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RESEARCH PAPER

A novel insulinotropic mechanism of whole grain-derived γ-oryzanol via the suppression of local dopamine D_2 receptor signalling in mouse islet

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BACKGROUND AND PURPOSE

γ-Oryzanol, derived from unrefined rice, attenuated the preference for dietary fat in mice, by decreasing hypothalamic endoplasmic reticulum stress. However, no peripheral mechanisms, whereby γ-oryzanol could ameliorate glucose dyshomeostasis were explored. Dopamine D_2 receptor signalling locally attenuates insulin secretion in pancreatic islets, presumably via decreased levels of intracellular cAMP. We therefore hypothesized that γ-oryzanol would improve high-fat diet (HFD)-induced dysfunction of islets through the suppression of local D_2 receptor signalling.

EXPERIMENTAL APPROACH

Glucose metabolism and regulation of molecules involved in D_2 receptor signalling in pancreatic islets were investigated in male C57BL/6J mice, fed HFD and treated with γ-oryzanol . In isolated murine islets and the beta cell line, MIN6 , the effects of γ-oryzanol on glucose-stimulated insulin secretion (GSIS) was analysed using siRNA for D_2 receptors and a variety of compounds which alter D_2 receptor signalling.

KEY RESULTS

In islets, γ-oryzanol enhanced GSIS via the activation of the cAMP/PKA pathway. Expression of molecules involved in D_2 receptor signalling was increased in islets from HFD-fed mice, which were reciprocally decreased by γ-oryzanol. Experiments with siRNA for D_2 receptors and D_2 receptor ligands *in vitro* suggest that γ-oryzanol suppressed D_2 receptor signalling and augmented GSIS.

CONCLUSIONS AND IMPLICATIONS

γ-Oryzanol exhibited unique anti-diabetic properties. The unexpected effects of γ-oryzanol on D_2 receptor signalling in islets may provide a novel; natural food-based, approach to anti-diabetic therapy.

Abbreviations

$[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; CCK-8, cholecystokinin-octapeptide; DAT, dopamine transporter; GLP-1, glucagon-like peptide 1; GSIS, glucose-stimulated insulin secretion; GTT, glucose tolerance test; HFD, high-fat diet; IHC, immunohistochemical; siRNA, small interfering RNA; TH, L-tyrosine hydroxylase; VMAT2, vesicular monoamine transporter 2

Tables of Links

TARGETS
GPCRs[a]
Dopamine D_2 receptor
GPR119
GPR120
Transporters[b]
DAT, dopamine transporter
VMAT2, vesicular monoamine transporter 2
Enzymes[c]
PKA
TH, tyrosine hydroxylase

LIGANDS	
cAMP	Haloperidol
CCK-8, cholecystokinin-octapeptide	Insulin
L-DOPA	Oleoylethanolamide
GLP-1, glucagon-like peptide 1	Palmitic acid
Glucagon	Quinpirole
GW 9508	Somatostatin
H-89	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c]Alexander *et al.*, 2013a,b,c).

Introduction

Dopamine is a major catecholamine neurotransmitter that controls a wide range of biological processes important in neurological, cardiovascular and metabolic homeostasis. Previous reports have demonstrated that in patients with Parkinson's disease, glucose metabolism was markedly impaired by treatment with L-DOPA, a dopamine precursor, in a dose-dependent manner (Sirtori *et al.*, 1972; Marsden and Parkes, 1977). Importantly, molecules involved in dopamine receptor signalling are expressed in both murine and human pancreatic islets (Rubi *et al.*, 2005; Simpson *et al.*, 2012). Notably, a recent study on isolated pancreatic islets from humans demonstrated that pancreatic islet-derived dopamine did attenuate insulin secretion in an autocrine or paracrine fashion via its receptors (Simpson *et al.*, 2012). In particular, studies in dopamine D_2 receptor knockout mice suggest a critical role of dopaminergic suppression in function and replication of pancreatic beta cells during development in mice (Garcia-Tornadu *et al.*, 2010).

It is well recognized that two distinct signalling pathways contribute to the control of insulin secretion from pancreatic beta cells, namely the ATP-sensitive K^+ channel-dependent pathway (triggering pathway) and the cAMP/PKA pathway (amplifying pathway) (Henquin, 2000; Kahn *et al.*, 2006). Two major incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide, are crucial regulators for glucose-stimulated insulin secretion (GSIS) through an increase in intracellular cAMP level, thereby activating the cAMP/PKA pathway. On the other hand, dopamine is known to substantially decrease intracellular cAMP level mainly via D_2 receptors in striatum and pituitary gland in the brain in rats, pigs and humans (Missale *et al.*, 1998; Vallone *et al.*, 2000).

Based on the notion that chronic feeding with a high fat diet (HFD) causes dysfunction of pancreatic islets and results in whole body glucose dysmetabolism (Giacca *et al.*, 2011), we hypothesized that dopamine receptor signalling would be activated locally in pancreatic islets from HFD-fed mice, thereby causing dyshomeostasis of islet functions, at least partly, through a decrease in intracellular cAMP level. On the other hand, it has been shown that expression of genes involved in D_2 receptor signalling in the brain reward system (e.g. striatum, ventral tegmental area) was considerably

decreased in HFD-induced obese rodents, resulting in profound addiction to fatty foods (Li *et al.*, 2009; Johnson and Kenny, 2010). This finding suggested that decreased local synthesis of dopamine in the brain could be relevant to this deviation in feeding behaviour.

γ-Oryzanol, derived from unrefined rice, is a unique bioactive substance, consisiting of a mixture of ferulic acid esters with phytosterols or triterpene alcohols (Lerma-Garcia *et al.*, 2009; Kozuka *et al.*, 2013). An earlier study in humans demonstrated that replacement of white rice by brown rice reduced the incidence of type 2 diabetes mellitus (Sun *et al.*, 2010). Based on this report and our interventional trial assessing the metabolically beneficial impact of brown rice on pre-diabetic obese humans (Sun *et al.*, 2010; Shimabukuro *et al.*, 2014), we recently reported in mouse experiments that γ-oryzanol acted directly on the hypothalamus and attenuated preference for dietary fat by decreasing hypothalamic endoplasmic reticulum (ER) stress, thereby ameliorating HFD-induced obesity (Kozuka *et al.*, 2012). We also demonstrated that long-term administration of γ-oryzanol considerably ameliorated HFD-induced glucose dyshomeostasis, independently of body weight and food intake (Kozuka *et al.*, 2012). Moreover, although γ-oryzanol (3.2 $mg \cdot g^{-1}$ body weight) given orally to mice was distributed predominantly to the brain (83.8 mg per 100 g tissue); it also accumulated particularly in the pancreas (3.5 mg per 100 g tissue) 1 h after supplementation (Kozuka *et al.*, 2015). However, the full mechanism whereby γ-oryzanol ameliorates glucose dysmetabolism throughout the body remained to be elucidated.

In rats, γ-oryzanol increased the dopamine content of the medial basal hypothalamus (Ieiri *et al.*, 1982). This effect was suppressed by an inhibitor of L-tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis (Ieiri *et al.*, 1982), suggesting a potential interaction of γ-oryzanol between dopamine metabolism and signalling via dopamine receptors. Based on all these findings, we tested if γ-oryzanol would improve dysfunction of pancreatic islets through the inhibition of D_2 receptor signalling in murine experimental models.

Methods

Animals

All animal care and experimental procedures were approved by the Animal Experiment Ethics Committee of the University of the Ryukyus (Nos. 5352, 5718 and 5943). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 204 animals were used in the experiments described here.

Eight-week-old male C57BL/6J mice obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) were housed at 24°C under a 12 h/12 h light/dark cycle. The mice were allowed free access to food and water.

Administration of γ-oryzanol

γ-Oryzanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in 0.5% methyl cellulose solution. γ-Oryzanol (20, 80 or 320 $\mu g \cdot g^{-1}$ body weight) was delivered into the stomach by a gavage needle every day during feeding with a HFD (Western Diet; Research Diets Inc., New Brunswick, NJ, USA) for 13 weeks. HFD and HFD containing 0.4% γ-oryzanol were manufactured as pellets by Research Diets (Research Diets Inc.). Daily intake of γ-oryzanol by mice, as estimated by mean food intake, was approximately 320 $\mu g \cdot g^{-1}$ body weight. The doses of γ-oryzanol were determined as described (Kozuka *et al.*, 2012).

Metabolic parameters

Whole blood was taken from the tail vein and blood glucose was measured using an automatic glucometer (Medisafe Mini; Terumo, Tokyo, Japan). Occasional blood samples were taken from the retro-orbital venous plexuses or tail vein. Plasma insulin, glucagon and active GLP-1 levels were measured using ELISA kits (Shibayagi Co. Ltd., Gunma, Japan; Wako Pure Chemical Industries, Ltd.; and Morinaga Institute of Biological Science, Inc., Tokyo, Japan). For glucose tolerance tests (GTTs), mice were intraperitoneally injected with 2.0 $g \cdot kg^{-1}$ glucose after an 18 h fast. Blood glucose levels were measured at the indicated times.

Sub-diaphragmatic vagotomy

Sub-diaphragmatic vagotomy, or sham surgery, was performed as described earlier (Miyamoto *et al.*, 2012) and mice were used for experiments 2 weeks after the surgery. To test the success of the vagotomy, we assessed the satiety induced by CCK-8 (Bachem, Bubendorf, Switzerland), which is mediated by the abdominal vagus nerves (Smith *et al.*, 1981; 1985). Sham-treated and vagotomized mice were injected i.p. with PBS or 8 $\mu g \cdot kg^{-1}$ CCK-8 after an 18 h fast.

Immunohistochemical (IHC) analyses

The pancreas was carefully dissected and fixed in 4% paraformaldehyde, embedded in paraffin and sectioned. The paraffin-embedded sections were stained with haematoxylin and eosin or immunostained for insulin (A0654; Dako Japan, Tokyo, Japan), glucagon (A0565; Dako Japan), somatostatin (AB5495; Merck Millipore, Billerica, MA), dopamine transporter (DAT) (AB1591P; Merck Millipore) and TH (AB152; Merck Millipore). The mean size and ratio of glucagon-positive α-cells, DAT-positive and TH-positive cell areas to the total islet area were calculated based on >100 islets per group using Photoshop (Adobe, San Jose, CA, USA).

Isolation of pancreatic islets and assessment of insulin/glucagon secretion

Pancreatic islets were isolated from mice by collagenase digestion (Liberase TL; Roche Diagnostics GmbH, Mannheim, Germany) and purified on a Histopaque gradient (Histopaque 1077; Sigma-Aldrich, St Louis, MO, USA) as described by Zmuda *et al.*, (2011). Insulin secretion from isolated islets and from a murine pancreatic beta cell line, MIN6 cells, (Miyazaki *et al.*, 1990), was measured as described earlier (Wei *et al.*, 2005). Briefly, the islets were incubated with or without γ-oryzanol (0.2, 2 or 20 $\mu g \cdot mL^{-1}$), forskolin (10 mM), Rp-8-Br-cAMPS (10 μM), H-89 (10 μM), haloperidol (1, 10 μM; Wako Pure Chemical Industries, Ltd.), a D_2 receptor antagonist, 10 μM L-DOPA, a dopamine precursor, or 5 μM quinpirole, a potent D_2 receptor agonist (Sigma-Aldrich), for 1 h, and

stimulated with glucose for an additional 1 h with or without γ-oryzanol, haloperidol, L-DOPA or quinpirole. The doses of each compound were decided as described (Simpson *et al.*, 2012). MIN6 cells and an α-cell line (α-TC cells) were seeded at a density of 2.0×10^5 cells·mL^{-1} on 24-well plates. After 48 h of culture, MIN6 cells were incubated with Krebs–Ringer bicarbonate buffer (KRB; composition; 119 mM NaCl, 4.74 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgCl_2$, 1.19 mM KH_2PO_4, 25 mM $NaHCO_3$, 0.5 % BSA, 25 mM HEPES, pH 7.4.) containing 2.5 mM glucose for 2 h, subsequently incubated in KRB with or without γ-oryzanol (0.2, 2 or 10 μg·mL^{-1}) for 1 h. The cells were also incubated with a series of insulin secretagogues with or without γ-oryzanol for 2 h. α-TC cells were incubated with KRB containing 16.7 mM glucose for 1 h, subsequently incubated with or without palmitic acid (0.25 or 0.5 mM; Sigma-Aldrich), γ-oryzanol (2 or 10 μg·mL^{-1}) or haloperidol (10 μM) for 2 h. Insulin or glucagon secretion was normalized by cellular protein content. Levels of cAMP and PKA activity were determined by the cyclic AMP EIA Kit (Cayman Chemical, Ann Arbor, MI, USA) and PKA kinase activity kit (Enzo Life Sciences, Farmingdale, NY, USA) respectively. To measure insulin content of islets, 10 islets were placed in 1 mL of acid-ethanol (90 mM HCl in 70% ethanol). Insulin was extracted overnight at –20°C after sonication, as previously described (Ariyama *et al.*, 2008). The acid-ethanol extract was neutralized with 1 M Tris (pH 7.5) and insulin levels were measured using an ELISA kit.

Measurement of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in isolated islets

$[Ca^{2+}]_i$ in isolated islets was measured by fura-2 microfluorometry as described (Nakata *et al.*, 2010). Briefly, islets on coverslips were incubated with 1 μM fura-2/acetoxymethylester (Dojin Chemical Co., Kumamoto, Japan) for 1 h at 37°C in KRB containing 2.8 mM glucose with or without γ-oryzanol or haloperidol. Islets were subsequently mounted in a chamber and superfused at a rate of 1 mL·min^{-1} at 37°C in KRB with or without γ-oryzanol or haloperidol. Fluorescence following excitation at 340 nm (F340) and that at 380 nm (F380) was measured, and $[Ca^{2+}]_i$ was expressed by the ratio (F340/F380).

RNA interference

The small interfering RNA (siRNA) for D_2 receptors (the *Drd2* gene) and a control scrambled siRNA were designed and purchased from Sigma-Aldrich. Pancreatic islets and MIN6 cells were transfected with each siRNA using Lipofectamine RNAi/MAX (Life technologies, Tokyo, Japan) according to the manufacturer's protocol. Insulin secretion from MIN6 cells was normalized against cellular DNA content.

Agonist activity assay

Recruitment of β-arrestin to GPCRs, induced by γ-oryzanol was tested by the PathHunter β-Arrestin Assay obtained from DiscoveRx (Fremont, CA, USA). Luminescence was analysed with Envision (PerkinElmer, Waltham, MA, USA) and % activity was expressed as the relative luminescence units of 10 μM γ-oryzanol in comparison with that of each positive ligand. Antagonist activity (% inhibition) was measured against approximately EC_{80} concentrations of agonists. Duplicate data were obtained. The *Z*-factor, a parameter of quality control in high throughput screening assays (Zhang *et al.*, 1999), was determined by the following equation: $Z\text{-factor} = 1 - 3(SD_{sample} + SD_{control})/|mean_{sample} - mean_{control}|$. SD_{sample} and $SD_{control}$ refer to standard deviation of sample and positive control regions respectively.

Western blotting

Western blotting was performed as described (Tanaka *et al.*, 2007) with antibodies against D_2 receptors (AB5084P; Merck Millipore), DAT, TH and β-actin (ab6276; Abcam, Cambridge, MA, USA).

Quantitative real-time PCR

Gene expression was examined as described (Kozuka *et al.*, 2012). Total RNA was extracted using Trizol reagent (Life technologies) and cDNA was synthesized using an iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Quantitative real-time PCR was performed using a StepOnePlus™ Real-Time PCR System and Fast SYBR Green Master Mix (Life Technologies). The mRNA levels were normalized against *Rn18s* (18S rRNA). The primer sets used for the quantitative real-time PCR analyses are summarized in Table 1.

Table 1

The primer sets used for quantitative real-time PCR analysis

Gene	GenBank Accession No.	Primer (5′–3′)
Drd2 (D2R)	NM_010077	*f* CCA TTG TCT GGG TCC TGT CC
		r GTG GGT ACA GTT GCC CTT GA
Drd3 (D3R)	NM_007877	*f* GCA GTG GTC ATG CCA GTT CAC TAT CAG
		r CCT GTT GTG TTG AAA CCA AAG AGG AGA GG
Slc6a3 (DAT)	NM_010020	*f* GCA CTA CTT CTT CTC CTC CT
		r CCT GAA GTC TTT ACT CCC TTC C
Th (TH)	NM_009377	*f* CCC TAC CAA GAT CAA ACC TAC C
		r GAG CGC ATG CAG TAG TAA GA
Slc18a2 (VMAT2)	NM_172523	*f* GTC TGT CTA TGG GAG TGT GTA T
		r GGG TAC GGC TGG ACA TTA TT
Rn18s (18S rRNA)	NR_003278	*f* TTC TGG CCA ACG GTC TAG ACA AC
		r CCA GTG GTC TTG GTG TGC TGA

Forward and reverse primers are designated by *f* and *r* respectively. D2R, dopamine D_2 receptor; D3R, dopamine D_3 receptor.

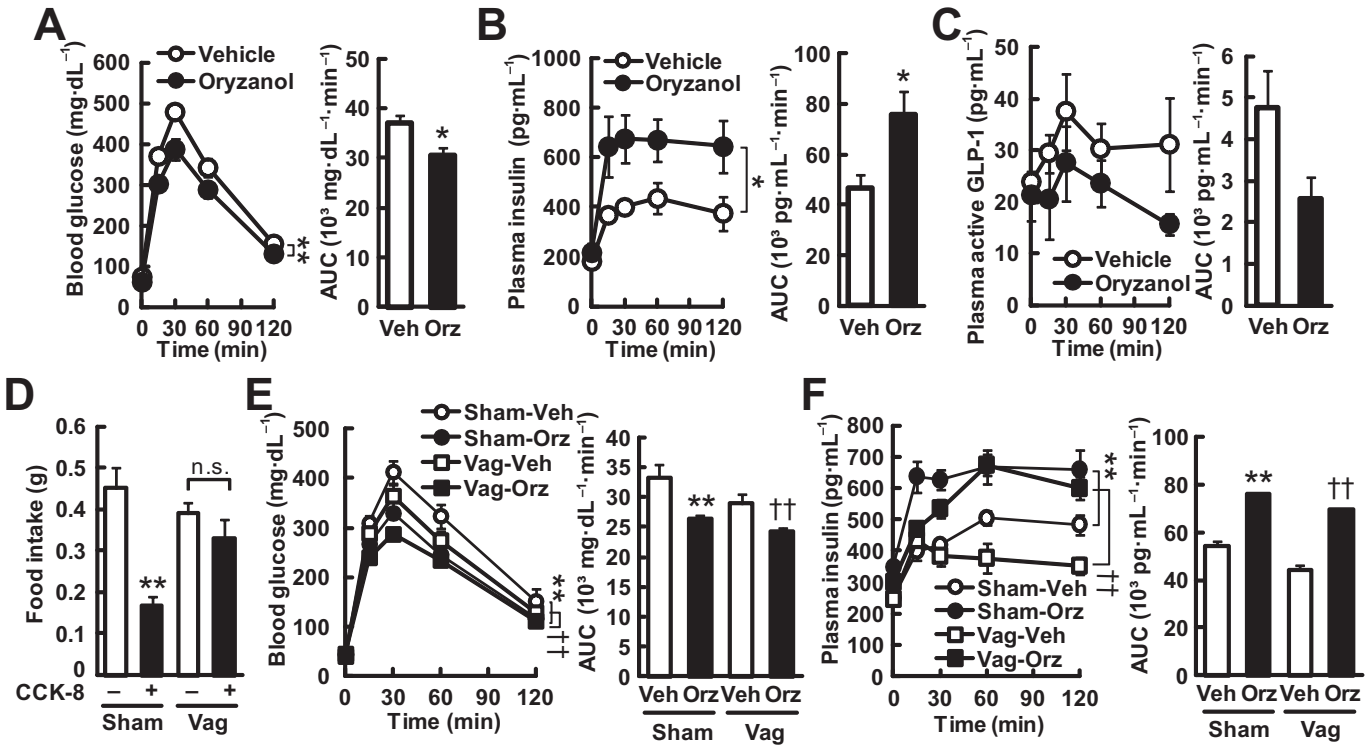

Figure 1

γ-Oryzanol enhances GSIS in mice. (A–C, E, F) Mice on a chow diet were treated with a single oral dose of γ-oryzanol (320 μg·g^{-1}). The concentrations and AUCs of blood glucose (A, E), plasma insulin (B, F) and plasma active GLP-1 (C) during ipGTTs (n = 8) are shown. Chow-fed mice (A–C) and vagotomized mice (Vag) (E, F) were analysed. (D) Satiety effects of CCK-8 were tested in sham-treated mice (Sham) and vagotomized mice (Vag). Sub-diaphragmatic vagotomy abolished the satiety effect of CCK-8. *P < 0.05, **P < 0.01 versus unoperated or sham-operated mice treated with vehicle (Vehicle or Sham-Veh). ††P < 0.01 versus vehicle-treated vagotomized mice (Vag-Veh). Data are expressed as means ± SEM.

Data analysis

Data are expressed as the mean ± SEM from n independent experiments. One-way ANOVA and repeated-measures ANOVA followed by multiple comparison tests (Bonferroni/Dunn method) were used where applicable. Student's t-test was used to analyse the differences between two groups. Differences were considered significant at P < 0.05.

Results

γ-Oryzanol acts directly on pancreatic islets and enhances GSIS in vivo

As a first step in exploring the effects of γ-oryzanol on GSIS in chow-fed mice, the effects of a single oral dose of γ-oryzanol (320 μg·g^{-1} body weight) on blood glucose and insulin levels were examined during i.p. GTTs (ipGTTs). γ-Oryzanol augmented GSIS and significantly enhanced glucose tolerance even in normal mice (Figure 1A,B). γ-Oryzanol showed a trend towards a decrease in the plasma GLP-1 level, but the change was not statistically significant (P = 0.11) (Figure 1C). To see if γ-oryzanol would enhance GSIS independently of GLP-1 receptors, we evaluated, using PathHunter β-arrestin assays, the agonist activities of γ-oryzanol on GLP-1 receptors and on two other GPCRs, GPR119 and GPR120, both of which potently stimulate GLP-1 secretion from intestine (Hirasawa *et al.*, 2005; Chu *et al.*, 2007; Lauffer *et al.*, 2009). γ-Oryzanol did not show agonist activities on these GPCRs [0% of exendin-4, a potent GLP-1 receptor agonist, Z-factor (a parameter of quality control in high throughput screening assays) (Zhang *et al.*, 1999) was 0.81; 9% of oleoylethanolamide, a potent GPR119 agonist, Z-factor was 0.41; –2% of GW 9508, a potent GPR120 agonist, Z-factor was 0.75 respectively].

To exclude the possibility that γ-oryzanol augments GSIS via a central mechanism, we carried out sub-diaphragmatic vagotomy in mice. Cholecystokinin-octapeptide (CCK-8) reduced the food intake in 1 h by 63% in sham-operated mice, while sub-diaphragmatic vagotomy abolished the satiety effect of CCK-8 (Figure 1D), indicating that the vagotomy was successful. In both sham-operated and vagotomized mice, a single oral dose of γ-oryzanol significantly lowered the blood glucose levels and the AUC of glucose during ipGTTs (Figure 1E). Noticeably, in both sham-operated and vagotomized mice, γ-oryzanol markedly increased plasma insulin levels and the AUC of insulin during ipGTTs (Figure 1F). These results suggest that γ-oryzanol acted directly on the pancreatic islets to enhance GSIS.

γ-Oryzanol enhances GSIS through activation of the cAMP/PKA pathway via the suppression of D_2 receptor signalling

In both isolated murine islets and MIN6 cells, γ-oryzanol markedly enhanced GSIS in a dose-dependent fashion (Figure 2A,E). Furthermore, in both cellular systems, γ-oryzanol significantly increased intracellular cAMP levels and PKA activity (Figure 2B,C,F,G). Similarly, augmentation of PKA activity by γ-oryzanol was abolished by H-89, a PKA inhibitor (Figure 2C,G). To explore the underlying mechanism, isolated murine islets and MIN6 cells were exposed to (i) forskolin, which increases intracellular cAMP level; (ii) Rp-8-Br-cAMPS, a cAMP antagonist; or (iii) H-89 respectively. In both cellular systems, γ-oryzanol augmented forskolin-enhanced insulin secretion (Figure 2D,H), while both Rp-8-Br-cAMPS and H-89 abolished such stimulatory effects of γ-oryzanol on GSIS (Figure 2I–K). These findings suggest that γ-oryzanol reinforces GSIS via the cAMP/PKA amplifying pathway in pancreatic islets.

On the other hand, haloperidol, a D_2 receptor antagonist, significantly enhanced GSIS (Figure 3A) through the elevation of intracellular cAMP (Figure 3B) but γ-oryzanol showed no additive effect with haloperidol (Figure 3C,D), supporting the notion that γ-oryzanol increased intracellular cAMP levels and enhanced GSIS through suppression of D_2 receptor signalling. Furthermore, both L-DOPA, a dopamine precursor, and quinpirole, a potent D_2 receptor agonist, abolished γ-oryzanol-induced enhancement of GSIS (Figure 3E–G). Of note, the inhibition by L-DOPA and quinpirole was concentration-dependent (Figure 3F,G). To further confirm the involvement of D_2 receptor signalling in enhancing GSIS by γ-oryzanol, *Drd2* was silenced *in vitro* by incubating the tissues or cells with specific siRNA for 2 days. In both pancreatic islets and MIN6 cells treated with *Drd2* siRNA, the expression of *Drd2* was attenuated by 71.4 ± 0.1% and 69.5 ± 0.1% compared with scrambled siRNA-treated cells respectively (Figure 3H,K). There were no significant changes in the expression of *Drd3* (dopamine D_3 receptor) in both systems (Figure 3H,K). Either γ-oryzanol or haloperidol enhanced GSIS accompanied by the elevation of intracellular cAMP level in cells treated with the scrambled siRNA. In contrast, in *Drd2* siRNA-treated cells, γ-oryzanol and haloperidol did not increase GSIS and intracellular cAMP level (Figure 3I,J,L). These results suggest that γ-oryzanol augments GSIS via the suppression of D_2 receptor signalling in pancreatic beta cells. Of note, data from the PathHunter β-arrestin assays suggested that there was no significant agonist or antagonist activities of γ-oryzanol for any of the dopamine receptors (Table 2).

γ-Oryzanol increases insulin biosynthesis and $[Ca^{2+}]_i$ in islets

Elevation of intracellular cAMP enhances the biosynthesis of insulin (Fehmann and Habener, 1992) and insulin secretion induced by increased $[Ca^{2+}]_i$ in the presence of insulinotropic glucose concentrations (Yada *et al.*, 1993). We therefore assessed the effect of γ-oryzanol and haloperidol on the biosynthesis of insulin and its secretion in response to increased $[Ca^{2+}]_i$ in murine-isolated islets. Both γ-oryzanol and haloperidol significantly increased intracellular insulin contents and the $[Ca^{2+}]_i$ response (Figure 4). Of note, both γ-oryzanol and haloperidol enhanced the first phase of $[Ca^{2+}]_i$ responses to high glucose (Figure 4B,C). These results also reinforce the notion that γ-oryzanol increases intracellular cAMP levels and subsequently enhances GSIS through suppression of D_2 receptor signalling.

γ-Oryzanol suppresses D_2 receptor signalling in pancreatic islets from HFD-fed mice

Following treatment of γ-oryzanol (320 $\mu g \cdot g^{-1}$ per body weight per day) for 13 weeks, glucose level in mice on a HFD was 1280 ± 50 $mg \cdot L^{-1}$, which was significantly decreased compared with those in mice on the HFD alone (1570 ± 80 $mg \cdot L^{-1}$, $P < 0.01$). Body weight in mice fed HFD with γ-oryzanol was 31.7 ± 0.8 g, which was comparable to that in mice fed HFD alone (30.4 ± 1.2 g). Areas of islet cells stained with antibody to TH, the rate-limiting enzyme of dopamine synthesis (Figure 5A), and antibody to DAT, which mediates dopamine uptake (Figure 5B), were increased in pancreatic islets from HFD-fed mice, whereas the stained areas were markedly decreased after treatment with γ-oryzanol. Consequently, the ratio of TH-positive or DAT-positive cell areas to the total islet area was significantly increased in HFD-fed mice, and was substantially decreased by the treatment with γ-oryzanol (Figure 5C,D). IHC analyses suggested that TH was localized in beta cells, while DAT was not confined to α-cells, beta cells or δ-cells (Figure 6).

We assessed protein and mRNA expression levels of genes involved in D_2 receptor signalling including D_2 receptors (*Drd2*), TH (*Th*), DAT (*Slc6a3*) and the vesicular monoamine transporter type 2 (VMAT2; *Slc18a2*), which transports dopamine into vesicles. In pancreatic islets from HFD-fed mice, the mRNA levels of *Drd2*, *Th* and *Slc6a3* were considerably elevated, while that of *Slc18a2*, also known as a functional marker of insulin production (Harris *et al.*, 2008), was markedly decreased (Figure 5E–H). Importantly, administration of γ-oryzanol depressed the mRNA levels of these genes (Figure 5E–H). In parallel with mRNA levels, protein levels of D_2 receptors, TH and DAT were concomitantly decreased by γ-oryzanol (Figure 5I–L).

γ-Oryzanol decreases glucagon secretion from murine islets

γ-Oryzanol significantly decreased glucagon levels in plasma of HFD-fed mice (Figure 7A) and in media of isolated islet cultures (Figure 7B). To test the possibility that γ-oryzanol directly acted on α-cells, a murine α-cell line, α-TC cells, was treated with γ-oryzanol. As shown in Figure 7C, glucagon secretion from α-TC cells was reduced, concentration-dependently, by glucose. It should be noted that mRNA level of *Drd2* in α-TC cells was extremely low, compared with those in isolated islets and MIN6 cells, while that of *Drd3* was about the same in the three types of cells (Figure 7D,E). In α-TC cells, γ-oryzanol and haloperidol did not affect glucagon secretion in both basal and palmitate-stimulated conditions (Figure 7F,G). IHC analyses of pancreatic islets from mice on a HFD demonstrated that γ-oryzanol augmented the intensity of insulin staining, while attenuating the average size of pancreatic islets, as well as the ratio of α-cells to the total islet area (Figure 7H–J). These results raised the possibility that γ-oryzanol reduced the increased secretion of glucagon via mechanisms independent of α-cells.

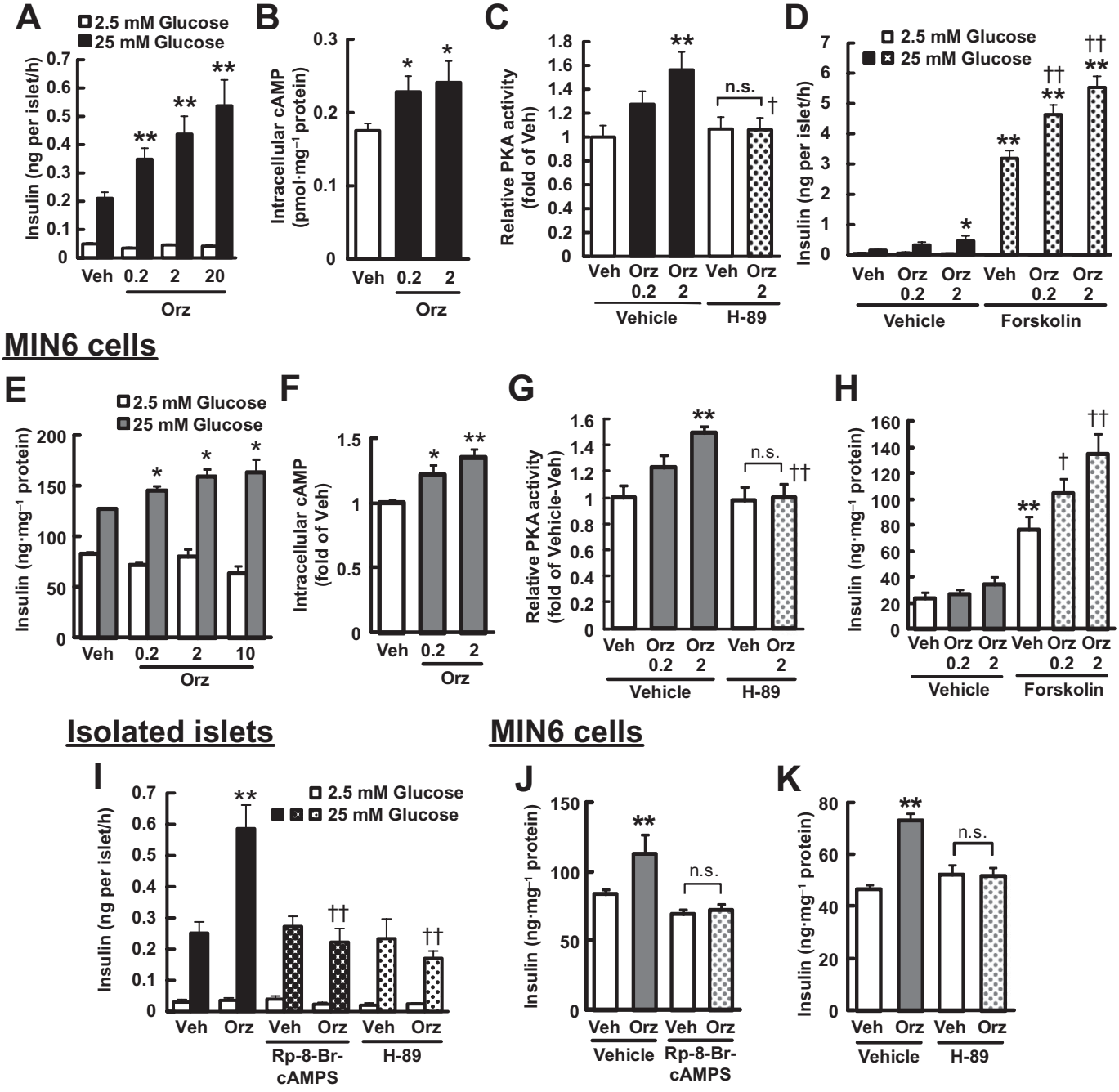

Figure 2

γ-Oryzanol enhances GSIS through activation of the cAMP/PKA pathway in murine isolated islets and MIN6 cells. Murine isolated islets (A–D, I) and MIN6 cells (E–H, J, K) were treated with the indicated concentrations of γ-oryzanol (Orz; 0.2, 2, 10 or 20 μg·mL^{-1}). (A, E) Insulin secretion was assessed following 25 mM glucose treated in murine-isolated islets (n = 10) (A) and MIN6 cells (n = 8) (E). (B, C, F, G) γ-Oryzanol (Orz; 0.2 or 2 μg·mL^{-1}) increased intracellular cAMP levels (B, F) and PKA activity (C, G) following 25 mM glucose in islets (n = 12) (B, C) and MIN6 cells (n = 8) (F, G). (D, H) Effects of γ-oryzanol (Orz; 0.2 or 2 μg·mL^{-1}) on insulin secretion enhanced by 10 μM forskolin in islets (n = 10) (D) and MIN6 cells following 2.5 mM glucose (n = 8) (H). (I–K) GSIS by 25 mM glucose was suppressed by 10 μM Rp-8-Br-cAMPS or 10 μM H-89 in islets (n = 10) (I) and MIN6 cells (n = 8) (J, K) treated with γ-oryzanol (Orz; 2 μg·mL^{-1}). Islets used in each experiment were isolated from eight mice, and they were pooled and divided into indicated number of groups. *P < 0.05, **P < 0.01 versus vehicle (Veh)-treated islets. $^{\dagger\dagger}P$ < 0.01 versus cells treated with vehicle (Veh) and γ-oryzanol (2 μg·mL^{-1}). n.s., not significant. Data are expressed as means ± SEM.

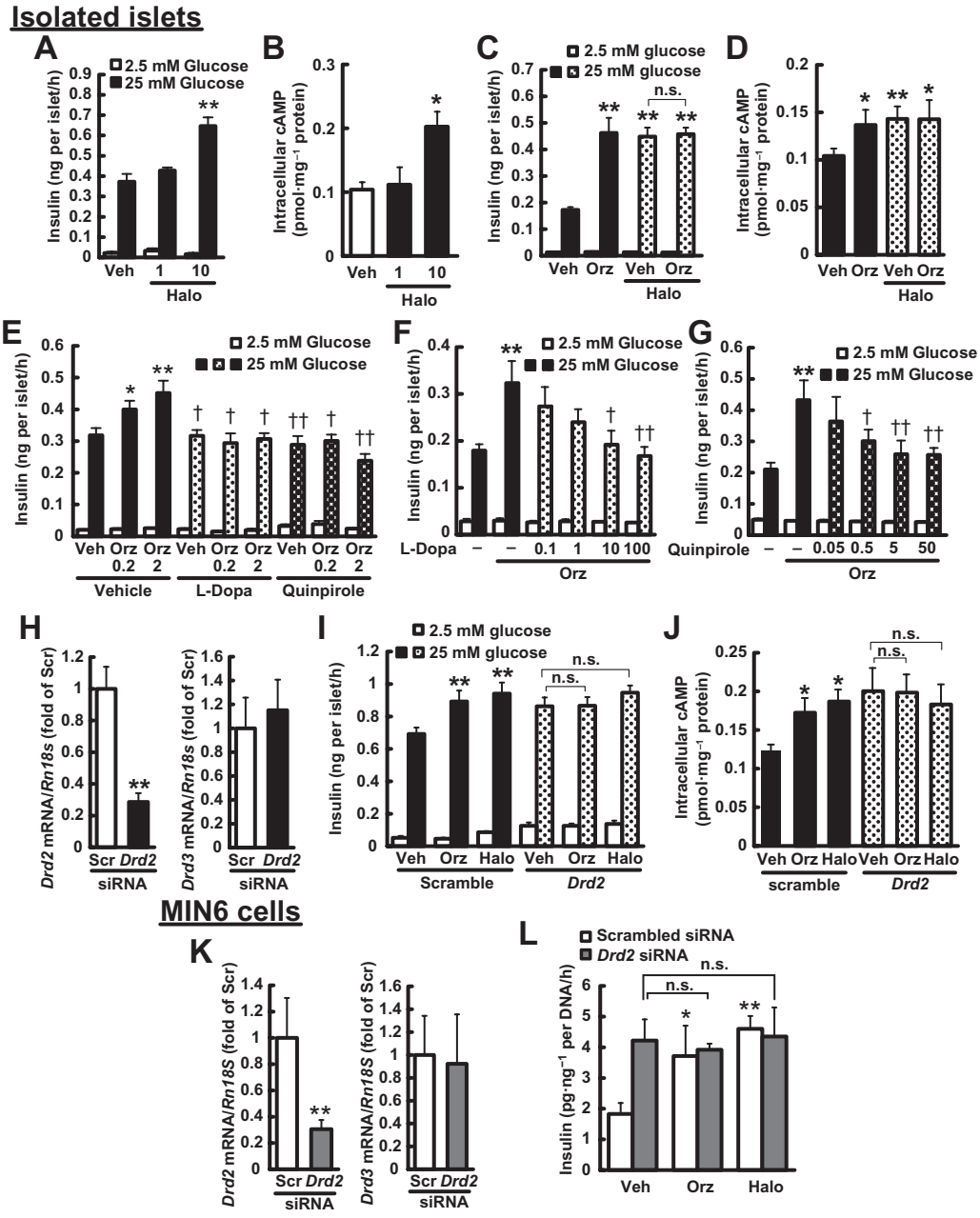

Figure 3

γ-Oryzanol enhances GSIS through the suppression of D_2 receptor signalling in murine isolated islets and MIN6 cells. (A, B) Haloperidol (1, 10 μM) increased insulin secretion (A) and intracellular cAMP levels (B) in isolated islets following 25 mM glucose (n = 12). (C, D) γ-Oryzanol (Orz; 2 μg·mL^{-1}) and haloperidol (10 μM) had no additive effect on insulin secretion (n = 12) (C) and intracellular cAMP levels (n = 24) (D) in isolated islets following 25 mM glucose. Islets used in each experiment were isolated from six mice, and they were pooled and divided into indicated number of groups. (E) Insulin secretion enhanced by the indicated concentrations of γ-oryzanol (Orz; 0.2 or 2 μg·mL^{-1}) was suppressed by 10 μM L-DOPA or 5 μM quinpirole and in isolated islets (n = 10; islets isolated from 12 mice were pooled and divided into indicated number of groups). (F, G) Insulin secretion in isolated islets treated with γ-oryzanol (Orz; 2 μg·mL^{-1}) was suppressed by the indicated concentrations of L-DOPA (0.1, 1, 10 or 100 μM) (F) or quinpirole (0.05, 0.5, 5 or 50 μM) (G) (n = 10–14; islets isolated from eight mice were pooled and divided into indicated number of groups). *P < 0.05, **P < 0.01 versus islets treated with vehicle (Veh). $^{\dagger}P$ < 0.05, $^{\dagger\dagger}P$ < 0.01 versus islets treated with vehicle (Veh) and γ-oryzanol. (H–L) Isolated pancreatic islets (H–J) and MIN6 cells (K, L) were treated with *Drd2* siRNA. (H, K) Level of mRNA expression for *Drd2* and *Drd3*. The levels were normalized against those of *Rn18s*. **P < 0.01 versus scrambled siRNA-transfected islets or cells (Scr). (I, L) Insulin secretion in siRNA-treated islets (I) and MIN6 cells (L) was not enhanced by γ-oryzanol (Orz; 2 μg·mL^{-1}) or haloperidol (10 μM) (n = 15–20). (J) γ-Oryzanol (Orz; 2 μg·mL^{-1}) and haloperidol (Halo; 10 μM) had no effect on intracellular cAMP levels in siRNA-treated islets (n = 10). Islets isolated from eight mice were pooled and divided into indicated number of groups. **P < 0.01 versus scrambled siRNA-transfected islets treated with vehicle (Veh). n.s., not significant. Amount of insulin secretion from MIN6 cells was normalized against the cellular protein content. Data are expressed as means ± SEM.

Table 2

Agonist or antagonist activities of γ-oryzanol for dopamine receptors (DRD1–DRD5)

	Agonist		Antagonist	
	% Activity	*Z*-factor	% Inhibition	*Z*-factor
DRD1	0	0.73	10	0.84
DRD2L	1	0.79	–5	0.81
DRD2S	2	0.81	5	0.91
DRD3	13	0.48	–13	0.79
DRD4	1	0.86	–2	0.77
DRD5	–2	0.75	9	0.87

Percentage of activity in γ-oryzanol for each dopamine receptor was calculated relative to the basal or maximal agonist values of dopamine. Percentage of inhibition by γ-oryzanol for each dopamine receptor was calculated relative to the basal or EC_{80} values for dopamine (antagonist activity). GPCR targets: DRD1, dopamine D_1 receptor; DRD2L, long form of the dopamine D_2 receptor; DRD2S, short form of the dopamine D_2 receptor; DRD3, dopamine D_3 receptor; DRD4, dopamine D_4 receptor; DRD5, dopamine D_5 receptor.

Discussion and conclusions

The major findings in the present study are summarized by the scheme shown in Figure 8. Here, we have demonstrated that, in mice, γ-oryzanol acted directly on pancreatic islets and enhanced GSIS *in vivo* and *in vitro* (Figures 1 and 2). Such a reinforcement of GSIS by γ-oryzanol was mediated by the local activation of the cAMP/PKA amplifying pathway (Figures 2 and 4). Along with chemical agonists for a variety of fatty acid receptors, cAMP/PKA amplifying pathways in pancreatic beta cells are promising drug targets for the treatment of type 2 diabetes (Drucker, 2006; Rayasam *et al.*, 2007; Ohishi and Yoshida, 2012). In this context, γ-oryzanol may be potentially useful as an alternative or a partner of combination therapies with incretin-related drugs.

To our knowledge, the present study is the first to demonstrate that protein and mRNA expression of molecules involved in D_2 receptor signalling was considerably elevated in pancreatic islets from mice fed on a HFD. Moreover, supplementation with γ-oryzanol corrected the dysregulation of these molecules *in vivo* (Figure 5). As increased signal transduction by D_2 receptors in pancreatic beta cells suppresses the secretion of insulin (Rubi *et al.*, 2005; Simpson *et al.*, 2012), such an effect of γ-oryzanol may be beneficial for individuals with glucose intolerance and type 2 diabetes. To date, how transcription of *Drd2* is regulated is largely undefined. It is possible that consensus element of NF-κB in the promoter region of *Drd2* (Bontempi *et al.*, 2007) is related to the HFD-induced dysregulation of D_2 receptors in isolated islets. Apart from the direct action of γ-oryzanol on pancreatic islets, it is also possible that improvement of hyperglycaemia *per se* may influence the expression of molecules involved in D_2 receptor signalling. In this context, further studies are necessary to elucidate fully the molecular mechanisms involved.

Intriguingly, in HFD-induced obese rodents, expression of genes involved in D_2 receptor signalling in the brain reward system (e.g. striatum, ventral tegmental area) was clearly decreased, resulting in a profound addiction to fatty foods (Li *et al.*, 2009; Johnson and Kenny, 2010). Furthermore, recent studies in rodents demonstrated that HFD-induced decrement in D_2 receptor expression in the brain reward system was closely associated with the hyper-methylation in the promoter region of the *Drd2* gene (Vucetic *et al.*, 2012). Studies are ongoing in our laboratory to investigate whether there is HFD-induced epigenetic dysregulation of the D_2 receptor signalling in pancreatic islets or beta cells.

In isolated islets and MIN6 cells, experiments with RNA interference for *Drd2* and with exogenous D_2 receptor ligands demonstrated that γ-oryzanol augmented GSIS via the suppression of D_2 receptor signalling (Figure 3). Enhancement of GSIS by γ-oryzanol was suppressed by L-DOPA (Figure 3E,F), while γ-oryzanol has neither agonist nor antagonist activities at D_2 receptors (Table 2). These findings suggest that γ-oryzanol has inhibitory effects on local dopamine synthesis.

In the pathophysiology of diabetes mellitus, exaggerated secretion of glucagon from pancreatic α-cells contributes to the vicious cycle of glucose dyshomeostasis (Holst, 2007). We demonstrated that γ-oryzanol substantially ameliorated the exaggerated secretion of glucagon in both HFD-fed mice and murine-isolated islets (Figure 7). As D_2 receptors are confined to beta cells in pancreatic islets in mice (Rubi *et al.*, 2005), our data raise the possibility that γ-oryzanol would not directly affect glucagon secretion from α-cells. To support this notion, we demonstrated in an α-cell line, α-TC cells, that γ-oryzanol and haloperidol did not affect glucagon secretion in either basal or palmitate-stimulated conditions (Figure 7F,G). The secretion of glucagon is known to be regulated by the central and peripheral nervous system as well as intra-islet paracrine factors including insulin, GABA and somatostatin (Ishihara *et al.*, 2003; Kawamori *et al.*, 2009; Walker *et al.*, 2011). For instance, postprandial glucagon release is strongly suppressed by GLP-1 and the effect of GLP-1 is mediated, at least partly, by somatostatin (Holst, 2007; Seino *et al.*, 2010). In this context, our results raise the possibility that γ-oryzanol may reduce increased secretion of glucagon via α-cell-independent, intra-islet paracrine factors.

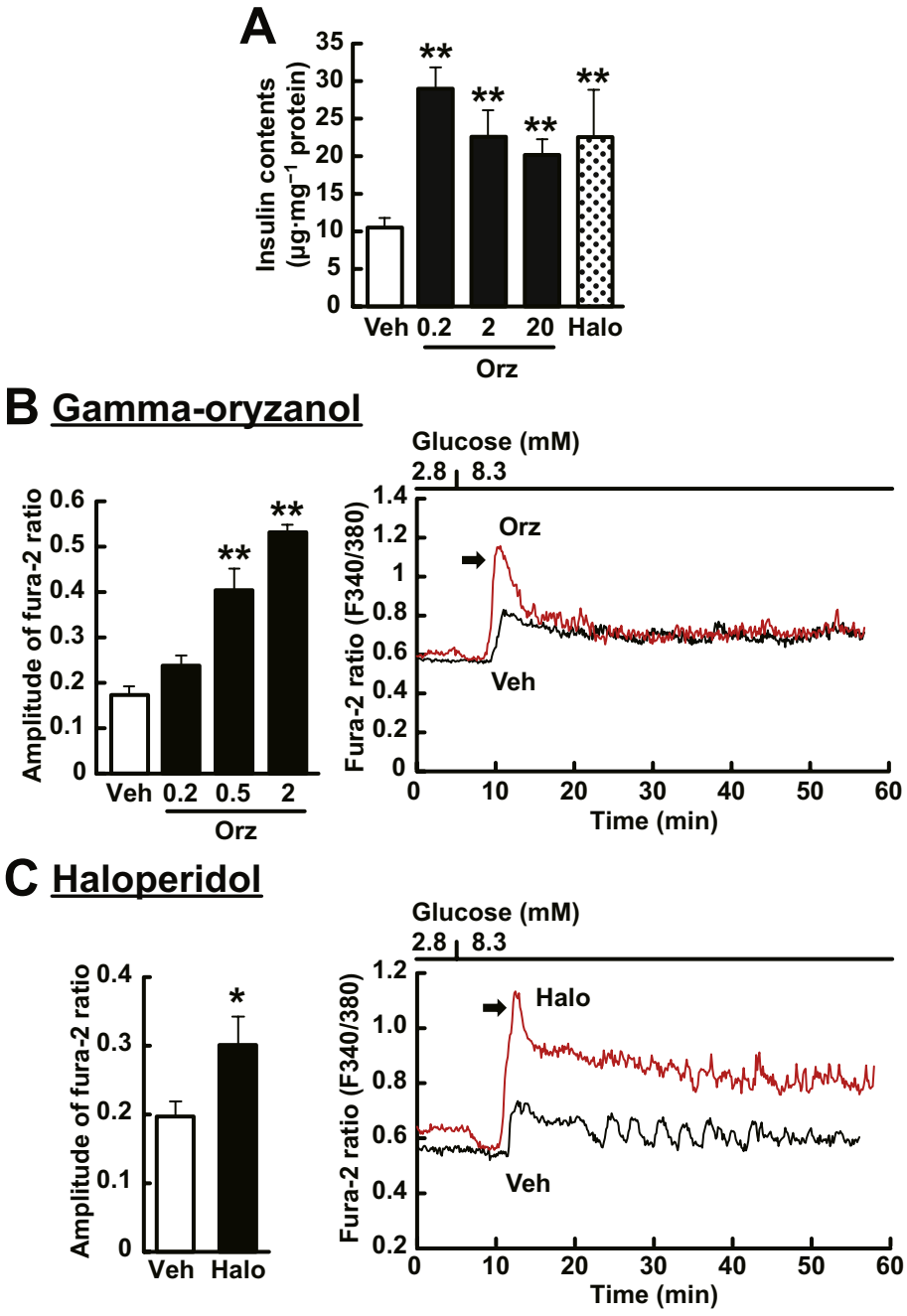

Figure 4

γ-Oryzanol increases intracellular insulin contents and $[Ca^{2+}]_i$ in murine isolated islets. (A) γ-Oryzanol (Orz; 0.2, 2 or 20 μg·mL^{-1}) and haloperidol (Halo; 10 μM) increased intracellular insulin contents ($n = 14$). (B, C) The representative $[Ca^{2+}]_i$ responses to 8.3 mM glucose in islets incubated with γ-oryzanol or haloperidol. Both 2 μg·mL^{-1} γ-oryzanol (B) and 10 μM haloperidol (C) potentiated the first-phase $[Ca^{2+}]_i$ response to 8.3 mM glucose in murine single islet. The peak amplitude of $[Ca^{2+}]_i$ responses was significantly enhanced by γ-oryzanol (Orz; 0.2, 0.5 or 2 μg·mL^{-1}) (B) (Veh, $n = 8$, Orz 0.2, $n = 12$, Orz 0.5, $n = 5$, Orz 2, $n = 3$; islets isolated from three mice were pooled and divided into indicated number of groups) and haloperidol (C) (Veh, $n = 12$, Halo, $n = 10$; islets isolated from two mice were pooled and divided into indicated number of groups). *$P < 0.05$, **$P < 0.01$ versus vehicle (Veh)-treated islets. Data are expressed as means ± SEM.

Regarding the effects of γ-oryzanol on food intake in mice, we previously reported that γ-oryzanol did not affect the total amount of food intake (chow: 16.8 ± 0.5 g per week, HFD: 16.4 ± 0.4 g per week, HFD + γ-oryzanol: 16.2 ± 0.5 g per week). However, γ-oryzanol does reduce the preference for fatty foods in mice (Kozuka *et al.*, 2012). Based on these findings, in the current experimental settings, the insulinotropic effects of γ-oryzanol on pancreatic islets should be

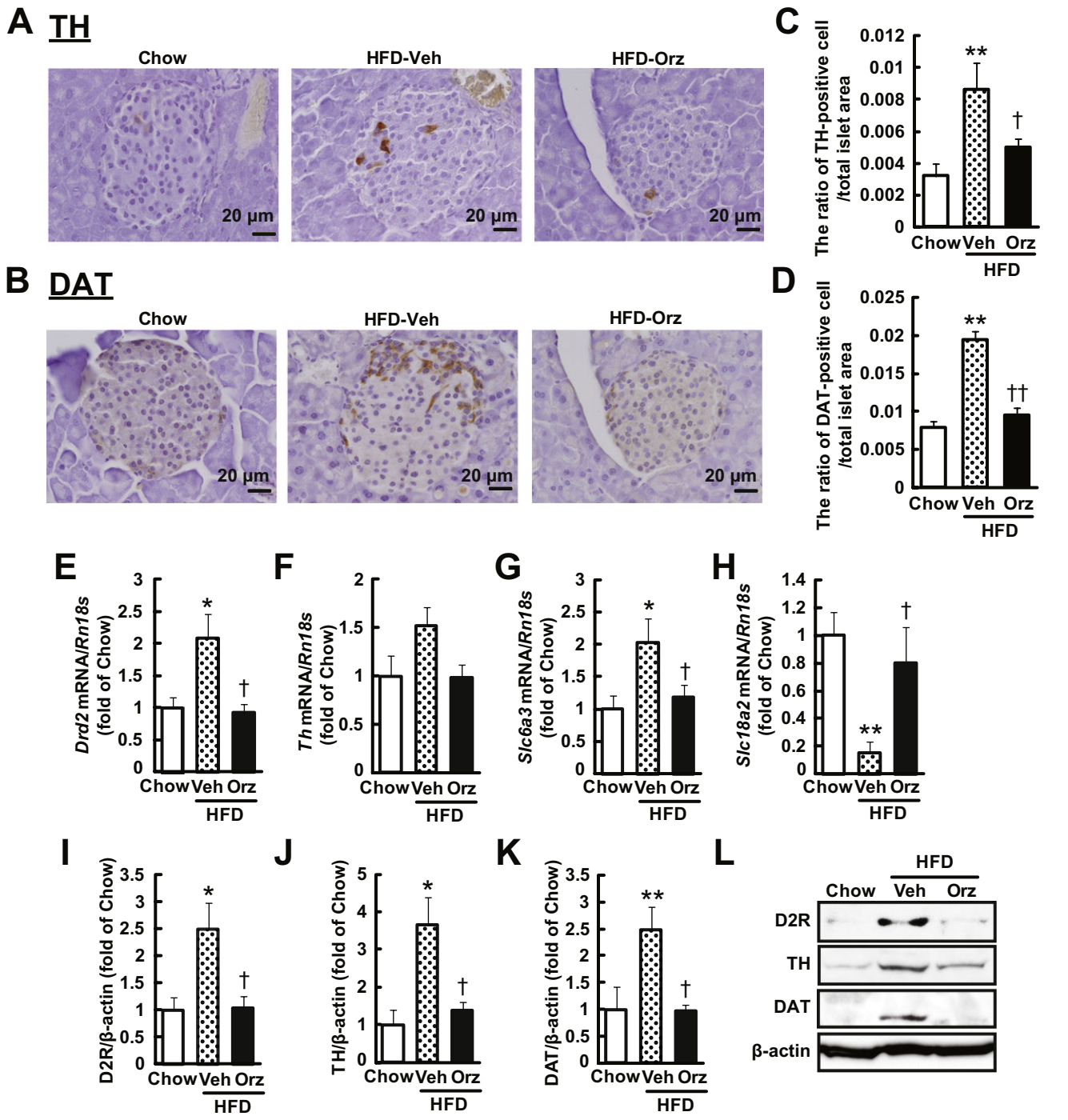

Figure 5

γ-Oryzanol suppresses the expression of molecules involved in D_2 receptor signalling in murine pancreatic islets from mice fed HFD. (A, B) IHC analyses of pancreatic islets from HFD-fed mice treated with γ-oryzanol (Orz). Paraffin-embedded sections were stained with anti-TH (A) or anti-DAT (B) antibodies. Scale bar, 20 μm; magnification, ×400. (C, D) The ratios of TH-positive (C) and DAT-positive (D) cell area to the total islet area were attenuated by the treatment with γ-oryzanol in HFD-fed mice (chow, $n = 6$, HFD-Veh, $n = 8$, HFD-Orz, $n = 8$). $^{**}P < 0.01$ versus chow-fed mice. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus vehicle (Veh)-treated HFD-fed mice. (E–H) Expression levels of *Drd2* (E), *Th* (F), *Slc6a3* (DAT) (G) and *Slc18a2* (VMAT2) (H) mRNAs in pancreatic islets from HFD-fed mice were decreased by γ-oryzanol (Orz; 320 $\mu g \cdot g^{-1}$ per body weight per day) ($n = 6$). The mRNA levels were determined by real-time PCR. The levels were normalized by those of *Rn18s* (18S rRNA). (I–L) Protein levels of D_2 receptors (I), TH (J) and DAT (K) in pancreatic islets from HFD-fed mice were decreased by γ-oryzanol ($n = 6$). Protein levels were determined by Western blotting. The values were normalized against those of β-actin protein. $^{*}P < 0.05$, $^{**}P < 0.01$ versus chow-fed mice. $^{\dagger}P < 0.05$ versus vehicle (Veh)-treated HFD-fed mice. Data are expressed as means ± SEM.

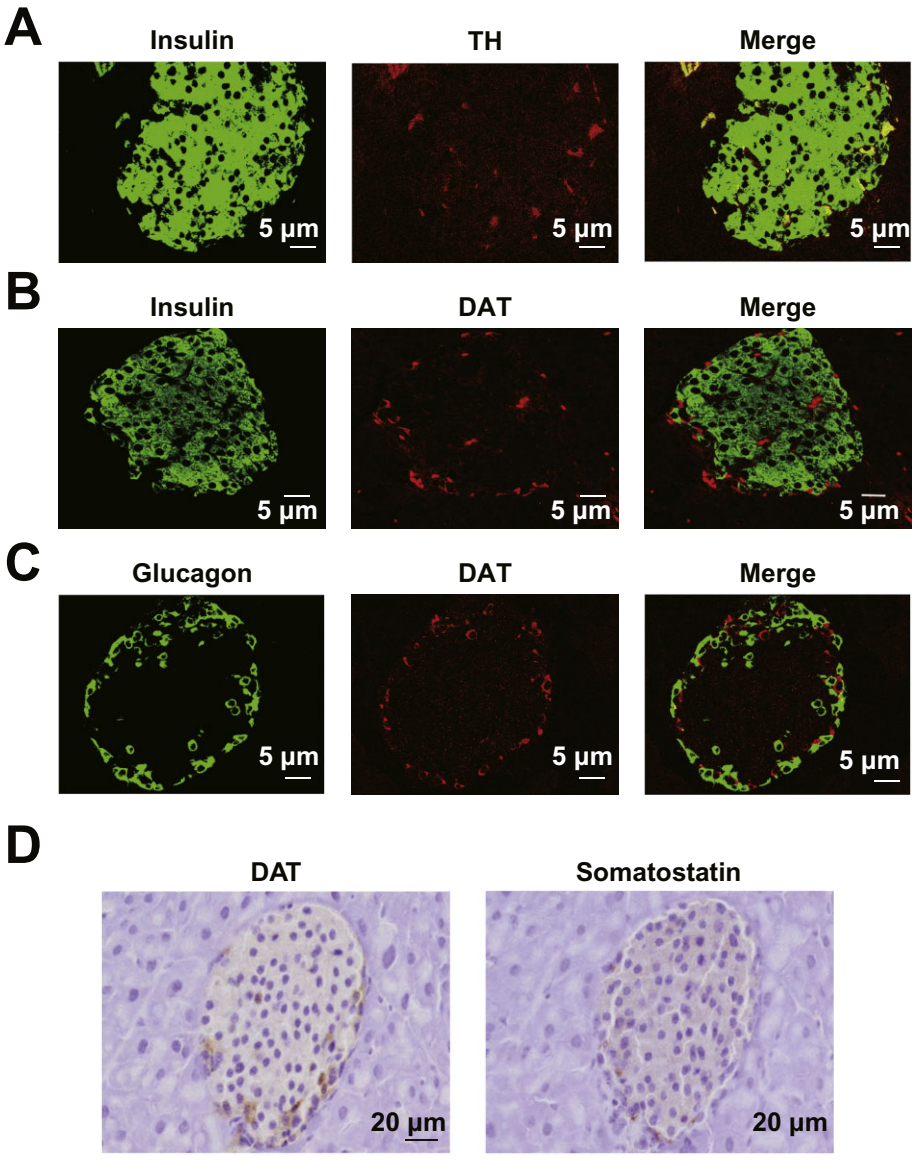

Figure 6

TH was localized in beta cells, whereas DAT was not confined to α-cells, beta cells or δ-cells. IHC analyses of pancreatic islets from HFD-fed mice. Paraffin-embedded sections were co-stained with anti-TH (red) and anti-insulin (green) (A), anti-DAT (red) and anti-insulin (green) (B), or anti-DAT (red) and anti-glucagon (green) (C) antibodies. Scale bar, 5 μm; magnification, ×600. (D) Serial paraffin-embedded sections were stained with anti-DAT and anti-somatostatin antibodies. Scale bar, 20 μm; magnification, ×400.

largely attributed to its direct mechanism. Moreover, as demonstrated in Figure 1, oral administration of γ-oryzanol to mice fed chow diet did not increase plasma GLP-1 level. The results of β-arrestin assays also support the notion that γ-oryzanol did not act as a ligand for GLP-1 receptor. Notably, secretion of GLP-1 is controlled strongly by a vagal nerve-mediated central mechanism (Drucker, 2006). However, even in vagotomized mice, γ-oryzanol markedly increased the plasma insulin levels during ipGTTs (Figure 1). These data suggest that γ-oryzanol acts directly on pancreatic islets and enhances GSIS independently of GLP-1 receptor signalling. Furthermore, we recently demonstrated that γ-oryzanol protects beta cells against ER stress-induced apoptosis in HFD-fed mice (Kozuka *et al.*, 2015). Taken together, γ-oryzanol exhibited metabolically beneficial effects on glucose homeostasis in a GLP-1 independent, unique insulinotropic manner.

The present study unveiled the mechanism, at least in part, whereby γ-oryzanol protects pancreatic islets against HFD-induced dysfunction and augments GSIS via the

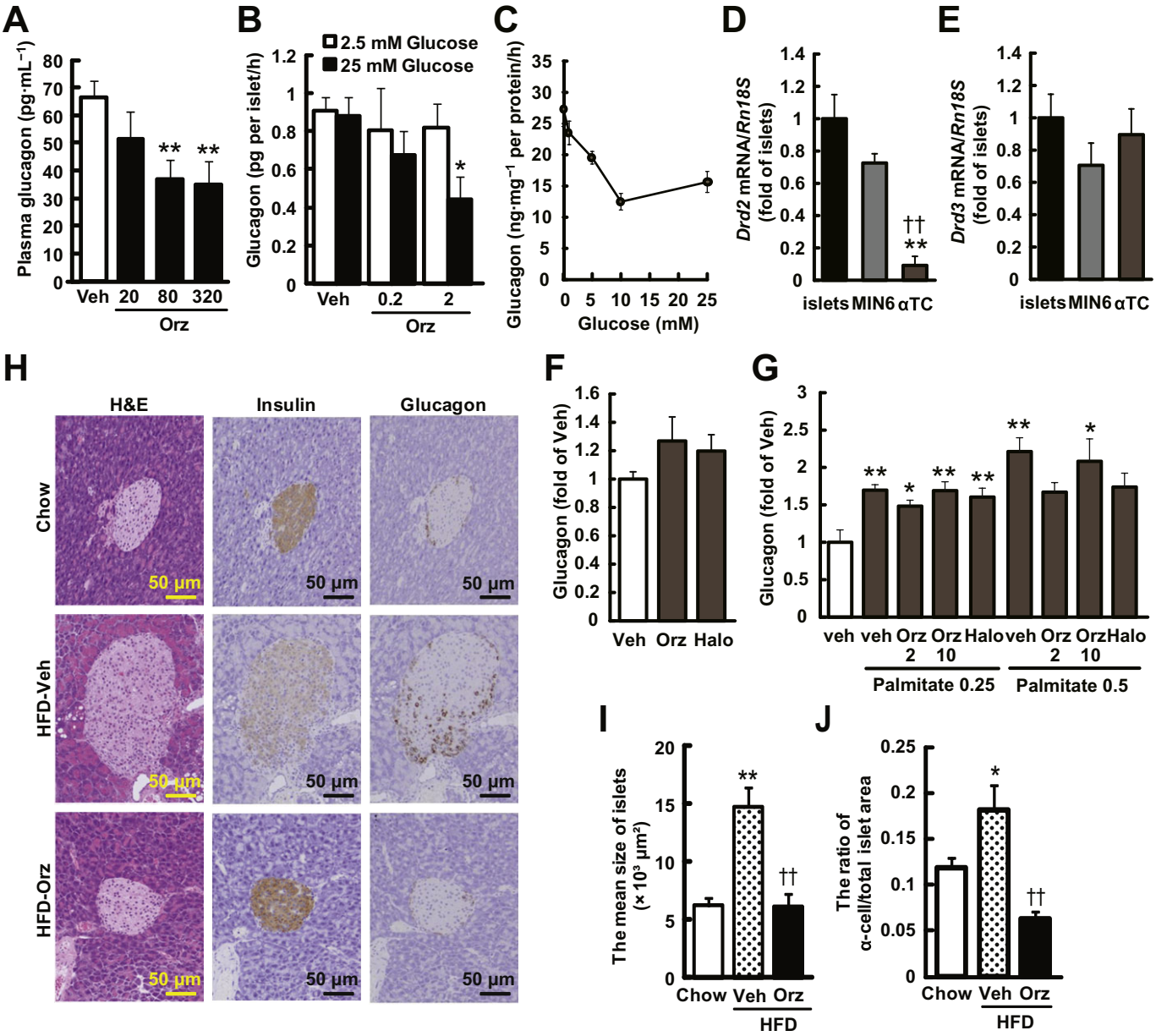

Figure 7

γ-Oryzanol ameliorates increased secretion of glucagon in HFD-fed mice and from murine isolated islets. (A) Plasma glucagon levels in HFD-fed mice treated with the indicated doses of γ-oryzanol (Orz) after a 4 h fast (20, 80 or 320 μg·g^{-1} per body weight per day; $n = 6$). (B) Glucagon secretion in isolated pancreatic islets was decreased by γ-oryzanol (Orz; 0.2 or 2 μg·mL^{-1}) following the exposure to 25 mM glucose ($n = 10$; islets isolated from three mice were pooled and divided into indicated number of groups). (C) Glucagon secretion was stimulated by indicated concentrations of glucose (0, 1, 5, 10 and 25 mM). Amount of glucagon secretion was normalized against the cellular protein content. (D, E) Expression level of *Drd2* and *Drd3* in isolated islets, MIN6 and α-TC cells. Levels of mRNA expression for *Drd2* (D) and *Drd3* (E) in three types of cells ($n = 12$). The mRNA levels were determined by real-time PCR. The levels were normalized against those of *Rn18s*. $^{**}P < 0.01$ versus islets, $^{††}P < 0.01$ versus MIN6 cells. (F, G) In α-TC cells treated with γ-oryzanol (Orz; 2 or 10 μg·mL^{-1}) or haloperidol (10 μM), glucagon secretion was assessed following 5 mM glucose (F) or palmitate (0.25 and 0.5 mM following 16.7 mM glucose (G). $^{*}P < 0.05$, $^{**}P < 0.01$ versus HFD-fed mice, islets, or α-TC cells treated with vehicle (Veh). (H) IHC analyses of isolated pancreatic islets from HFD-fed mice treated with γ-oryzanol (Orz; 320 μg·g^{-1}·day^{-1}). Serial paraffin-embedded sections were stained with haematoxylin and eosin (H&E) (upper panel) or anti-insulin (middle panel), anti-glucagon (lower panel) antibodies. Scale bar, 50 μm; magnification, ×200. (I, J) The mean sizes of islets (I) and ratios of glucagon-positive α-cell areas to the total islet area (J) were calculated ($n = 6$–8). $^{*}P < 0.05$, $^{**}P < 0.01$ versus chow-fed mice. $^{††}P < 0.01$ versus HFD-fed mice treated with vehicle (Veh). Data are expressed as means ± SEM.

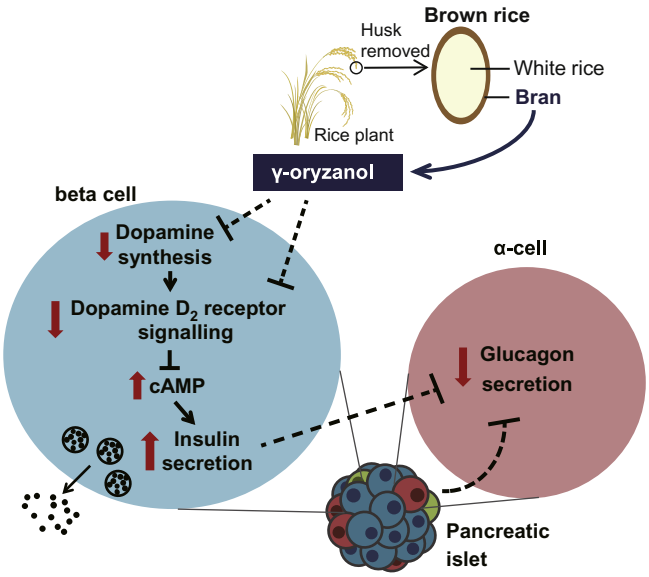

Figure 8

Scheme illustrating the effects of γ-oryzanol on pancreatic islets in mice. In pancreatic islets and beta cells, γ-oryzanol suppresses D_2 receptor signalling, at least partly via the inhibition of local dopamine synthesis, leading to an increase in the intracellular cAMP level. Consequently, GSIS is augmented via the cAMP/PKA pathway (amplifying pathway). On the other hand, γ-oryzanol ameliorates exaggerated secretion of glucagon from pancreatic α-cells, not via the direct action on α-cells, but presumably via some intra-islet paracrine factors.

attenuation of local D_2 receptor signalling in mice. This series of unexpected actions of γ-oryzanol may lead to a novel, natural food-based preventive treatment for type 2 diabetes.

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Author contributions

C. K., C. S.-O. and M. N. performed the research. C. K. and H. M. designed the research study. S. S., R. U., M. H., Y. O., H. T., C. S.-O., C. T., M. M., M. T., S. I., M. N., T. Y., J. M., S. O. and M. S. provided invaluable advice on research design and data interpretation. J. M. and S. O. contributed essential reagents or tools. C. K. analysed the data. C. K. and H. M. wrote the paper.

Conflict of interest

We declare that we have no conflict of interest.

References

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013a). The concise guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013b). The concise guide to PHARMACOLOGY 2013/14: Transporters. Br J Pharmacol 170: 1706–1796.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013c). The concise guide to PHARMACOLOGY 2013/14: Enzymes. Br J Pharmacol 170: 1797–1867.

Ariyama Y, Tanaka Y, Shimizu H, Shimomura K, Okada S, Saito T *et al.* (2008). The role of CHOP messenger RNA expression in the link between oxidative stress and apoptosis. Metabolism 57: 1625–1635.

Bontempi S, Fiorentini C, Busi C, Guerra N, Spano P, Missale C (2007). Identification and characterization of two nuclear factor-kappaB sites in the regulatory region of the dopamine D2 receptor. Endocrinology 148: 2563–2570.

Chu ZL, Jones RM, He H, Carroll C, Gutierrez V, Lucman A *et al.* (2007). A role for beta-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. Endocrinology 148: 2601–2609.

Drucker DJ (2006). The biology of incretin hormones. Cell Metab 3: 153–165.

Fehmann HC, Habener JF (1992). Insulinotropic hormone glucagon-like peptide-I(7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. Endocrinology 130: 159–166.

Garcia-Tornadu I, Ornstein AM, Chamson-Reig A, Wheeler MB, Hill DJ, Arany E *et al.* (2010). Disruption of the dopamine d2 receptor impairs insulin secretion and causes glucose intolerance. Endocrinology 151: 1441–1450.

Giacca A, Xiao C, Oprescu AI, Carpentier AC, Lewis GF (2011). Lipid-induced pancreatic beta-cell dysfunction: focus on in vivo studies. Am J Physiol Endocrinol Metab 300: E255–E262.

Harris PE, Ferrara C, Barba P, Polito T, Freeby M, Maffei A (2008). VMAT2 gene expression and function as it applies to imaging beta-cell mass. J Mol Med (Berl) 86: 5–16.

Henquin JC (2000). Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49: 1751–1760.

Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M *et al*. (2005). Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med 11: 90–94.

Holst JJ (2007). The physiology of glucagon-like peptide 1. Physiol Rev 87: 1409–1439.

Ieiri T, Kase N, Hashigami Y, Kobori H, Nakamura T, Shimoda S (1982). [Effects of gamma-oryzanol on the hypothalamo-pituitary axis in the rat]. Nihon Naibunpi Gakkai Zasshi 58: 1350–1356.

Ishihara H, Maechler P, Gjinovci A, Herrera PL, Wollheim CB (2003). Islet beta-cell secretion determines glucagon release from neighbouring alpha-cells. Nat Cell Biol 5: 330–335.

Johnson PM, Kenny PJ (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat Neurosci 13: 635–641.

Kahn SE, Hull RL, Utzschneider KM (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444: 840–846.

Kawamori D, Kurpad AJ, Hu J, Liew CW, Shih JL, Ford EL *et al*. (2009). Insulin signaling in alpha cells modulates glucagon secretion in vivo. Cell Metab 9: 350–361.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: Reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Kozuka C, Yabiku K, Sunagawa S, Ueda R, Taira SI, Ohshiro H *et al*. (2012). Brown rice and its component, gamma-oryzanol, attenuate the preference for high-fat diet by decreasing hypothalamic endoplasmic reticulum stress in mice. Diabetes 61: 3084–3093.

Kozuka C, Yabiku K, Takayama C, Matsushita M, Shimabukuro M, Masuzaki H (2013). Natural food science based novel approach toward prevention and treatment of obesity and type 2 diabetes: recent studies on brown rice and γ-oryzanol. Obes Res Clin Pract 7: e165–e172.

Kozuka C, Sunagawa S, Ueda R, Higa M, Tanaka H, Shimizu-Okabe C *et al*. (2015). Gamma-oryzanol protects pancreatic beta-cells against endoplasmic reticulum stress in male mice. Endocrinology 156: 1242–1250.

Lauffer LM, Iakoubov R, Brubaker PL (2009). GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. Diabetes 58: 1058–1066.

Lerma-Garcia MJ, Herrero-Martinez JM, Simo-Alfonso EF, Mendonca CRB, Ramis-Ramos G (2009). Composition, industrial processing and applications of rice bran gamma-oryzanol. Food Chem 115: 389–404.

Li Y, South T, Han M, Chen J, Wang R, Huang XF (2009). High-fat diet decreases tyrosine hydroxylase mRNA expression irrespective of obesity susceptibility in mice. Brain Res 1268: 181–189.

Marsden CD, Parkes JD (1977). Success and problems of long-term levodopa therapy in Parkinson's disease. Lancet 1: 345–349.

McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998). Dopamine receptors: from structure to function. Physiol Rev 78: 189–225.

Miyamoto L, Ebihara K, Kusakabe T, Aotani D, Yamamoto-Kataoka S, Sakai T *et al*. (2012). Leptin activates hepatic 5′ AMP-Activated Protein Kinase through sympathetic nervous system and alpha1 adrenergic receptor: a potential mechanism for improvement of fatty liver in lipodystrophy by leptin. J Biol Chem 287: 40441–40447.

Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y *et al*. (1990). Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 127: 126–132.

Nakata M, Shintani N, Hashimoto H, Baba A, Yada T (2010). Intra-islet PACAP protects pancreatic beta-cells against glucotoxicity and lipotoxicity. J Mol Neurosci 42: 404–410.

Ohishi T, Yoshida S (2012). The therapeutic potential of GPR119 agonists for type 2 diabetes. Expert Opin Investig Drugs 21: 321–328.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al*.; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledge base of drug targets and their ligands. Nucl Acids Res 42 (Database Issue): D1098–D1106.

Rayasam GV, Tulasi VK, Davis JA, Bansal VS (2007). Fatty acid receptors as new therapeutic targets for diabetes. Expert Opin Ther Targets 11: 661–671.

Rubi B, Ljubicic S, Pournourmohammadi S, Carobbio S, Armanet M, Bartley C *et al*. (2005). Dopamine D2-like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion. J Biol Chem 280: 36824–36832.

Seino Y, Fukushima M, Yabe D (2010). GIP and GLP-1, the two incretin hormones: similarities and differences. J Diabetes Investig 1: 8–23.

Shimabukuro M, Higa M, Kinjo R, Yamakawa K, Tanaka H, Kozuka C *et al*. (2014). Effects of the brown rice diet on visceral obesity and endothelial function: the BRAVO study. Br J Nutr 111: 310–320.

Simpson N, Maffei A, Freeby M, Burroughs S, Freyberg Z, Javitch J *et al*. (2012). Dopamine-mediated autocrine inhibitory circuit regulating human insulin secretion in vitro. Mol Endocrinol 26: 1757–1772.

Sirtori CR, Bolme P, Azarnoff DL (1972). Metabolic responses to acute and chronic L-dopa administration in patients with parkinsonism. N Engl J Med 287: 729–733.

Smith GP, Jerome C, Cushin BJ, Eterno R, Simansky KJ (1981). Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science 213: 1036–1037.

Smith GP, Jerome C, Norgren R (1985). Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. Am J Physiol 249: R638–641.

Sun Q, Spiegelman D, van Dam RM, Holmes MD, Malik VS, Willett WC *et al*. (2010). White rice, brown rice, and risk of type 2 diabetes in US men and women. Arch Intern Med 170: 961–969.

Tanaka T, Masuzaki H, Yasue S, Ebihara K, Shiuchi T, Ishii T *et al*. (2007). Central melanocortin signaling restores skeletal muscle AMP-activated protein kinase phosphorylation in mice fed a high-fat diet. Cell Metab 5: 395–402.

Vallone D, Picetti R, Borrelli E (2000). Structure and function of dopamine receptors. Neurosci Biobehav Rev 24: 125–132.

Vucetic Z, Carlin JL, Totoki K, Reyes TM (2012). Epigenetic dysregulation of the dopamine system in diet-induced obesity. J Neurochem 120: 891–898.

Walker JN, Ramracheya R, Zhang Q, Johnson PR, Braun M, Rorsman P (2011). Regulation of glucagon secretion by glucose: paracrine, intrinsic or both? Diabetes Obes Metab 13 (Suppl. 1): 95–105.

Wei FY, Nagashima K, Ohshima T, Saheki Y, Lu YF, Matsushita M *et al*. (2005). Cdk5-dependent regulation of glucose-stimulated insulin secretion. Nat Med 11: 1104–1108.

Yada T, Itoh K, Nakata M (1993). Glucagon-like peptide-1-(7–36) amide and a rise in cyclic adenosine 3′,5′-monophosphate increase cytosolic free Ca2+ in rat pancreatic beta-cells by enhancing Ca2+ channel activity. Endocrinology 133: 1685–1692.

Zhang JH, Chung TDY, Oldenburg KR (1999). A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J Biomol Screen 4: 67–73.

Zmuda EJ, Powell CA, Hai T (2011). A method for murine islet isolation and subcapsular kidney transplantation. J Vis Exp 50: e2096.