance and food intake in mice**

Intraperitoneal administration of GLP-2 (25 nmol/kg bw) to NIH Swiss mice in combination with glucose had no significant effect on blood glucose levels and corresponding glucose-induced plasma insulin concentrations (Fig. 4A-D). In contrast, the same dose GLP-1 significantly decreased individual (P<0.05 to P<0.01) and 0-90 min overall AUC (P<0.05) glucose levels (Fig. 4A,B), and also increased (P<0.05) corresponding overall plasma insulin
concentrations (Fig. 4D). Both GLP-1 and GLP-2 significantly ($P<0.05$ to $P<0.01$) reduced food intake at 30 and 60 min post-injection in overnight fasted mice, with the significant appetite suppressive effects of GLP-1 also extending to 90 ($P<0.01$) and 120 ($P<0.05$) minutes post-injection (Fig. 4E).

Effects of streptozotocin and hydrocortisone induced insulin-deficiency and insulin-resistance on islet and intestinal GLP-2 immunoreactivity

In agreement with previous studies, streptozotocin treatment significantly reduced ($P<0.01$) and hydrocortisone increased ($P<0.001$) beta-cell area (Fig. 5D). Streptozotocin mice also had increased ($P<0.001$) alpha-cell area, which was not evident in hydrocortisone-treated mice (Fig. 5D). Representative images showing immunoreactivity of GLP-2 in islets are shown in Figure 5A,B. Induction of insulin deficiency or insulin resistance by streptozotocin and hydrocortisone injection, respectively, significantly ($P<0.01$) decreased the co-localisation of GLP-2 with glucagon in the endocrine pancreas (Fig. 5A,E). In contrast, hydrocortisone decreased ($P<0.01$) co-localisation of GLP-2 with glucagon (Fig. 5A,E). Interestingly, both treatment interventions significantly ($P<0.05$) increased co-localisation of GLP-2 with somatostatin (Fig. 5B,F). We also assessed GLP-2 immunoreactivity in the mucosal area of the small intestine (Fig. 5C), where streptozotocin decreased ($P<0.05$) GLP-2 cell count per mm² mucosal area, whilst hydrocortisone treatment was without effect (Fig. 5C,G). In agreement, streptozotocin also decreased ($P<0.05$) total intestinal L-cell, but this was unaltered by hydrocortisone treatment (data not shown).

Effects of GLP-2 on rodent BRIN BD11 and human 1.1B4 beta-cell proliferation
Culture of BRIN BD11 beta-cells with GLP-1 or GLP-2 (both at 10^{-6} M) for 16 h significantly ($P<0.001$ and $P<0.01$, respectively) boosted proliferation frequency (Fig. 6A). Whilst GLP-2 had no effect on 1.1B4 beta-cell proliferation, GLP-1 induced a significant ($P<0.001$) proliferative effect in these cells (Fig. 6B). Representative images of Ki-67 stained beta-cells are shown in Figure 6C.

**Protective effects of GLP-2 on streptozotocin-induced DNA damage in rodent BRIN BD11 and human 1.1B4 beta-cells**

GLP-1 and GLP-2 both fully countered the unfavourable effects of streptozotocin (5 mM) on BRIN BD11 beta-cell viability (Fig. 7A). Similar positive observations were made in human 1.1B4 cells, but GLP-2 was unable to completely reverse ($P<0.01$) the detrimental effects of streptozotocin on cell viability in this cell line (Fig. 7B). Similarly, co-culture of BRIN BD11 or 1.1B4 beta-cells with either GLP-1 or GLP-2 completely neutralised the damaging effects of streptozotocin on % tail DNA (Fig. 7C,D). The negative effect of 5 mM streptozotocin on olive tail moment in BRIN BD11 cells was partially countered ($P<0.001$) by GLP-1 and GLP-2, but levels were still elevated ($P<0.001$) compared to respective control cells (Fig. 7E). GLP-2 had an essentially similar beneficial effect on olive tail moment in human 1.1B4 cells, whilst GLP-1 returned olive tail moment to control levels following streptozotocin insult in these cells (Fig. 7F). Representative comet images are shown in Figure 7G.

**Discussion**

Evidence of pancreatic islet alpha-cell expression of PC1/3 [29,31,41], together with confirmed significant positive actions of intra-islet GLP-1 [25], suggests a potential role of other PC1/3
proglucagon gene products, such as GLP-2, in the regulation of islet function. Thus, in accordance with previous work showing presence of the GLP-2 receptor in the alpha-cell cytoplasm and membrane [10], GLP-2 receptor gene expression was demonstrated in both rodent alpha-cells and isolated islets in the present study. Previous observations in human islets revealed GLP-2 receptor expression to be particularly strong in alpha-cells [1]. However, we have also clearly shown detectable GLP-2 receptor mRNA levels and immunoreactive GLP-2 staining in rodent beta-cells and human 1.1B4 cells, consistent with a role for GLP-2 in islet and beta-cell function. Although, confirmation of GLP-2 receptor protein expression by methods other than immunocytochemistry in pancreatic beta-cells would help support these observations.

In agreement with the current observations, plasma glucose has been shown not to change following GLP-2 administration in rodents [4,5]. However, GLP-2 is known to play a role in glucagon secretion [24], and pharmacological GLP-2 levels are associated with increased glucagon levels in humans [28], and the perfused rat pancreas [10]. Thus, it may have been interesting to assess glucagon levels within the current study. However, effects of GLP-2 on glucagon have been shown to not ultimately affect circulating glucose concentrations [4]. Unlike the recognised insulin secretory actions of GLP-1 [35], GLP-2 did not increase insulin levels in mice, isolated islets or cultured rodent and human 1.1B4 cells clonal beta-cells [23]. Furthermore, GLP-2 did not alter BRIN BD11 beta-cell membrane potential and \([\text{Ca}^{2+}]_i\), or GLP-1 induced insulin secretion, in harmony with limited effects on beta-cell secretory function and low levels of receptor expression in these cells. Despite this, previous studies have highlighted a role for GLP-2 in the control of metabolism. Thus, in harmony with previous studies [7,9], both GLP-1 and GLP-2 possessed clear appetite suppressive effects in overnight fasted mice. Effects of both peptides were transient in nature, in keeping with short biological half-lives due to N-terminal truncation by dipeptidyl peptidase 4 [18]. The ability of GLP-1 to
suppress food intake is known to be mediated via stimulation of GLP-1 receptors in the hypothalamus [3]. The fundamental anorectic mechanism(s) of GLP-2 remain to be elucidated [37], although an indirect effect of GLP-1 receptor signalling has been suggested [36].

As such, genetic knockout of GLP-2 receptors does not result alteration of glucose homeostasis or islet architecture in mice [4]. However, GLP-2 receptor knockdown in genetically obese diabetic (ob/ob) mice, leads to a deterioration of glucose tolerance, alpha-cell hyperplasia and reduced beta-cell mass, with impaired islet cell proliferation [4]. In full agreement, more recent studies in high fat fed mice have shown significantly improved glucose tolerance and insulin sensitivity, coupled with increased beta-cell mass following prolonged treatment with the long-acting GLP-2 receptor agonist, (Gly\(^2\))GLP-2 [6]. Moreover, Roux-en-Y gastric bypass (RYGB) surgery, that is associated with dramatic and rapid improvements in beta-cell function, has been shown to increase GLP-2 levels [20]. Therefore, we next assessed GLP-2 islet expression and location in mouse models of islet cell cytotoxic stress and insulin resistance, to evaluate the role of GLP-2 in situations of beta cell destruction and growth.

Animals treated with streptozotocin and hydrocortisone had typical expected changes in islet morphology, including dramatic changes in alpha- and beta-cell areas, as demonstrated previously [22]. We detected co-localisation of GLP-2 within alpha-cells, in accord with previous observations [31]. We were also able to evidence immunofluorescent GLP-2 staining in somatostatin containing delta-cells, which does contrast with earlier studies in rats and humans [10]. However, this phenotype could simply be species related, but confocal microscopy would be needed to confirm this finding. Both insulin-deficient streptozotocin and insulin-resistant hydrocortisone treated mice presented with decreased co-immunoreactivity of GLP-2 within glucagon containing cells. This could suggest a possible contributory role for GLP-2 in cellular adaptations to beta-cell stress, and is especially interesting in streptozotocin mice where alpha-cell area is substantially increased [22]. Previous studies in our laboratory
have shown that GLP-1 co-localisation in alpha-cells also decreases following streptozotocin treatment [39]. Thus, PC1/3 proglucagon derived gene products may have comparable effects in islet cell adaptations to beta-cell destruction, that requires further detailed study. We also observed decreased GLP-2 positive intestinal cells in streptozotocin mice, and gut barrier function has been shown to be impaired in GLP-2 receptor knockout mice [5]. This enteroendocrine adaptation could be important in controlling intestinal integrity [21], although it should be noted that streptozotocin did decrease total intestinal L-cell count.

Increased co-localisation of GLP-2 with somatostatin in situations of both beta-cell growth and destruction is notable, especially since delta-cells are known to be PDX-1 positive. As such, in the present study GLP-2 significantly augmented BRIN BD11 beta-cell proliferation, in a similar manner to GLP-1 [3]. Indeed, proliferative actions of GLP-2 in the intestine have already been suggested [2,8,12]. Effects of GLP-2 on human 1.1B4 cell proliferation were less obvious, despite presence of the GLP-2 receptor on these cells and use of reasonably high peptide doses, which may require further study. It has been proposed that GLP-2 also has important anti-apoptotic actions [2,43]. In the current study GLP-2 was equally as effective as GLP-1 in terms of protecting beta-cells from streptozotocin-induced DNA damage [38]. In agreement, the long-acting GLP-2 analogue (Gly²)GLP-2 has been shown to reverse the cytotoxic damage of dextran sulfate-induced colitis in mice [13]. This could suggest an important role for GLP-2 in adaptations to situations of severe beta-cell destruction. Examining the direct effects of GLP-2 in primary human islets/beta-cells would also be of interest, but availability of such tissue is a major obstacle.

In conclusion, the current observations add to the evidence that GLP-2 plays a role in directly regulating the endocrine pancreas, particularly in response to islet stress. The observed alteration of GLP-2 localisation in situations of insulin resistance and deficiency, together with
benefits on beta-cell survival, suggest that modulation of GLP-2 receptor signalling could represent a novel target for the treatment of diabetes.

Conflict of interest statement

The authors declare that no conflicting interests exist.

Acknowledgements

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References


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<td>Mouse</td>
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<td>Abcam, ab6995</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Guinea pig</td>
<td>1:200</td>
<td>Raised in-house PCA2/4</td>
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<td>GLP-2</td>
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<td>1:200</td>
<td>Santa Cruz, sc-7781</td>
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<tr>
<td>SST</td>
<td>Rat</td>
<td>1:500</td>
<td>Biorad, 8330-009</td>
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<tr>
<td>Ki-67</td>
<td>Rabbit</td>
<td>1:200</td>
<td>Abcam, ab15580</td>
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Table 1. Target, host and source of primary antibodies employed for immunoflourescent islet histological studies
Pancreatic and intestinal tissues were sectioned (8 μm) and selected for analysis at intervals of 10 sections. After deparaffinising, sections were rehydrated using a series of decreasing strength ethanol solutions. Antigen retrieval was carried out using a citrate buffer (pH 6.0) at 94°C for 20 min, and sections were incubated with primary antibodies in blocking buffer (2% BSA) overnight at 4°C. The following primary antibodies were used: anti-insulin (Abcam), anti-glucagon (raised in-house), anti-glucagon-like-peptide-2 (Santa Cruz), anti-somatostatin (Biorad) and anti-Ki67 (Abcam).

Table 2. Target, host, reactivity and source of secondary antibodies employed for immunofluorescent islet histological studies

<table>
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<th>Reactivity</th>
<th>Dilution</th>
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<tr>
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<td>IgG</td>
<td>Donkey</td>
<td>Goat</td>
<td>1:400</td>
<td>Alexa Fluor 488, Invitrogen, UK</td>
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Following primary antibody incubation, sections were incubated with appropriate secondary antibodies for 1 hr at 37°C, and stained with nuclear DAPI staining. The secondary antibodies used were goat anti-mouse, goat anti-guinea pig, goat anti-rabbit, goat anti-rat and donkey anti-goat (Invitrogen and Abcam). Slides were mounted with anti-fade mounting medium, viewed using Olympus system fluorescent microscope and photographed with a DP70 camera adapter system.
Figure Legends

**Fig. 1.** mRNA expression of the GLP-2 receptor in rodent and human cultured clonal beta-cells, isolated mouse islets and rodent alpha-cells. GLP-2 receptor mRNA expression in (A) rodent BRIN BD11 beta-cells, (B) human 1.1B4 beta-cells, (c) isolated C57BL/6 mouse islets and (D) rodent TC1.9 alpha-cells. mRNA expression was normalised relative to β-actin expression and also compared against expression of the classic islet-cell GPCR, GIPR. Values are mean ± SEM (n = 4-6). **P<0.01 compared to GIPR expression. All mRNA expression was normalised to Actb/ACTB expression.

**Fig. 2.** Effects of GLP-2 on insulin release from rodent BRIN BD11 beta-cells, human 1.1B4 beta-cells and isolated mouse islets. (A,B,E) BRIN BD11, (C) 1.1B4 cells or (D) isolated mouse islets were incubated (A-C 20 mins; D 60 mins) with either 5.6 or 16.7 mM glucose, as appropriate, and the effects of GLP-2 (10⁻⁸ – 10⁻⁶ M) determined. GLP-1 (10⁻⁸ – 10⁻⁶ M) was included as a direct comparator. (E) Effects of GLP-2 (10⁻⁶ M) on GLP-1 induced (10⁻⁸ M) insulin secretion were also determined in BRIN BD11 cells. (A-C,E) Values are mean ± SEM
(n=8). **P<0.01, ***P<0.001 compared to respective control. (D) Values are mean ± SEM (n=4). *P<0.05, **P<0.01, ***P<0.001 compared to 16.7 mM glucose.

**Fig. 3.** Effects of GLP-2 on membrane potential and [Ca^{2+}]_i in rodent BRIN BD11 cells. (A,B) Cells were incubated with 5.6 mM glucose in the presence of GLP-2 (10^{-6} M) and membrane potential or [Ca^{2+}]_i assessed over a 5 minute period, with KCl (15 mM) or alanine (10 mM) as positive controls, respectively. (C,D) area under curve data is also shown. Values are mean ± SEM (n=6). **P<0.001 compared to 5.6 mM glucose. ΔΔΔP<0.001 compared to respective positive control.

**Fig. 4.** Acute effects of GLP-2 on glucose tolerance, insulin response to glucose and food intake in overnight fasted mice. (A) Blood glucose and (C) plasma insulin levels were assessed immediately before and after intraperitoneal administration of GLP-2 (25 nmol/kg bw) together with glucose (18 mmol/kg bw). Respective (B) blood glucose and (D) plasma insulin area under curve data are also shown. (E) Cumulative food intake was assessed after intraperitoneal administration of saline vehicle (0.9% NaCl), or GLP-2 (25 nmol/kg bw) in overnight fasted mice. GLP-1 (25 nmol/kg bw) was included as a direct comparator for all studies. Values are mean ± SEM (n=6 mice). *P<0.05 and **P<0.01 compared to saline treated mice.

**Fig. 5.** Effects of streptozotocin and hydrocortisone treatment of mice on GLP-2 islet expression and location, as well as intestinal mucosal expression. C57BL/6 mice received daily injections of streptozotocin (50 mg/kg bw) or hydrocortisone (70 mg/kg bw) for 5 or 10 days respectively before examination of pancreatic histology on day 10. (A-C) Representative
images showing islet GLP-2 (red) staining with (A) glucagon and (B) somatostatin (green) and (C) small intestinal GLP-2 positive cells of control, streptozotocin and hydrocortisone treated mice. Nuclei are demonstrated using DAPI staining (blue). (A,B) Arrows indicate cells that are positive for both GLP-2 and glucagon or somatostatin. (C) Arrows indicate cells that are positive for GLP-2. (D) Alpha- and beta-cell areas in control, streptozotocin and hydrocortisone mice were determined using the ‘closed polygon’ tool in Olympus Cell^F analysis software. (E,F) Quantification of co-localisation of GLP-2 with (E) glucagon, (F) somatostatin is also shown, along with (G) intestinal GLP-2 cell count (count per mm² mucosal area). Values are mean ± SEM (n=6 mice). *P<0.05, **P<0.01, ***P<0.001 compared to control mice.

**Fig. 6.** Effects of GLP-2 on clonal rodent BRIN BD11 and human 1.1B4 cell proliferation. (A,B) Proliferation frequency in (A) BRIN BD11 and (B) 1.1B4 cells cultured with GLP-2 (10⁻⁶ M) for 16 h. GLP-1 (10⁻⁶ M) is included as a direct comparator. (C) Representative images showing proliferating beta-cells in the presence (16 h) of GLP-1 and GLP-2 (both at 10⁻⁶ M). Arrows indicate proliferating cells Values are mean ± SEM (n=4). *P<0.05, ***P<0.001 compared to control.

**Fig. 7.** Effects of GLP-2 on protection of clonal rodent BRIN BD11 cells and human 1.1B4 cell from streptozotocin induced cellular stress. (A,B) Cell viability, (C,D) % tail DNA and (E,F) olive tail moment were assessed in response to 2 h exposure to 5 mM streptozotocin with and without co-culture with GLP-2 (10⁻⁶ M) in (A,C,E) BRIN BD11 and (B,D,F) 1.1B4 beta-cells. GLP-1 (10⁻⁶ M) is included as a direct comparator. (G) Representative images showing comets of control, streptozotocin alone and in combination with GLP-1 or GLP-2 (both at 10⁻
M) in both cell types. Arrows indicate cells with comet tails. Values are mean ± SEM (n=4). **P<0.01, ***P<0.001 compared to control. ΔΔΔP<0.001 compared to streptozotocin treated cells.

Figure 1

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Figure 2

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Figure 7

A: BRIN BD11 cells
B: 1.1B4 cells
C:
D:
E:
F:

G: BRIN BD11 beta-cells
Control
Streptozotocin
GLP-1 (10^{-6} M)

1.1B4 beta-cells
Control
Streptozotocin
GLP-1 (10^{-6} M)

GLP-2 (10^{-6} M)