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ORIGINAL ARTICLE

Stimulating beta cell replication and improving islet graft function by GPR119 agonists

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Conflicts of Interest
All authors declare no conflict of interest.

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Summary

G protein-coupled receptor 119 (GPR119) is predominantly expressed in β cells and intestinal L cells. In this study, we investigated whether oleoylethanolamide (OEA), a GPR119 endogenous ligand, and PSN632408, a GPR119 synthetic agonist, can stimulate β-cell replication *in vitro* and *in vivo* and improve islet graft function in diabetic mice. We found that OEA and PSN632408 significantly increased numbers of insulin$^+$/5-bromo-2′-deoxyuridine (BrdU)$^+$ β cells in cultured mouse islets in a dose-dependent manner. All diabetic recipient mice, given marginal syngeneic islet transplants with OEA or PSN632408 or vehicle, achieved normoglycemia at 4 weeks after transplantation. However, normoglycemia was achieved significantly faster in OEA- or PSN632408-treated diabetic mice than in vehicle-treated diabetic mice ($P < 0.05$). The percentage of insulin$^+$/BrdU$^+$ β cells in islet grafts in OEA- and PSN632408-treated mice was significantly higher than in vehicle-treated mice ($P < 0.01$). Our data demonstrated that OEA and PSN632408 can stimulate β-cell replication *in vitro* and *in vivo* and improve islet graft function. Targeting GPR119 is a novel therapeutic approach to increase β-cell mass and to improve islet graft function by stimulating β-cell replication.

Introduction

Islet transplantation can restore normoglycemia in some patients with type 1 diabetes [1,2]. However, multiple-donor pancreases are usually required to obtain an islet mass sufficient for achieving normoglycemia. Although some transplant centers have performed single-donor islet transplantation and have shown that diabetes could be reversed with islets isolated from a single-donor pancreas [3–6], the islet mass from single donors is often not sufficient to restore normoglycemia. Nevertheless, because of the pancreas donor shortage, single-donor islet transplantation is a very attractive goal. Indeed, successful single-donor islet transplantation would not only increase the availability of human islet transplantation but also reduce the risks and costs of islet transplantation. Therefore, strategies to increase β-cell mass before and after transplantation and to improve the function of transplanted

β-cells need to be developed to facilitate successful single-donor islet transplantation.

G protein-coupled receptor 119 (GPR119) was first identified as an orphan G protein-coupled receptor in a number of mammalian species [7]. It is predominantly expressed in the pancreatic β cells and β-cell lines [8,9]. In addition, expression of GPR119 was found in glucagon-like peptide 1 (GLP-1) secreting intestinal endocrine L cells [10]. Phospholipid lysophosphatidylcholine and oleoylethanolamide (OEA) have been identified as 2 endogenous ligands for GPR119 [8,11]. Both of them can increase intracellular cAMP levels and result in glucose-dependent insulin secretion. However, OEA is significantly more potent than phospholipid lysophosphatidylcholine in activating GPR119 [11]. PSN632408, a selective small-molecular GPR119 agonist, can increase intracellular cAMP levels in a GPR119-dependent manner and reduce food intake and body weight gain in rats [11]. Furthermore, it can stimulate insulin secretion *in vitro* by activating GPR119.

GPR119 agonists have been shown to enhance glucose-dependent insulin secretion *in vivo* and improve oral glucose tolerance in wild-type mice, but not in GPR119-deficient mice [9]. A greater effect of GPR119 agonist on insulin secretion when given orally, compared with intravenously, suggests action via incretin-based mechanisms. Indeed, GPR119 is expressed on intestinal L cells and GPR119 agonists stimulate GLP-1 secretion from enteroendocrine cell lines *in vitro* and GLP-1 *in vivo* [10,12–14]. Activation of GPR119 is essential for OEA-induced GLP-1 secretion from intestinal enteroendocine L-cell *in vitro* and *in vivo* [14]. GLP-1 is a potent glucose-dependent insulinotropic peptide hormone that stimulates insulin secretion, promotes β-cell regeneration, and prevents β-cell apoptosis [15,16]. These data indicate that GPR119 agonists have not only a direct effect on β cells but also an indirect effect on β cells through stimulating GLP-1 release.

As one of the leading targets for next generation of anti-diabetic therapy, several GPR119 agonists have been identified as insulinotropic agents for treating type 2 diabetes and are currently in clinical trials [17–20]. Although GPR119 agonists can directly stimulate insulin secretion from β cells, whether they can stimulate β-cell replication in islets remains unknown. In this study, we investigated the effect of OEA and PSN632408 on β-cell replication in cultured islets and in transplanted islet grafts.

Materials and methods

Animals

Male retired breeder C57BL/6 mice (more than 36 weeks old) and B6/129S4-*Ppara*^{tm1Gonz}/J mice (more than 40 weeks old) and young C57BL/6 mice (7–8 weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). These mice were housed in pathogen-free animal facilities at the University of Minnesota. All experiments related to these mice were performed according to the protocol approved by the Institutional Animal Care and Use Committee. The retired breeder C57BL/6 mice and B6/129S4-*Ppara*^{tm1Gonz}/J mice served as islet donors for islet culture and transplantation. Young C57BL/6 mice with diabetes served as recipients for islet transplantation. The recipient mice were given streptozotocin (STZ) through the intraperitoneal at 200 mg/kg to induce diabetes. Diabetes was diagnosed when the non-fasting blood glucose was >400 mg/dl on two consecutive measurements. The blood glucose was measured by a GLUCOMETER ELITE blood glucose monitoring system (BayerCo, Elkhart, IN, USA). These diabetic mice underwent daily insulin treatment for at least 2 weeks before transplantation.

Islet isolation and culture

The islets were isolated according to a protocol that is similar to a previously published isolation protocol [21,22]. Briefly, 2.5 ml of Hank's balanced salt solution containing 2 mg/ml collagenase from Clostridium histolyticum (Serva, Heildelberg, Germany) was injected into the pancreatic duct. The distended pancreas was removed and incubated at 37 °C for 16 min. The islets were purified by centrifugation on gradients comprising three different densities (1.134, 1.110 and 1.070 g/cm^3). After centrifugation, the distinct layer of islets was collected and washed. Islets free of acinar cells, vessels, lymph nodes, and ducts were used for culture and transplantation.

For studying β-cell replication, we used exendin-4, a GLP-1 receptor agonist, as a control. Total 120 islets with or without exendin-4 (Bachem Bioscience, King of Prussia, PA, USA), OEA (Cayman Chemical, Ann Arbor, MI, USA), PSN632408 (Cayman Chemical) were cultured in each 6 cm culture dish (Costar, Cambridge, MA) in 5 ml of RPMI1640 with 10% horse serum, 6 mM glutamine, 10 mM HEPES, and 1% antibiotic-antimycotic (Sigma, St. Louis, MO) at 37 °C in a humidified atmosphere of 5% CO_2 in air for 4 days, according to the previous published islet culture protocol for studying β-cell replication [23,24]. Culture medium with or without exendin-4, OEA and PSN632408 was changed daily. To label replicated cells, 5-bromo-2′-deoxyuridine (BrdU; Sigma-Aldrich, St Louis, MO, USA) was added into culture medium on day 3 to a level of 10 μM and left on the cultures overnight [20]. Islets were then harvested for double insulin and BrdU immunofluorescence staining. At least three separate experiments were performed for each treatment.

Islet transplantation and in vivo treatment

Islet transplantation was performed as described previously [21,22]. A total of 100 islets were transplanted into each recipient mouse. Briefly, the left kidney of recipient mice was exposed through a lumbar incision under sterile conditions. PE-50 polyethylene tubing (BectonDickinson, Parsippany, NJ, USA) containing 100 islets was inserted beneath the kidney capsule and gently pushed from the lower pole to the upper pole. The recipient mice were randomly divided into four groups and treatments started from the day of transplantation. Mice in group I were orally treated with vehicle for OEA (containing dimethyl sulfoxide) daily. Mice in group II were orally treated with OEA at 10 mg/kg/day. Mice in group III were orally treated with vehicle for PSN632408 (containing ethanol) daily. Mice in group IV were orally treated with PSN632408 at 10 mg/kg/day. All recipient mice were also intraperitoneally treated with BrdU at 100 mg/kg/day for 4 weeks. Daily nonfasting blood glucose of each recipient was measured to monitor islet graft function during the first week, then twice a week. After 4 weeks' treatment, nephrectomy was performed and all left kidneys bearing primary islet grafts were collected.

Immunofluorescence

Double immunofluorescence staining was performed for insulin and BrdU in cultured islets. After culture for 4 days, islets were washed in HBSS, fixed in 4% paraformaldehyde, and treated for 20 min with 0.5 N HCl. For BrdU labeling, islets were incubated with a mouse monoclonal anti-BrdU antibody (Caltag Laboratories, Inc., San Francisco, CA, USA), followed by Cy3-conjugated donkey anti-mouse immunoglobulin (IgG) (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) as a secondary antibody. For insulin labeling, islets were incubated with a guinea pig anti-swine insulin (Dako Cytomation, Carpinteria, CA, USA), and followed by FITC-conjugated goat anti-guinea pig IgG (Dako Cytomation) as a secondary antibody. To determine islet β-cell replication, the number of $BrdU^+$ (red nuclei) with $Insulin^+$ (green cytoplasm) per islet was counted by using Bio-Rad Lasersharp 1024 Confocal Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). $Insulin^+$ and $BrdU^+$ β cell per islet were calculated as the mean ± SD.

Double immunofluorescence staining for insulin and BrdU in islet grafts was performed on 10% formalin fixed and paraffin-embedded sections, as previously described [25,26]. For insulin labeling, sections were first deparaffinized, rehydrated, and incubated with guinea pig anti-swine insulin (Dako). Then sections were incubated in the dark with FITC-conjugated, goat anti-guinea pig immunoglobulin. For BrdU labeling, sections were steamed in Retrievagen A and were incubated with biotinylated anti-BrdU and then with Streptavidin-AlexaFluor-594. Nuclear staining was performed using TOPRO-3 (Molecular Probes, Eugene, OR, USA). $BrdU^+$ (red nuclei overlapping with TOPRO-3) and $BrdU^-$ (only blue TO-PRO-3 nuclei) nuclei in $insulin^+$ (green cytoplasm) β cells were counted using the confocal microscope. All images were taken with a plan apochromat 63× lens. $Insulin^+$ and $BrdU^+$ β cell ratios were calculated as the mean ± SD of both $insulin^+$ and $BrdU^+$ β cells over the total of $insulin^+$ β cells in islets.

Plasma GLP-1 active immunoassay

Young C57BL/6 mice were orally treated with vehicles or OEA at 10 mg/kg or PSN632408 at 10 mg/kg. At 30 min after treatment, blood samples were obtained by cardiac puncture. Plasma samples were stored at −80 °C until analyzed. Active GLP-1 in the plasma was measured by using mouse GLP-1 ELISA kit (Millipore Corporation, Billerica, MA) that is specific for the GLP-1[7–36] amide form, the majority of circulating biologically active GLP-1. The assay was performed according to the manufacturer's protocol.

Statistics

The significance of differences between the control and treatment groups was determined by the Kaplan-Meier analysis, one-way analysis of variance and the Student's *t*-test. A P-value <0.05 was considered statistically significant.

Results

OEA stimulated β cell replication in cultured mouse islets

After 4 days' culture, $insulin^+/BrdU^+$ β cells could be detected in untreated and OEA-treated islets by confocal microscopy. However, more $insulin^+$ $/BrdU^+$ β cells were found in OEA-treated islets (Fig. 1A). Islet cells showed both nuclear BrdU label (red) and cytoplasmic insulin (green) staining, thus confirming that replicated cells in the islets are truly β cells. As shown in one representative experiment (Fig. 1B), without treatment, the mean of $insulin^+/BrdU^+$ β cells was 9.8 ± 5.5 in each cultured islet. With 0.1 μM exendin-4, there were 21.2 ± 11.3 $insulin^+/BrdU^+$ β cells in each islet, which was significantly higher than the untreated control ($P < 0.01$, vs. untreated islets). OEA treatment was also associated with a significant increase in $insulin^+/BrdU^+$ β cells at each dose level. At 0.1 μM OEA, each islet contained 21.3 ± 14.1 $insulin^+$/

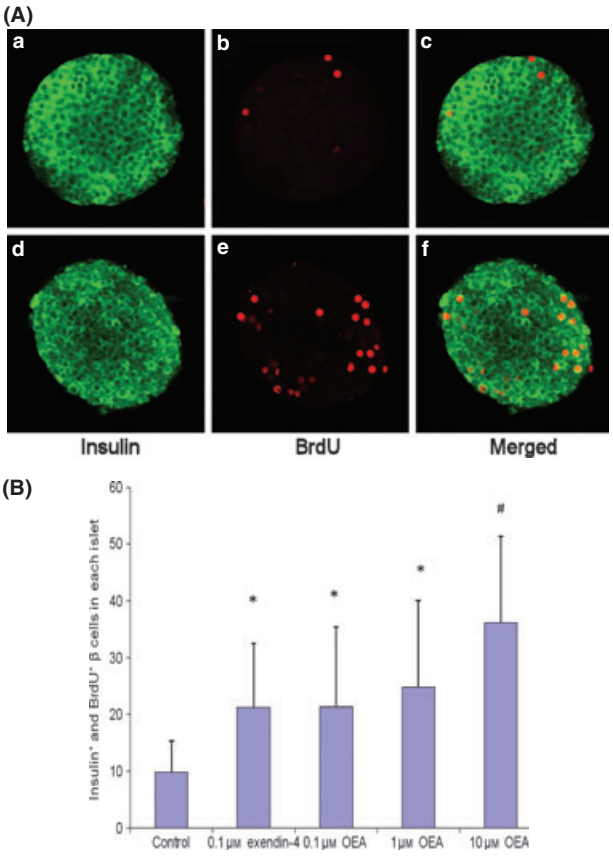

Figure 1 (A) Confocal microscopy of insulin and BrdU double immunofluorescence stained islets after 4 days culture with and without OEA. (a) untreated control islet stained with insulin (green); (b) untreated control islet stained with BrdU (red); (c) untreated control islet stained with insulin and BrdU (merged); (d) OEA-treated islet stained with insulin (green); (e) OEA-treated islet stained with BrdU (red); (f) OEA-treated islet stained with insulin and BrdU (merged). (B) Insulin$^+$ and BrdU$^+$ β cells in each cultured mouse islet treated with different dose of OEA in one representative experiment. Exendin-4 was used as a control. Data are shown as mean ± SD. The number of insulin$^+$/BrdU$^+$ β cells in exendin-4 or OEA treated islets was significantly higher than in untreated islets (*$P < 0.01$). The number of insulin$^+$/BrdU$^+$ β cells in islets treated with 10 μM OEA was significantly higher than in islets treated with 0.1 μM and 1.0 μM OEA (#$P < 0.01$).

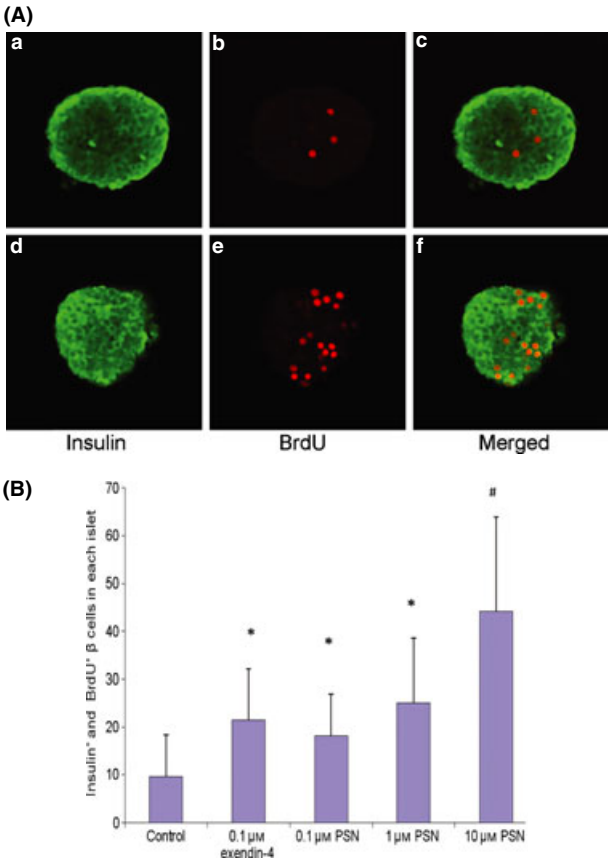

Figure 2 (A) Confocal microscopy of insulin and BrdU double immunofluorescence stained islets after 4 days' culture with and without PSN632408. (a) untreated control islet stained with insulin (green); (b) untreated control islet stained with BrdU (red); (c) untreated control islet stained with insulin and BrdU (merged); (d) PSN632408-treated islet stained with insulin (green); (e) PSN632408-treated islet stained with BrdU (red); (f) PSN632408-treated islet stained with insulin and BrdU (merged). (B) Insulin$^+$ and BrdU$^+$ β cells in each cultured mouse islet treated with different dose of PSN632408. Exendin-4 was used as a control. Data are shown as mean ± SD. The number of insulin$^+$/BrdU$^+$ β cells in exendin-4 or PSN632408-treated islets was significantly higher than in untreated islets (*$P < 0.01$). The number of insulin$^+$/BrdU$^+$ β cells in islets treated with 10 μM PSN632408 was significantly higher than in islets treated with 0.1 μM and 1.0 μM PSN632408 (#$P < 0.01$).

BrdU$^+$ β cells; at 1.0 μM OEA, each islets contained 24.9 ± 15.2 insulin$^+$/BrdU$^+$ β cells; and at 10 μM OEA, each islets contained 36.1 ± 15.2 insulin$^+$/BrdU$^+$ β cells ($P < 0.01$ vs 0.1 μM and 1 μM OEA-treated islets). Similar results were also observed in two additional separate experiments. Fold increase of insulin$^+$/BrdU$^+$ β cells in each islet in all three separate experiments was 1.8 ± 1.0 in 0.1 μM exendin-4 treated islets ($P < 0.01$, vs. untreated islets), 1.8 ± 1.2 in 0.1 μM OEA-treated islets, 2.2 ± 1.3 in 1 μM OEA-treated islets, and 3.3 ± 1.7 in 10 μM OEA-treated islets ($P < 0.01$ vs. 0.1 μM and 1 μM OEA-treated islets). Thus, OEA stimulated β-cell replication in cultured islets in a dose-dependent manner.

PSN632408 stimulated β cell replication in cultured mouse islets

Although insulin$^+$/BrdU$^+$ β cells were detected in untreated islets, more insulin$^+$/BrdU$^+$ β cells were found in PSN632408-treated islets (Fig. 2A). As shown in one representative experiment (Fig. 2B), a significant increase in

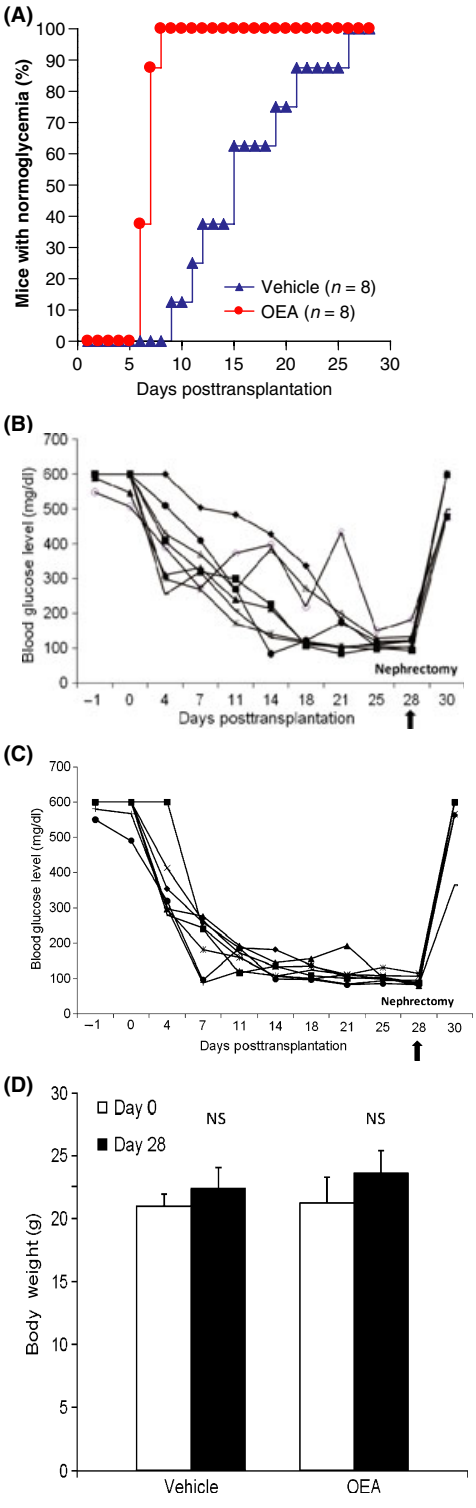

Figure 3 (A) Percentage of diabetic C57BL/6 mice achieved normoglycemia after receiving 100 C57BL/6 islets with or without OEA treatment. At 2 weeks post-transplantation, all OEA-treated recipient mice achieved normoglycemia, although only 38% of vehicle-treated recipient mice achieved normoglycemia. (B) Blood glucose levels in diabetic C57BL/6 mice that received 100 C57BL/6 islets and vehicle treatment. Each line represents a single recipient mouse. (C) Blood glucose levels in diabetic C57BL/6 mice that received 100 C57BL/6 islets and OEA treatment. Each line represents a single recipient mouse. (D) Body weight of C57BL/6 mice that received 100 islets and with or without OEA treatment, before and 4 weeks after transplantation. Data are presented as the mean ± SD. Both vehicle-treated and OEA-treated mice showed an increase in body weight at 4 weeks post-transplantation, but this change was not significant (NS: $P > 0.05$).

insulin$^+$/BrdU$^+$ β cells was found in both exendin-4 and PSN632408-treated islets, compared with untreated islets. Without treatment, each islet contained 9.6 ± 8.8 insulin$^+$/BrdU$^+$ β cells. With 0.1 μM exendin-4, there were 21.5 ± 10.6 insulin$^+$/BrdU$^+$ β cells in each islet ($P < 0.01$, vs. untreated islets). As with OEA treatment, treatment with PSN632408 was associated with a significant increase in insulin$^+$/BrdU$^+$ β cells at each dose level. At 0.1 μM PSN632408, islets contained 18.1 ± 8.8 insulin$^+$/BrdU$^+$ β cells ($P < 0.01$ vs. untreated islets); at 1.0 μM PSN632408, islets contained 25.1 ± 13.5 insulin$^+$/BrdU$^+$ β cells ($P < 0.01$ vs. untreated islets); and at 10 μM PSN632408, islets contained 44.2 ± 19.7 insulin$^+$/BrdU$^+$ β cells ($P < 0.01$ vs. 0.1 μM or 1 μM PSN632408-treated islets). Similar results were also observed in three additional experiments. The fold increase of insulin$^+$/BrdU$^+$ β cells in each islet in all four separate experiments was 2.0 ± 0.3 in 0.1 μM exendin-4 treated islets ($P < 0.01$, vs. untreated islets), 1.4 ± 0.5 in 0.1 μM PSN632408-treated islets, 2.3 ± 0.4 in 1 μM PSN632408-treated islets ($P < 0.01$, vs. untreated islets), and 3.7 ± 0.8 in 10 μM PSN632408-treated islets ($P < 0.01$, vs. 0.1 μM PSN632408-treated islets; $P < 0.05$, vs. 1 μM PSN632408-treated islets). Thus, our data indicated that PSN632408 stimulates β-cell replication in cultured islets in a dose-dependent manner.

OEA mediates satiety by activation of peroxisome proliferator-activated receptor-α (PPAR-α) [27]. PPAR-α is also expressed on β cells [28]. To rule out the possibility that the effect of OEA on β-cell replication may also be mediated by activating PPAR-α, we isolated islets from PPAR-α knockout *Ppara*^{tm1Gonz}/J mice and directly compared the effect of OEA and PSN632408 on β-cell replication in these cultured islets. Without treatment, the mean of insulin$^+$/BrdU$^+$ β cells was 6.8 ± 4.7 in each islet. With treatment, the mean insulin$^+$/BrdU$^+$ β cells was 15.7 ± 8.3 in each islet treated with 10 μM OEA ($P < 0.01$, vs. untreated islets) and 16.8 ± 10.5 in each islet treated with PSN632408 ($P < 0.01$, vs. untreated islets). Similar results were also observed in two additional experiments. Thus, our data indicated that OEA is as effective as PSN632408 on stimulating β-cell replication in cultured islets without activating PPAR-α.

OEA improved mouse islet graft function in diabetic mice

At 2 weeks post-transplantation, all OEA-treated recipient mice achieved normoglycemia, although only 38% of vehicle-treated recipient mice achieved normoglycemia (Fig. 3 A). At 4 weeks post-transplantation, normoglycemia was achieved in all C57BL/6 diabetic mice with or without OEA treatment. However, normoglycemia was achieved in OEA-treated mice significantly earlier than in vehicle-treated mice. The vehicle-treated mice achieved normoglycemia in 16 ± 5 days ($n = 8$), whereas OEA-treated mice required only 7 ± 1 days ($n = 8$, $P < 0.01$). Although blood glucose levels were reduced in vehicle-treated and OEA-treated mice after transplantation, OEA-treated mice after returned to normoglycemia more quickly than vehicle-treated mice (Fig. 3B and C). To confirm islet graft function, we removed the left kidney bearing islet grafts in recipient mice, which had islet graft function at 4 weeks post-transplantation. The return of hyperglycemia in these mice after left nephrectomy confirmed islet graft function. Thus, OEA improved mouse islet graft function in diabetic mice.

Body weight in recipient mice was measured at the day of transplantation and at the beginning of treatment. Both vehicle-treated and OEA-treated mice showed an increase in body weight at 4 weeks post-transplantation, but this change was not significant (Fig. 3D, $P > 0.05$). At the day of pretransplantation, the mean body weight was 21.0 ± 0.9 g in vehicle-treated mice and 21.3 ± 2.1 g in OEA-treated mice. At 4 weeks post-transplantation, the mean percentage of body weight was 22.3 ± 1.7 g in vehicle-treated mice and 23.6 ± 1.8 g in OEA-treated mice. Thus, OEA at 10 mg/kg/day did not reduce the body weight in recipient mice.

PSN632408 improved mouse islet graft function in diabetic mice

At 2 weeks post-transplantation, 88% PSN632408-treated recipient mice achieved normoglycemia although none of vehicle-treated recipient mice achieved normoglycemia (Fig. 4A). As we observed in OEA-treated mice, normoglycemia was achieved in all C57BL/6 diabetic mice with or without PSN632408 treatment at 4 weeks post-transplantation. However, normoglycemia was achieved in PSN632408-treated mice significantly earlier than in vehicle treated mice. Vehicle-treated mice achieved normoglycemia in 19 ± 7 days ($n = 8$), while PSN632408-treated mice required only 8 ± 5 days ($n = 8$, $P < 0.05$). Nonfasting blood glucose levels were significantly reduced in PSN632408-treated mice after transplantation (Fig. 4B). The mean nonfasting blood glucose level was 579 ± 59 mg/dl in vehicle-treated mice and 546 ± 47 mg/

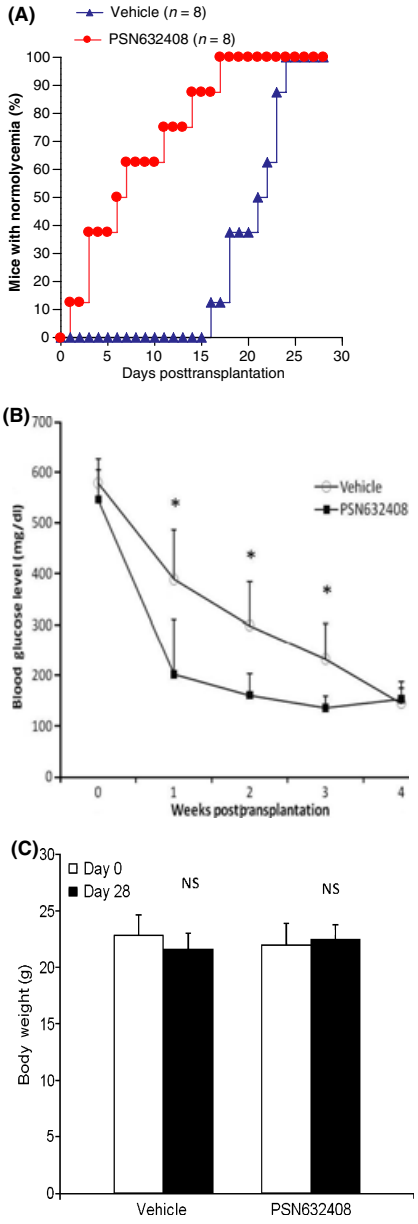

Figure 4 (A) Percentage of mice with normoglycemia in diabetic C57BL/6 mice that received 100 C57BL/6 islets with or without PSN632408 treatment. At 2 weeks post-transplantation, 88% PSN632408-treated recipient mice achieved normoglycemia, although none of the vehicle-treated recipient mice achieved normoglycemia. (B) Blood glucose levels in diabetic C57BL/6 mice that received 100 C57BL/6 islets with or without PSN632408 treatment. The mean nonfasting blood glucose level in PSN632408-treated mice was significantly lower than vehicle-treated mice at 1 week, 2 weeks, and 3 weeks post-transplantation (*$P < 0.01$). (C) Body weight of C57BL/6 mice that received 100 islets and with or without PSN632408 treatment, before and 4 weeks after transplantation. Data are presented as the mean ± SD. There was no significant change in body weight between vehicle-treated and PSN632408-treated mice and showed an increase in body weight at 4 weeks post-transplantation, but this change was not significant (NS: $P > 0.05$).

dl in PSN632408-treated mice before transplantation. The mean nonfasting blood glucose level was 389 ± 97 mg/dl in vehicle-treated mice and 202 ± 108 mg/dl in PSN632408-treated mice at 1 week post-transplantation ($P < 0.01$); 299 ± 86 mg/dl in vehicle-treated mice and 161 ± 42 mg/dl in PSN632408-treated mice at 2 week post-transplantation ($P < 0.01$); 232 ± 70 mg/dl in vehicle-treated mice and 136 ± 23 mg/dl in PSN632408-treated mice at 3 weeks post-transplantation ($P < 0.01$). Thus, PSN632408 improved mouse islet graft function in diabetic mice.

Vehicle-treated mice showed a slight decrease in body weight and PSN632408-treated mice showed a slight increase in body weight at 4 weeks post-transplantation (Fig. 4C, $P > 0.05$). At the day of pretransplantation, the mean body weight was 22.9 ± 1.8 g in vehicle-treated mice and 22.0 ± 1.9 g in PSN632408-treated mice. At 4 weeks post-transplantation, the mean percentage of body weight was 21.6 ± 1.4 g in vehicle-treated mice and 22.5 ± 1.9 g in PSN632408-treated mice. Thus, PSN632408 at 10 mg/kg/day did not reduce the body weight in recipient mice.

OEA stimulated β cell replication in mouse islet grafts

Insulin$^+$/BrdU$^+$ β cells were observed in islet grafts from both vehicle-treated mice and OEA-treated mice. However, more insulin$^+$/BrdU$^+$ β cells were found in islet grafts from OEA-treated mice (Fig. 5A). Rates of β-cell replication were significantly higher in islet grafts from OEA-treated mice than in islet grafts from vehicle-treated mice (Fig. 5B). At 4 weeks post-transplantation, the mean percentage of insulin$^+$/BrdU$^+$ β cells was 5.9 ± 3.7% in islet grafts from vehicle-treated mice ($n = 4$) and 17.2 ± 3.8% in islet grafts from OEA-treated mice ($n = 4$, $P < 0.01$). Thus, β cells in islet grafts from mice over

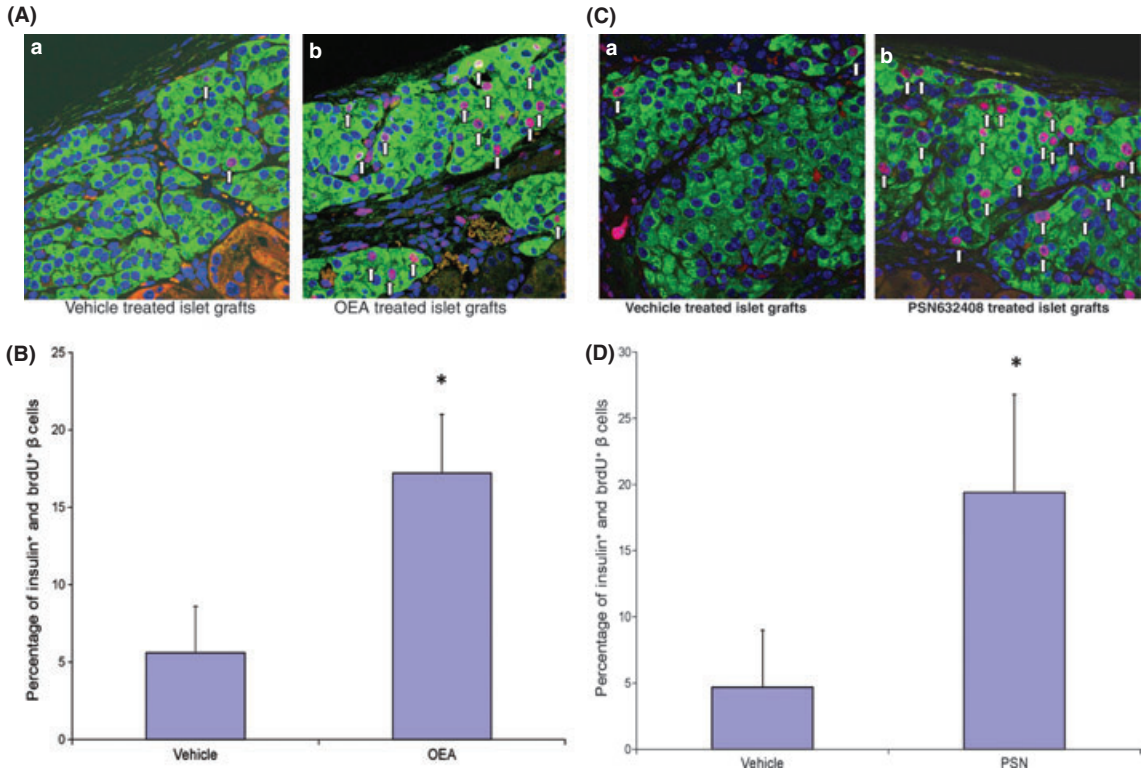

Figure 5 (A) Double immunofluorescent staining for insulin (green color) and BrdU (red color) in islet grafts in diabetic mice treated with vehicle (a); and in diabetic mice treated with OEA (b). Insulin$^+$ and BrdU$^+$ β cells (arrowheads) in the islet grafts can be seen. (B) Percentage of insulin$^+$ and BrdU$^+$ β cells in total insulin$^+$ β cells in islet grafts from vehicle-treated and OEA-treated mice at 4 weeks post-transplantation. Data are presented as the mean ± SD. The percentage of insulin$^+$/BrdU$^+$ β cells in islet grafts from OEA-treated mice was significantly higher than in islet grafts from vehicle-treated mice (*$P < 0.01$). (C) Double immunofluorescent staining for insulin and BrdU in islet grafts in diabetic mice treated with vehicle (a); and in diabetic mice treated with PSN632408 (b). Insulin$^+$ and BrdU$^+$ β cells (arrowheads) in the islet grafts can be seen. (D) Percentage of insulin$^+$ and BrdU$^+$ β cells in total insulin$^+$ β cells in islet grafts from vehicle-treated and PSN632408-treated mice at 4 weeks post-transplantation. Data are presented as the mean ± SD. The percentage of insulin$^+$/BrdU$^+$ β cells in islet grafts from PSN632408-treated mice was significantly higher than in islet grafts from vehicle-treated mice (*$P < 0.01$).

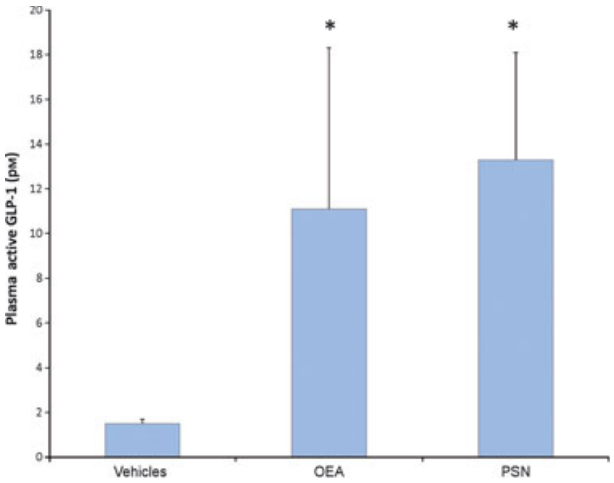

Figure 6 Active GLP-1 levels in plasma of C57BL/6 mice at 30 min after treatment with vehicles, OEA, and PSN632408. Data are presented as the mean ± SD. The plasma GLP-1 levels in OEA-treated mice and in PSN632408-treated mice were significantly higher than in vehicle-treated mice (*$P < 0.01$).

40 weeks-of-age were able to replicate and, furthermore, OEA treatment stimulated additional β-cell replication in these islet grafts from older mice.

PSN632408 stimulated β cell replication in mouse islet grafts

Similarly, more insulin$^+$/BrdU$^+$ β cells were present in islet grafts from PSN632408-treated mice compared with vehicle-treated mice (Fig. 5C). The percentage of replicated β cells was significantly higher in islet grafts from PSN632408-treated mice than in islet grafts from vehicle-treated mice (Fig. 5D). At 4 weeks post-transplantation, the mean percentage of insulin$^+$/BrdU$^+$ β cells was 4.7 ± 4.3% in islet grafts from vehicle-treated mice ($n = 8$) and 19.4 ± 7.4% in islet grafts from PSN632408-treated mice ($n = 7$, $P < 0.01$). We also studied whether there are insulin$^+$/BrdU$^+$ β cells in native pancreases. Few insulin$^+$/BrdU$^+$ β cells were found native islets and none was found in pancreatic ducts (data not shown). Thus, our data indicated that PSN632408 treatment also stimulated β-cell replication in islet grafts.

OEA and PSN632408 treatment increased plasma active GLP-1 levels in mice

We measured the plasma active GLP-1 concentrations in C57BL/6 mice 30 min after OEA or PSN632408 treatment. The plasma active GLP-1 levels were significantly increased at 30 min compared with the control mice (Fig. 6). At 30 min after treatment, the plasma GLP-1 level was 1.5 ± 0.2 pM in both vehicle-treated mice ($n = 6$), 11.1 ± 7.2 pM in OEA-treated mice ($n = 6$, $P < 0.01$), and 13.3 ± 4.8 pM in PSN632408-treated mice ($n = 6$, $P < 0.01$). Thus, OEA and PSN632408 treatment increased the plasma active GLP-1 levels.

Discussion

In this study, we found that both OEA and PSN632408 can stimulate β-cell replication in cultured mouse islets. We also used islets from old donors for islet culture and transplantation as we found that exendin-4 can stimulate β-cell replication in mouse islet grafts from both young and old donors [29]. Although aging correlates with a decreased capacity for β-cell replication [30–32], our data showed that OEA and PSN632408 could stimulate β-cell replication in cultured islets isolated from old donors. In single-donor islet transplantation, human islets have been cultured for 2 days before transplantation [3,4]. Therefore, simulating β-cell replication to increase β-cell mass in cultured islets by GPR119 agonists is an attractive approach and could be beneficial to islet transplantation.

OEA is an endogenous lipid produced in the intestine that reduces food intake, promotes lipolysis, and decreases body weight gain in rodents [33]. GPR119 is essential for OEA-induced GLP-1 secretion from intestinal enterodenocrine L-cells in vitro and in vivo [14]. Although OEA can activate PPAR-α [27], which is also expressed on β cells [28], we ruled out the possibility that the effect of OEA on β-cell replication is mediated by activating PPAR-α. We found that OEA is as effective as PSN632408 on stimulating β-cell replication in cultured islets isolated from PPAR-α knockout mice. PSN632408 is a selective GPR119 agonist and does not activate PPAR-α, PPAR-γ, cannabinoid receptor 1, cannabinoid receptor 2 and other feeding-related receptors [11]. Therefore, OEA or PSN632408-stimulated β-cell replication in culture is mediated by GPR119 activation.

We found that OEA or PSN632408 treatment significantly enhanced the reversal of diabetes in mice that received a marginally therapeutic dose of islets. Reversal of diabetes depended on the function of the islet grafts, as hyperglycemia reoccurred after removal of the left kidney bearing islet grafts. Furthermore, we found that OEA or PSN632408 treatment significantly increased β-cell replication in islet grafts. The effect of OEA or PSN632408 is probably due to activation of GPR119 on islets and/or activation of GPR119 on intestinal enteroendocine L-cells. GPR119 agonist directly stimulates insulin secretion from β cells *in vitro*, improves glucose tolerance, and enhances glucose-dependent insulin release *in vivo* [9]. Our in vitro studies showed that OEA and PSN632408 can directly stimulate β-cell replication in cultured islets. These data

suggest that OEA and PSN632408 may improve islet graft function and stimulate β-cell replication in islet grafts by direct activation of GPR119 on islets. OEA can stimulate GLP-1 secretion from intestinal enteroendocine L-cells through activation of GPR119 *in vitro* and *in vivo,* and we found that OEA and PSN632408 treatment increased the plasma GLP-1 level in mice. Therefore, it is possible that OEA and PSN632408-stimulated β-cell replication in islet grafts is also due to increased plasma GLP-1 concentrations. Whether the effect of OEA or PSN632408 on improving islet graft function and stimulating β-cell replication in islet grafts is mediated by directly activating GPR119 on islets and/or activating GPR119 on intestinal enteroendocine L-cells can only be addressed by using GLP-1 receptor knockout mice and GPR119 knockout mice. Nevertheless, our data indicate that using GPR119 agonists to improve islet graft function and to stimulate β-cell replication is a novel therapeutic approach that can potentially be used for human islet transplantation. As human patients who still have some β-cell mass remaining at the onset of type 1 diabetes, GPR119 agonists may also be used to restore normoglycemia by stimulating β-cell replication and increasing β-cell mass, if autoimmunity was halted in these patients.

β-cell neogenesis from precursors in the ductal epithelium of the pancreas has been demonstrated in several diabetic models [34,35]. Exenin-4 has been shown to stimulate not only β-cell replication but also β-cell neogenesis [36]. However, we found few insulin$^+$/BrdU$^+$ β cells in islets and no insulin$^+$/BrdU$^+$ β cells in ducts of native pancreases in these recipient mice. Given the paucity of β cells in the pancreas following STZ treatment, it is not surprising that few β cells could be found in these recipient mice. At the time of transplantation and GPR119 agonist treatment, all recipient mice were more than 10 weeks old. Our data indicate that GPR119 agonists cannot stimulate β-cell neogenesis from ductal cells in mice greater than 10 weeks old. However, we cannot rule out the possibility that GPR119 agonists can stimulate β-cell neogenesis from ductal cells in younger mice. Further studies are needed to determine the effect of GPR119 agonists on β-cell neogenesis in younger mice.

We found that only some but not all β cells in islets replicate in response to GRP119 agonist treatment. It has been shown that pre-existing β-cells are the major source of new β cells during adult life in mice [37] and that all β cells contribute equally to islet growth and maintenance [38]. Further studies are needed to explore whether these replicated β cells are from progenitor cells and to identify specific pathways that govern β-cell replication in islets treated with GPR119 agonists.

It has been shown that OEA at 30 mg/kg and PSN632408 at 100 mg/kg could significantly reduce food intake in normal rats. Administration of PSN632408 daily at the dose of 100 mg/kg also significantly reduced the body weight in diet-induced obese rats [11]. The doses of OEA and PSN632408 used in that study were much higher than the doses used in the present study. No loss of body weight was found in the present study in mice treated for 4 weeks with OEA (10 mg/kg/day) or PSN632408 (10 mg/kg/day), compared with vehicle-treated control mice. In fact, the mean body weight in both the OEA-treated recipient mice and PSN632408-treated recipient mice increased slightly, although this was not statistically significant. The increased body weight in the treated mice was probably due to the rapid reversal of diabetes after islet transplantation, which prevented the loss of body weight often seen in uncontrolled diabetes.

GLP-1 has an extremely short half-life after secretion because of rapid degradation by dipeptidyl peptidase IV (DPP-IV). Therefore, inhibition of DPP-IV activity could potentially improve the therapeutic effectiveness of GLP-1 [3]. Although either a selective GPR119 agonist or a DPP-IV inhibitor could enhance plasma GLP-1 levels and improve oral glucose tolerance, combing GPR119 agonist with DPP-IV inhibitor is significantly better than either one alone [10]. Our earlier studies have shown that a DPP-IV inhibitor can stimulate β-cell replication in nonobese diabetic mice [26]. Therefore, combing GPR119 agonist with DPP-IV inhibitor may be a more effective therapeutic approach to stimulate β-cell replication and improve islet graft function.

In summary, our data demonstrate that GPR119 agonists can stimulate β-cell replication in islets *in vitro* and *in vivo* and can improve islet graft function. Targeting GPR119 is a novel therapeutic approach to increase β-cell replication and to improve islet graft function. Based on these findings, further investigation of the effects of GPR119 agonists in human islets is now warranted to determine the applicability of this mode of therapy to human clinical trials.

Authorship

JG and LT: contributed equally to this study and share the first authorship. ZG, JL, and RLS: participated in research design. JG, LT, GW, and NVB: participated in the performance of the research. JG, LT, ZG, and TDO'B: participated in data analysis, JG, ZG, and TDO'B: participated in the writing of the paper.

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