Insulin exocytosis in Goto-Kakizaki rat β-cells subjected to long-term glinide or sulfonylurea treatment

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Sulfonylurea and glinide drugs display different effects on insulin granule motion in single β-cells in vitro. We therefore investigated the different effects that these drugs manifest towards insulin release in an in vivo long-term treatment model. Diabetic GK (Goto-Kakizaki) rats were treated with nateglinide, glibenclamide or insulin for 6 weeks. Insulin granule motion in single β-cells and the expression of SNARE (soluble N-ethylmaleimidesensitive factor-attachment protein receptor) proteins were then analysed. Perifusion studies showed that decreased first-phase insulin release was partially recovered when GK rats were treated with nateglinide or insulin for 6 weeks, whereas no first-phase release occurred with glibenclamide treatment. In accord with the perifusion results, TIRF (total internal reflection fluorescence) imaging of insulin exocytosis showed restoration of the decreased number of docked insulin granules and the fusion events from them during first-phase release for nateglinide or insulin, but not glibenclamide, treatment; electron microscopy results confirmed the TIRF microscopy data. Relative to vehicle-treated GK β-cells, an increased number of SNARE clusters were evident in nateglinide- or insulin-treated cells; a lesser increase was observed in glibenclamide-treated cells. Immunostaining for insulin showed that nateglinide treatment better preserved pancreatic islet morphology than did glibenclamide treatment. However, direct exposure of GK β-cells to these drugs could not restore the decreased first-phase insulin release nor the reduced numbers of docked insulin granules. We conclude that treatment of GK rats with nateglinide and glibenclamide varies in long-term effects on β-cell functions; nateglinide treatment appears overall to be more beneficial.

Key words: exocytosis, glinide, insulin, pancreatic β-cell, sulfonylurea, total internal reflection fluorescence (TIRF).

INTRODUCTION

Considering the prevalence in the number of Type 2 diabetes cases with a high risk of cardiovascular disease [1,2], prevention of diabetes onset and strict blood glucose control in diabetic patients are expedient. Treatments for Type 2 diabetic patients with characteristically impaired insulin release [3–5] aim to restore insulin release from β-cells, and include pharmaceutical agents targeting KATP (ATP-sensitive K⁺) channels [6–9]. Sulfonylurea drugs, such as glibenclamide, and glinide drugs, such as mitiglinide, inhibit KATP channel functions and are common treatments [10–14]. Although both drug types elicit insulin secretion via KATP channel closure, the mechanisms of action for these drugs differ. Our previous TIRF (total internal reflection fluorescence) imaging results showed that these drugs have disparate effects on insulin granule motion in single β-cells in vitro [15]. The question remains of how long-term treatment in vivo with these drugs affect insulin exocytosis.

Use of sulfonylureas such as glibenclamide corresponds with a high frequency of hypoglycaemia, and long-term use of glibenclamide yields a progressive decrease in its efficacy. Although this decreased efficacy conceivably results from decreased insulin-producing capacity by the pancreatic β-cells, the mechanism of secondary failure is still unknown [13]. Because glibenclamide binds tightly to SUR (sulfonylurea receptor) sites at the pancreatic β-cell membrane and has long-acting metabolites [16,17], glibenclamide is effective in a once-daily dose [18]. In contrast, glinides such as nateglinide bind quickly and reversibly to SUR sites [19], resulting in a rapid and short insulin response resembling the physiological pattern of postprandial insulin release [20,21]. Unlike glibenclamide, nateglinide appears not to promote hypoglycaemic attack. Thus it is thought that long-term treatment with these two drug types in vivo in diabetic GK rats may display different effects on β-cell function, particularly insulin exocytosis. To address this issue, in the present study, we administered glibenclamide, nateglinide or control insulin to diabetic GK (Goto-Kakizaki) rats for 6 weeks. We then examined β-cell functions by TIRF imaging of insulin exocytosis, perfusion analysis and expression analysis of SNARE (soluble N-ethylmaleimide-sensitive factor-attachment protein receptor) proteins. Our results demonstrated that long-term nateglinide treatment in GK rats is more effective than glibenclamide in improving the expression of SNAREs, the docking status of insulin granules and fusion events from these granules.

Abbreviations used: AUC, area under the curve; CCD, charge-coupled device; EM, electron microscopy; GFP, green fluorescent protein; GK, Goto-Kakizaki; IL-1β, interleukin 1β; KATP, ATP-sensitive K⁺; KRB, Krebs–Ringer buffer; SNAP-25, synaptosome-associated protein of 25 kDa; SNARE, soluble N-ethylmaleimide-sensitive factor-attachment protein receptor; SUR, sulfonylurea receptor; TIRF, total internal reflection fluorescence; TIRFM, TIRF microscopy; t-SNARE, target membrane SNARE.

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EXPERIMENTAL

Animals

Diabetic GK rats and non-diabetic male Wistar rats were obtained from a commercial breeder (Oriental Yeast, Tokyo, Japan). Rats were housed under a 12 h light/12 h dark cycle and given free access to food and water until the start of experiments, which were conducted with 8-week-old male rats. The body weights of GK rats were not statistically different from controls. For 6 weeks from the experimental start date, GK rats were fed twice daily (9:00 h and 16:00 h) for 1 h, and vehicle (0.5% methylcellullose), nateglinide (50 mg/kg) or glibenclamide (2 mg/kg) was administered by oral gavage just before feeding. For insulin administration, human insulin (2 units of Humulin\(^\text{®}\); Eli Lilly and Co.) was injected subcutaneously just before feeding. Blood samples were collected from tail veins at the indicated times, and blood glucose levels were measured by Glutest\(^\text{®}\) (Sanwa Kagaku Kenkyusho).

TIRFM (TIRF microscopy)

At the end of the 6-week treatments, pancreatic islets of Langerhans were isolated by collagenase digestion as described by Nagamatsu et al. [22], with some modifications. Isolated islets were dissociated into single cells by incubation in Ca\(^{2+}\)-free KRB (Kreb’s–Ringer buffer) containing 1 mM EGTA, and cultured on fibronectin-coated (KOKEN Co.) high-refractive-index glass (Olympus) in RPMI 1640 medium (Gibco BRL) supplemented with 10% (v/v) fetal bovine serum (Gibco BRL), 200 units/ml penicillin and 200 µg/ml streptomycin as described in [23]. Cells were maintained at 37 °C in an atmosphere of 5% CO\(_2\). To label the insulin secretory granules, pancreatic β-cells were infected with the recombinant adenovirus Adex1CA insulin–GFP (green fluorescent protein) [23]. The Olympus total internal reflection system was used with a high-aperture objective lens [Apo 100× OHR; NA (numerical aperture) 1.65; Olympus]. To observe GFP and Alexa Fluor\(^\text{®}\) 488 (Molecular Probes) labels, we used a 488 nm laser line for excitation and a 515 nm long-pass filter for the barrier. Images were then projected on to a cooled CCD (charge-coupled device) camera (DV887DCSBV; ANDOR) operated with Metamorph version 6.3 (Universal Imaging). Images were acquired at 300 ms intervals. The space constant for the exponential decay of the evanescent field was approx. 43 nm. For real-time images of GFP-tagged insulin granule motion, β-cells were placed on the high-refractive-index glass, mounted in an open chamber, and incubated for 30 min at 37 °C in KRB containing 110 mM NaCl, 4.4 mM KCl, 1.45 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 2.3 mM calcium gluconate, 4.8 mM NaHCO\(_3\), 2.2 mM glucose, 10 mM Hepes (pH 7.4) and 0.3% BSA. Cells were then transferred to the thermostat-controlled stage (37 °C) of TIRFM, and stimulation with glucose was achieved by the addition of 52 mM glucose/KRB into the chamber (22 mM final concentration of glucose). Most analyses, including tracking (single projection of differing images) and area calculations, were performed using Metamorph software. To analyse the data, fusion events were selected manually, and the average fluorescence intensity of individual granules in a 1 µm × 1 µm square placed over the granule centre was calculated. The number of fusion events was counted manually while looping approx. 5000 frame time-lapses.

Insulin-release assay

At the end of the 6-week treatments, pancreatic islets were isolated and β-cells were housed in a small chamber (approx. 5 × 10\(^5\) cells/ chamber) and perfused with KRB (2.2 mM glucose) for 60 min at a flow rate of 0.5 ml/min at 37 °C before collecting fractions. Insulin release was stimulated by 16.7 mM glucose. Fractions were collected at 1 min intervals. Insulin release in 5 µl aliquots of medium was measured by an insulin ELISA kit (Morinaga).

EM (electron microscopy)

EM was carried out by conventional methods as described previously [24]. Tissues were fixed in phosphate-buffered 2.5% glutaraldehyde (pH 7.4), post-osmicated, dehydrated with graded alcohols and embedded in Epon 812. After staining with uranyl acetate and lead citrate, ultrathin sections were examined using a transmission electron microscope TEM-1010C (JEOL). In EM, granules at their shortest distance of < 10 nm from the plasma membrane were qualified as morphologically docked granules [25].

Immunohistochemical analysis

For TIRF analysis of syntaxin 1A (also known as HPC-1) and SNAP-25 (synaptosome-associated protein of 25 kDa) clusters.
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Figure 2 Insulin release in response to 16.7 mM glucose in perfused β-cells from control Wistar rat and 6-week-treated GK rat β-cells

(A) At the end of 6 weeks of treatment, pancreatic β-cells were isolated and perfused with basal medium containing 2.2 mM glucose for 30 min. Cells were then stimulated with 16.7 mM glucose for 45 min. (B) An enlargement of the shaded area in (A) shows insulin released from vehicle-, nateglinide- or glibenclamide-treated β-cells. Results are mean ± S.E.M. insulin release (n = 3–4).

Figure 3 TIRF imaging of insulin exocytosis in single β-cells

After GK rats were treated with vehicle, nateglinide, glibenclamide or insulin for 6 weeks, pancreatic β-cells were isolated. Cells were infected with Adex1CA insulin–GFP, then TIRF imaging of insulin exocytosis with 22 mM glucose stimulation was performed as described in the Experimental section. The histogram shows the number of fusion events per 200 μm² at 1 min intervals post-stimulation. The closed bars represent fusion of previously docked granules; the open bars represent fusion of newcomer granules.

Figure 4 TIRF imaging of insulin granules morphologically docked at the plasma membrane

(A) β-Cells from control Wistar rats and 6-week-treated GK rats were fixed with paraformaldehyde and immunostained with anti-insulin antibody. The surrounding lines represent the outline of cells attached to the coverslip. (B) The number of fluorescent spots (docked insulin granules) was counted manually (per 200 μm²). Results are mean ± S.E.M. percentages of measurements compared with the control. *P < 0.05, **P < 0.01 compared with GK-vehicle by Dunnett’s test; †P < 0.01, GK-nateglinide compared with GK-glibenclamide by Tukey–Kramer’s test; Wistar control, n = 7; GK-vehicle, n = 8; GK-nateglinide, n = 6; GK-glibenclamide, n = 6; GK-insulin, n = 9.

Statistical analysis

Results are means ± S.E.M. for at least three different experiments performed independently. Statistical analysis was performed by ANOVA followed by Dunnett’s test or Tukey–Kramer’s test using the Statview software for Windows version 5.0 (SAS Institute, Cary, NC, U.S.A.).
RESULTS

Effects of nateglinide and glibenclamide in vivo in GK rats

We first examined how short-term administration of nateglinide and glibenclamide before feeding affects GK rat blood glucose levels. The concentration of each drug to be used was chosen based on the total area under the curve (AUC) generated by administering drug to fasted rats, feeding them for 1 h beginning at 09:00 h, then tracking glucose levels from 09:00 to 15:00 h (glucose AUC_{09:00−15:00}). Because there was no significant difference in the total glucose AUC_{09:00−15:00} for 50 mg/kg nateglinide and 2 mg/kg glibenclamide (Figure 1A), we used these concentrations of drugs in the present study. Therefore the dosage of nateglinide used was nearly 25-fold higher than that of glibenclamide. In fact, in an ex vivo study, the maximal insulinoicotropic effects of nateglinide and glibenclamide on isolated rat pancreatic islets were observed at concentration of approx. 10 \mu M and 300 nM respectively [27], suggesting that the effective concentration of nateglinide was approx. 30-fold higher than that of glibenclamide. As shown in Figure 1(A), nateglinide adminis-

![Figure 5 Status of docked insulin granules observed by EM](image)

(A) Typical EM images of the plasma membrane area facing the blood capillary are shown. Arrowheads show morphologically docked insulin granules at their distance of < 10 nm from the plasma membrane. Scale bar, 500 nm. (B) Quantification of the number of docked granules. Results are means ± S.E.M. (n = 6 each); **P < 0.01 compared with GK-vehicle by Dunnett’s test; #P < 0.05, GK-nateglinide compared with GK-glibenclamide by Tukey–Kramer’s test. NS, not significant.
(n = 10 each), \( P < 0.01 \) compared with vehicle treatment, at 3 weeks after treatment; 198.2 ± 6.1 mg/dl in vehicle treatment compared with 141.2 ± 2.5 mg/dl in insulin treatment (n = 10 each), \( P < 0.01 \) compared with vehicle treatment, at 6 weeks after treatment]. However, glibenclamide treatment did not decrease 1 h postprandial blood glucose levels (Figure 1C).

**Nateglinide and insulin treatments partially recover first-phase glucose-induced insulin release**

After the 6-week treatment with drug or insulin, we isolated pancreatic \( \beta \)-cells from treated GK rats. Cells were perfused, and insulin release was subsequently measured by ELISA. ELISA data showed partial recovery of first-phase release in insulin-treated \( \beta \)-cells relative to control (Wistar rat) cells. Second-phase release was similar in insulin-treated and control levels (Figure 2A). Because the levels of insulin release are low in nateglinide- and insulin-treated cells, the scale in the graph was enlarged as shown in Figure 2B. Nateglinide-treated GK \( \beta \)-cells showed a biphasic glucose-induced insulin release pattern. In contrast, there was no obvious first phase in glibenclamide-treated \( \beta \)-cells.

**TIRF imaging analysis of insulin exocytosis**

We explored the effects of nateglinide, glibenclamide or insulin treatment on the dynamic motion of single insulin granules by TIRF imaging. As shown in our previous study [23,28], in rat \( \beta \)-cells fusion of insulin granules during first-phase release mainly involved previously docked granules, whereas fusion from newcomer granules was observed during second-phase release (Supplementary Movie S1 at http://www.BiochemJ.org/bj/412/bj4120093add.htm and Figure 3, Wistar control). There was no fusion from previously docked granules in vehicle-treated GK \( \beta \)-cells (Supplementary Movie S2 at http://www.BiochemJ.org/bj/412/bj4120093add.htm). However, nateglinide-treated (Supplementary Movie S3 at http://www.BiochemJ.org/bj/412/bj4120093add.htm) and insulin-treated (Supplementary Movie S4 at http://www.BiochemJ.org/bj/412/bj4120093add.htm) GK \( \beta \)-cells showed fusion events from previously docked granules under 22 mM glucose stimulation (Figure 3). There was little fusion observed from previously docked granules in glibenclamide-treated GK \( \beta \)-cells (Supplementary Movie S5 at http://www.BiochemJ.org/bj/412/bj4120093add.htm and Figure 3). We then counted the number of fusion events manually, and performed statistical analysis. The total number of fusion events during first-phase release (5 min after stimulation, per 200 \( \mu \)m²) was increased in both insulin-treated and nateglinide-treated GK \( \beta \)-cells (14.8 ± 0.9 in vehicle-treated cells (n = 5); 19.5 ± 2.2 in insulin-treated cells (n = 6), \( P < 0.01 \) compared with GK-vehicle; 13.7 ± 1.3 in nateglinide-treated cells (n = 8), \( P < 0.01 \) compared with GK-vehicle), but the number of fusion events in glibenclamide-treated GK \( \beta \)-cells was not increased (9.6 ± 2.7 in glibenclamide-treated cells, no significant difference from GK-vehicle).

We then examined the docking status of insulin granules using TIRFM with immunostaining for insulin (Figure 4). In agreement with our previous findings [23], we rarely observed morphologically docked granules in diabetic GK \( \beta \)-cells. The number of docked insulin granules in vehicle-treated GK rat \( \beta \)-cells markedly decreased to 31.2 ± 1.9% of control numbers (Figure 4B). In contrast, nateglinide (66.0 ± 3.1% of control, \( P < 0.01 \) compared with GK-vehicle) and insulin (80.4 ± 3.5% of control, \( P < 0.01 \) compared with GK-vehicle) caused a considerable increase in the number of docked insulin granules,
Glibenclamide was less effective than nateglinide. In order to confirm the TIRFM data, we used EM to examine the status of docked insulin granules. As shown in Figure 5(A), the number of docked insulin granules was reduced in vehicle-treated GK β-cells, but nateglinide and insulin treatments increased the number of docked granules. We found that the number of insulin granules within 10 µm of the plasma membrane (10.0 ± 1.2 and 1.8 ± 0.7 granules per 10 µm of plasma membrane in Wistar control and GK-vehicle treatment samples respectively) was increased by nateglinide and insulin treatments (GK-nateglinide 5.0 ± 0.8, P < 0.01 compared with GK-vehicle; GK-insulin 4.5 ± 0.4, P < 0.01 compared with GK-vehicle), whereas the effect of glibenclamide was minor (GK-glibenclamide 2.6 ± 0.2, no significant difference compared with GK-vehicle; P < 0.05, GK-nateglinide compared with GK-glibenclamide) (Figure 5B).

Decreased number of t-SNARE (target membrane SNARE) clusters partially restored by nateglinide and insulin treatments

Since the expression and number of syntaxin 1A clusters on the plasma membrane regulate first-phase insulin release [28], we examined the effect of 6-week treatments with these drugs on SNARE clusters. To quantify the number of t-SNARE clusters on the plasma membrane, pancreatic β-cells were immunostained and observed by TIRFM. The number of syntaxin 1A clusters (26.5 ± 1.0% of control) and SNAP-25 clusters (22.5 ± 1.0% of control) markedly decreased in vehicle-treated GK rat islets (Figure 6). Both nateglinide and insulin treatments caused recovery of the decreased number of syntaxin 1A and SNAP-25 clusters (for syntaxin 1A, GK-nateglinide showed 61.4 ± 2.6% of control, P < 0.01 compared with GK-vehicle; GK-insulin 72.9 ± 2.9% of control, P < 0.01 compared with GK-vehicle; for SNAP-25, GK-nateglinide showed 47.4 ± 1.6% of control, P < 0.01 compared with GK-vehicle; GK-insulin 51.2 ± 1.8% of control, P < 0.01 compared with GK-vehicle). However, glibenclamide treatment only slightly increased the number of clusters relative to that in the GK-vehicle and GK-nateglinide samples (syntaxin 1A, 34.4 ± 4.3% of control, no significant difference compared with GK-vehicle; SNAP-25, 28.9 ± 1.0% of control, P < 0.05 compared with GK-vehicle), indicating that glibenclamide was less effective than nateglinide.

Morphology of the pancreatic islets

We examined the morphology of the pancreatic islets in GK rats by immunostaining for insulin at the end of the 6-week treatment with drug or insulin. As shown in Figure 7, GK rat islets displayed an irregular structure, with cells separated from each other. Nateglinide and insulin treatments restored such irregularity to the structure similarly to Wistar control samples. Whereas insulin immunoreactivity was much weaker in vehicle-treated GK rats, nateglinide and insulin treatments caused strong insulin immunoreactivities that were not observed in glibenclamide-treated islets.

Direct exposure of GK β-cells to drug failed to recover the decreased first-phase insulin release or the reduced numbers of docked insulin granules

Finally, we examined the direct effect of nateglinide and glibenclamide on pancreatic β-cells isolated from GK rats to determine whether acute exposure to these drugs is sufficient to restore the decreased first-phase insulin release or the reduced numbers of docked insulin granules. GK rat β-cells were pre-incubated for 1 h with 10 µM nateglinide or 300 nM glibenclamide, concentrations which, as described above, should have maximal effects on isolated rat pancreatic β-cells. After these drugs were removed, treated cells were then stimulated with 22 mM glucose. As shown in Figure 8(A) using TIRFM, neither nateglinide nor glibenclamide could restore the decreased first-phase insulin release. We also conducted TIRFM with immunostaining for insulin to observe the docking status of insulin granules in these treated cells (Figure 8B). The number of docked granules in vehicle-pre-incubated GK rat β-cells was reduced to 27.9 ± 3.2% of control numbers, in agreement with results shown in Figure 4(B). Similarly, the pre-incubation with nateglinide (29.8 ± 3.4% of control) and glibenclamide (25.1 ± 3.3% of control) had no effect on the number of docked granules. These results indicate that direct exposure of GK β-cells to these drugs...
Insulin exocytosis in nateglinide-treated GK rat β-cells

DISCUSSION

We have reported previously that sites of action for glinides in pancreatic β-cells are different from those for sulfonylureas, on the basis of evidence that only mitiglinide, but not glibenclamide, causes the fusion of insulin granules in SURI-KO mice β-cells [15]. Thus different mechanisms underlying glinide and sulfonylurea drug activities are expected to variably affect diabetic β-cell functions when these drugs are administered long-term in vivo. We found that nateglinide, but not glibenclamide, treatment in GK rat β-cells caused a restoration of both decreased t-SNARE clusters and the number of docked insulin granules, increasing fusion events from docked insulin granules under glucose stimulation. However, direct exposure of GK β-cells to these drugs could not restore the decreased first-phase insulin release nor the reduced numbers of docked insulin granules. Notably, although both nateglinide and glibenclamide could equally reduce the blood glucose levels during a 6 h period after feeding, only nateglinide treatment could restore the impaired insulin exocytosis. Thus our data indicate that the beneficial effect of nateglinide treatment may be due not only to release of glucotoxicity, but also to an indirect effect as a consequence of its protection against pancreatic islet damage. The finding that nateglinide, but not glibenclamide, could rapidly reduce blood glucose levels after feeding indicates distinct effects on β-cell functions. Insulin treatment also suppressed the blood glucose levels promptly after feeding, permitting effective recovery of first phase of insulin release and fusion from previously docked granules. It has been proposed that the restoration of both the early phase of insulin release and postprandial hyperglycaemia have potentially significant implications in improving metabolic control and reducing macrovascular complications [29]. Therefore blood glucose levels just after feeding play an important role not only in the recovery of impaired insulin exocytosis, but also in rationalizing the therapeutic approach. Of course, it remains possible that alteration of fatty acid or other metabolite levels elicited by glinide treatment is affecting insulin exocytosis.

Postprandial hyperglycaemia is determined, in large part, by peripheral glucose utilization and the severity of insulin resistance [30]; however, hepatic glucose output and glucose utilization are more sensitive to suppression by insulin [31]. Insulin released during the early first phase has a more likely role in acute hepatic glucose output [32]. Therefore the partial recovery of first-phase insulin release by nateglinide and insulin treatments may effectively suppress glucose output from the liver, rapidly decreasing postprandial hyperglycaemia. At present, the reason such an acute suppression of blood glucose levels just after feeding eventually recovers the docking status of insulin granules, as well as SNARE protein expression, is unknown. It is possible that IL-1β (interleukin 1β) production contributes to this mechanism. Hyperglycaemia may be associated with IL-1β production in β-cells [33,34], although it is still controversial [35]. We demonstrated previously that the exposure of β-cells to IL-1β decreased SNARE expression and fusion of insulin granules [36]. If nateglinide treatment could alter IL-1β production in β-cells, such an alteration could regulate SNARE expression and the docking status of insulin granules. Although there is no report regarding nateglinide and IL-1β to date, it appears worthwhile to investigate whether nateglinide treatment may inhibit IL-1β production.
We also reported previously that the effect of glinide on insulin exocytosis is very rapid and quickly decays; in contrast, glibenclamide acts on insulin exocytosis continuously [15]. Mechanistic differences between these drugs may be due to their different binding affinity towards SURs. The short action of glinide may benefit β-cell survival; continuous stimulation of β-cells by glibenclamide may contribute to β-cell exhaustion. In agreement with our data, Lagmich et al. [37] reported that when both normal and GK rats were exposed to nateglinide and glibenclamide for 7 days, insulin in islets, the secretary response to high-glucose and basal biosynthetic activity were better in those animals which received nateglinide. Taken together, these data suggest that short-acting nateglinide preserves β-cell functions better than the long-acting glibenclamide does.

An alternative speculation is that autocrine signals induced by first-phase insulin release may regulate β-cell functions. Large amounts of insulin released in a time course resembling the first phase of glucose-stimulated insulin release may be required for binding to insulin receptors on β-cells. Autocrine signalling by insulin in islet β-cells is now well known to be important for islet mass, affecting β-cell apoptosis and proliferation [38–40]. It has typically been observed that nateglinide treatment can prevent destruction of GK rat islets [41,42]. We noted that the insulin immunostaining pattern in nateglinide-treated islets was almost similar to those of control islets; we concur that nateglinide probably inhibits apoptosis or promotes proliferation directly. It has been reported that glibenclamide, but not nateglinide, induced β-cell apoptosis in human islets [43], corroborating the suggestion that short-acting insulin secretagogues may be preferred to long-acting ones. In addition, it is of note that insulin treatment has the most beneficial effects on restoring biphasic insulin exocytosis. Although the precise reason is unknown, it is conceivable that insulin signalling itself makes islets survive and maintains the physiological functions of β-cells. In conclusion, long-term nateglinide treatment in GK rats is more beneficial than glibenclamide in improving insulin exocytosis.

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