Sub-chronic stimulation of glucocorticoid receptor impairs and mineralocorticoid receptor protects cytosolic Ca^{2+} responses to glucose in pancreatic β -cells

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Abstract

The development of diabetes associated with stress, obesity, and metabolic syndrome involves elevated plasma glucocorticoid levels. It has been shown that short-term (<1 day) exposure to glucocorticoids reduces insulin secretion from pancreatic islets by affecting several steps of glucose signaling in β -cells. However, longer term direct effects of glucocorticoids on β -cells remain to be established. In this study, single β -cells isolated from rat islets were treated with glucocorticoids, mineralocorticoids, and their receptor agonists/antagonists for 3 days in culture, followed by assessment of the β -cell responsiveness to glucose by measuring cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) using fura-2. Following treatment with corticosterone at 10–500 ng/ml for 3 days, the first-phase $[\text{Ca}^{2+}]_i$ response to 8.3 mM glucose in β -cells was suppressed. Simultaneous administration of RU-486, a glucocorticoid receptor (GR) antagonist, prevented this suppression. RU-486

by itself promoted the β -cell $[\text{Ca}^{2+}]_i$ response to glucose. Conversely, dexamethasone (1000 ng/ml), a highly selective GR agonist, impaired β -cell $[\text{Ca}^{2+}]_i$ responses to glucose. A mineralocorticoid receptor (MR) antagonist spironolactone, co-administered with corticosterone, further depressed $[\text{Ca}^{2+}]_i$ responses to glucose, while an MR ligand aldosterone attenuated the corticosterone inhibition of $[\text{Ca}^{2+}]_i$ responses. Neither spironolactone nor aldosterone by itself affected $[\text{Ca}^{2+}]_i$ responses. These results indicate that long-term treatment with corticosterone impairs β -cell $[\text{Ca}^{2+}]_i$ responses to glucose. This effect is mediated by GR and attenuated partially by simultaneous MR stimulation by corticosterone. The results show a novel function of MR to protect islet β -cells against deteriorating glucocorticoid action via GR.

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Introduction

Induction and progression of diabetes under stress conditions involve elevated plasma glucocorticoid levels, owing to activation of the hypothalamic–pituitary–adrenal axis and sympathetic nervous system (Chan *et al.* 2002, Tsigos & Chrousos 2002, Kyrou *et al.* 2006). In obesity and metabolic syndrome, excessive plasma glucocorticoid level is considered a factor that leads to diabetes (Kyrou *et al.* 2006, Dallman *et al.* 2007). Cushing's syndrome with elevated plasma glucocorticoid level and treatment with therapeutic doses of glucocorticoid often cause impaired glucose tolerance and type 2 diabetes. Furthermore, type 1 11 β -hydroxysteroid dehydrogenase, an enzyme that converts inactive forms to active glucocorticoids, is expressed in pancreatic islets and elevated in type 2 diabetes in rodents (Davani *et al.* 2000, Duplomb *et al.* 2004). Thus, glucocorticoid excess under several pathophysiological conditions predisposes to the development of type 2 diabetes, and this process is mediated by impairment of insulin secretion from pancreatic islets, peripheral insulin resistance, and increased hepatic glucose output (Hoogwerf & Danese 1999, Ullrich *et al.*

2005). Therefore, the effect of elevated glucocorticoids on islet β -cells is an issue that is causally related to type 2 diabetes and hence need to be fully clarified. It has been shown that short-term (<1 day) exposure to high levels of glucocorticoid suppresses glucose-induced insulin secretion (Billaudel *et al.* 1984, Khan *et al.* 1992, Ogawa *et al.* 1992, Delaunay *et al.* 1997, Gremlich *et al.* 1997, Lambillotte *et al.* 1997, Jeong *et al.* 2001, Ullrich *et al.* 2005) and affects the related steps in islet β -cells, which include glucose transporter type 2 (Ogawa *et al.* 1992, Gremlich *et al.* 1997), glucose cycling (Khan *et al.* 1992), serum- and glucocorticoid-inducible kinase 1 (Ullrich *et al.* 2005), voltage-gated K^+ channels (Ullrich *et al.* 2005), Ca^{2+} influx (Billaudel *et al.* 1984), and cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) oscillations (Lambillotte *et al.* 1997, Ullrich *et al.* 2005). However, longer term direct effects of elevated glucocorticoids on islet β -cells, which are more relevant to the *in vivo* and clinical situations, remain to be clarified.

This study aimed to clarify longer term effects of glucocorticoids on β -cells. Isolated rat islet β -cells were treated with corticosterone for 3 days in primary culture, followed by assessment of their responsiveness to glucose by measuring

[Ca²⁺]_i. We employed [Ca²⁺]_i for evaluating the responsiveness to glucose in islet β-cells, since [Ca²⁺]_i is a key mediator of glucose signaling and insulin secretion in β-cells and [Ca²⁺]_i measurements are carried out in single β-cells whose activity, unlike that of islets, is fairly well kept after a long-term culture for 3 days. Corticosterone stimulates not only glucocorticoid receptor (GR) but also mineralocorticoid receptor (MR). To dissect out the role of each receptor, we used relatively GR-preferential agonist and antagonist, dexamethasone and RU-486 respectively, as well as relatively MR-preferential agonist and antagonist, aldosterone and spironolactone. We found that the semi-chronic exposure to corticosterone and dexamethasone impairs [Ca²⁺]_i responses to glucose in β-cells via GR, and that simultaneous stimulation of MR can counteract the GR-mediated inhibitory effect of corticosterone and thereby protect β-cell responses to glucose.

Materials and Methods

Preparation and culture of single islet cells

Islets of Langerhans were isolated from Wistar rats aged 8–12 weeks by collagenase digestion. Islets were collected and immediately dispersed into single cells in HEPES-added Krebs Ringer bicarbonate buffer (HKRB) without Ca²⁺ (Yanagida *et al.* 2002). The single cells were plated on cover slip and maintained in culture for 3 days under control (Eagle’s minimum essential medium (MEM) containing 5.6 mM glucose and 10% fetal bovine serum) and test conditions added with corticosterone, RU-486, dexamethasone, spironolactone, and aldosterone (Fig. 1).

Solutions and chemicals

Measurements were carried out in HKRB composed of (in mM) 129 NaCl, 5.0 NaHCO₃, 4.7 KCl, 1.2 KH₂PO₄, 2.0 CaCl₂, 1.2 MgSO₄, and 10 HEPES at pH 7.4 supplemented

with 0.1% BSA. Fura-2 and fura-2/acetoxymethyl ester were obtained from Dojin Chemical (Kumamoto, Japan). All other chemicals were purchased from Sigma.

Measurements of [Ca²⁺]_i

Following culture for 3 days under control and test conditions, the cells were first incubated in HKRB containing 2.8 mM glucose for 30 min for stabilization and fura-2 loading, and then subjected to [Ca²⁺]_i measurements (Fig. 1) as reported previously (Yanagida *et al.* 2002). [Ca²⁺]_i was measured by fura-2 microfluorometry according to the reported procedure (Yada *et al.* 1994). Briefly, the cells on cover slips were incubated with 1 μM fura-2/acetoxymethyl ester in HKRB for 30 min. Cells were then mounted in a chamber and superfused with HKRB at a rate of 1 ml/min at 37 °C. Cells were excited at 340 and 380 nm alternately every 2.5 s, emission signals at 510 nm (F340 and F380 respectively) were detected with an intensified charge-coupled device camera, and ratio (F340/F380) images were produced by an Argus-50 imaging system (Hamamatsu Photonics, Hamamatsu, Japan). Ratio values were converted to [Ca²⁺]_i according to calibration curves obtained from the relationship between free Ca²⁺ concentration and the ratio determined in a cytosol-mimicking solution using Ca-EGTA buffer and fura-2 free acid (Yada *et al.* 1995).

Selection of single β-cells and criteria for glucose responses

β-Cells were selected according to the previously reported procedure (Yada *et al.* 1995). Single islet cells on cover slips that had a diameter of 12.5–17.5 μm and responded to tolbutamide (300 μM) with the increases in [Ca²⁺]_i were found to be immunocytochemically positive for insulin. Data were taken from the cells that fulfilled these morphological and physiological criteria for β-cells.

Figure 1 Protocols for culture of islet cells and [Ca²⁺]_i measurements. Single cells isolated from rat islets were cultured for 3 days under control conditions with 5.6 mM glucose and those with corticosterone (1, 10, 50, and 500 ng/ml), RU-486 (500 ng/ml), corticosterone (500 ng/ml) and RU-486 (500 ng/ml), dexamethasone (1000 ng/ml), spironolactone (5000 ng/ml), corticosterone (500 ng/ml) and spironolactone (5000 ng/ml), aldosterone (5 ng/ml), and corticosterone (500 ng/ml) and aldosterone (5 ng/ml). After the culture, the cells were first incubated for 30 min in HKRB with 2.8 mM glucose for stabilization and fura-2 loading, and then subjected to measurements of [Ca²⁺]_i. Tolb, tolbutamide; 2.8G, 2.8 mM glucose; 8.3G, 8.3 mM glucose.

Only the $[\text{Ca}^{2+}]_i$ increase that took place within 10 min upon stimulation with 8.3 mM glucose and whose amplitude was >80 nM was considered as the response.

Protocol to examine long-term effects of glucocorticoids and related agents on glucose responses in β -cells

Single cells isolated from islets were cultured for 3 days in control conditions and those with corticosterone, RU-486, dexamethasone, spironolactone, and aldosterone (Fig. 1). After the culture, the cells were first incubated for 30 min in HKRB with 2.8 mM glucose for stabilization and fura-2 loading, and then subjected to measurements of $[\text{Ca}^{2+}]_i$. Stimulation with 8.3 mM glucose induced the first-phase $[\text{Ca}^{2+}]_i$ increase followed by the second-phase $[\text{Ca}^{2+}]_i$ increase superimposed with $[\text{Ca}^{2+}]_i$ oscillations in β -cells, as reported previously (Yada *et al.* 1995, Dezaki *et al.* 2004). During the 3-day period of control culture, the incidence and amplitude of the first-phase $[\text{Ca}^{2+}]_i$ increase decreased to a marginal degree and in a consistent manner, while those of $[\text{Ca}^{2+}]_i$ oscillations fell down substantially. In this study, effects of glucocorticoids and related substances on the first-phase $[\text{Ca}^{2+}]_i$ increases were exclusively investigated.

Statistical analysis

The calculated values are expressed as mean $\pm$ S.E.M. (n =number of observations). The statistical analysis was carried out by unpaired t -test and χ^2 -test.

Results

Concentration-dependent effects of corticosterone treatment on $[\text{Ca}^{2+}]_i$ responses to glucose in β -cells

Physiological glucocorticoid hormone released from adrenal cortex is corticosterone in rats, while it is cortisol in humans. The plasma corticosterone level is below 50 ng/ml under physiological conditions and ranges from 50 to 500 ng/ml under stress conditions (Raone *et al.* 2007). Therefore, the present study used 1, 10, 50, and 500 ng/ml corticosterone administered in culture.

Isolated islet cells were cultured for 3 days in the medium without or with corticosterone at varying concentrations of 1–500 ng/ml. Following the culture, the single islet cells were subjected to $[\text{Ca}^{2+}]_i$ measurements by fura-2 fluorescence imaging under superfusion conditions. As shown in Fig. 2A, in control experiments after culture without glucocorticoids for 3 days, a rise in the superfusate glucose concentration from 2.8 to 8.3 mM induced a rapid increase in $[\text{Ca}^{2+}]_i$ that corresponds to the first-phase secretory response to glucose (Billaudel *et al.* 1984), and it occurred in 590 out of 820 β -cells examined (72.0%; Fig. 3A). The incidence of the glucose-induced first-phase $[\text{Ca}^{2+}]_i$ increase was decreased in the β -cells after treatment with corticosterone in culture: 309 out of 460 cells (67.2%) with corticosterone at 1 ng/ml, 166 out of

Figure 2 $[\text{Ca}^{2+}]_i$ responses to glucose in single β -cells after treatment with corticosterone for 3 days in culture. $[\text{Ca}^{2+}]_i$ responses to 8.3 mM glucose and 300 μM tolbutamide (Tolb) in β -cells following culture for 3 days under (A) control conditions and those added with (B) corticosterone at 10 ng/ml and (C) 500 ng/ml. $[\text{Ca}^{2+}]_i$ responses to 8.3 mM glucose, but not to Tolb, were impaired in β -cells after culture with 500 ng/ml corticosterone. Cells were superfused with HKRB containing 2.8 mM (2-8G) or 8.3 mM glucose (8-3G) at 37 °C. Tolb was added to superfusate at the periods specified by the bars above tracings.

264 cells (62.9%) at 10 ng/ml, 190 out of 312 cells (60.9%) at 50 ng/ml, and 234 out of 391 cells (59.8%) at 500 ng/ml (Fig. 3A). Thus, corticosterone concentration dependently lowered the incidence of the β -cell $[\text{Ca}^{2+}]_i$ responses to glucose, in which significant differences were observed between control group and either of 10, 50, or 500 ng/ml corticosterone groups. Treatment with corticosterone also decreased the amplitude of $[\text{Ca}^{2+}]_i$ responses to glucose:

Figure 3 Concentration-dependent effects of corticosterone treatment on incidence and amplitude of $[Ca^{2+}]_i$ responses to glucose in β -cells. (A) Incidence and (B) mean amplitude of $[Ca^{2+}]_i$ responses to glucose in β -cells after culture under control conditions and those with corticosterone at 1, 10, 50, and 500 ng/ml. Incidence is expressed by the number of β -cells that responded to glucose over those responded to Tolb (%). In (A), the numbers above each bar indicate the number of β -cells that responded to glucose over that examined. In (B), the numbers above each bar indicate the number of β -cells examined. * $P < 0.05$ versus control, # $P < 0.05$ versus control.

192.8 nM in control, 180.6 nM with corticosterone at 1 ng/ml, 174.7 nM at 10 ng/ml, 173.9 nM at 50 ng/ml, and 164.8 nM at 500 ng/ml (Figs 2A–C and 3B). There was a significant difference between control and 500 ng/ml corticosterone groups (Fig. 3B). The addition of 300 μ M tolbutamide (Tolb) induced $[Ca^{2+}]_i$ increases in β -cells, and their amplitude was not significantly altered by corticosterone at any concentrations in culture (Fig. 2).

Effects of co-administration of RU-486 with corticosterone on $[Ca^{2+}]_i$ responses to glucose in β -cells

$[Ca^{2+}]_i$ responses to glucose in β -cells were suppressed by 500 ng/ml corticosterone and this suppression was attenuated by 500 ng/ml RU-486, a GR antagonist, added simultaneously in culture (Fig. 4A, C, and D). Incidence of $[Ca^{2+}]_i$ responses to glucose (control; 216 out of 389 cells (55.5%)) was reduced with 500 ng/ml corticosterone (205 out of 419 cells (48.9%)) and this reduction was significantly restored

with 500 ng/ml RU-486 (239 out of 406 cells (58.8%), $P < 0.05$; Fig. 5A). Amplitude of $[Ca^{2+}]_i$ responses (control; 170.8 ± 7.9 nM) was also suppressed with corticosterone (133.4 ± 6.9 nM) and this suppression was significantly restored with RU-486 (166.5 ± 7.6 nM, $P < 0.05$; Fig. 5B). In addition, RU-486, when added to control culture without corticosterone, significantly enhanced $[Ca^{2+}]_i$ responses to glucose (Fig. 4B versus A) both in incidence (262 out of 379 cells (69.0%)) with RU-486 versus 216 out of 389 cells (55.5%) in control, $P < 0.05$ and in amplitude (210.7 ± 8.4 nM with RU-486 versus 170.8 ± 7.9 nM in control, $P < 0.05$; Fig. 5).

Effects of dexamethasone treatment on $[Ca^{2+}]_i$ responses to glucose in β -cells

Treatment for 3 days with dexamethasone at 1000 ng/ml, but not 10 ng/ml, suppressed $[Ca^{2+}]_i$ responses to glucose in β -cells (Fig. 6A and B). Incidence of $[Ca^{2+}]_i$ responses to glucose (control; 151 out of 270 cells (55.9%)) was reduced with 1000 ng/ml dexamethasone (77 out of 173 cells (44.5%), $P < 0.05$) but unaltered with 10 ng/ml dexamethasone (127 out of 224 cells (56.7%)) (Fig. 6C). Amplitude of $[Ca^{2+}]_i$ increases (control; 158.6 ± 9.3 nM) was significantly lowered with dexamethasone at 1000 ng/ml (117.9 ± 9.7 nM, $P < 0.05$) but not at 10 ng/ml (148.6 ± 9.4 nM; Fig. 6D). The extent of suppression of $[Ca^{2+}]_i$ responses by 1000 ng/ml dexamethasone was somewhat greater than that by 500 ng/ml corticosterone in both the incidence and amplitude.

Effects of co-administration of spironolactone with corticosterone on $[Ca^{2+}]_i$ responses to glucose in β -cells

$[Ca^{2+}]_i$ responses to glucose in β -cells were suppressed by 500 ng/ml corticosterone and this suppression was amplified by 5000 ng/ml spironolactone, an MR antagonist, added simultaneously in culture (Fig. 7A, C, and D). Incidence of $[Ca^{2+}]_i$ responses to glucose (control; 180 out of 276 cells (65.2%)) was reduced with corticosterone (137 out of 233 cells (58.8%)) and this reduction was markedly enhanced by spironolactone (137 out of 286 cells (47.9%), $P < 0.05$; Fig. 8A). Amplitude of $[Ca^{2+}]_i$ responses (control; 184.4 ± 8.7 nM) was also suppressed with corticosterone (160.8 ± 8.0 nM) and this suppression was significantly enhanced with spironolactone (138.0 ± 7.6 nM, $P < 0.05$; Fig. 8B). On the other hand, spironolactone, when added to control culture without corticosterone, had no effect on $[Ca^{2+}]_i$ responses to glucose (Figs 7B and 8).

Effects of co-administration of aldosterone with corticosterone on $[Ca^{2+}]_i$ responses to glucose in β -cells

$[Ca^{2+}]_i$ responses to glucose occurred in 161 out of 243 cells (66.3%) in controls and 167 out of 264 cells (63.3%) following treatment with 5 ng/ml aldosterone, an MR agonist

(DeLoof *et al.* 2000, Mazzocchi *et al.* 2000, Madsen *et al.* 2003; Fig. 9). Neither the amplitude nor the pattern of $[\text{Ca}^{2+}]_i$ responses to glucose was altered by aldosterone. Incidence of $[\text{Ca}^{2+}]_i$ responses to glucose was reduced with corticosterone

(187 out of 322 cells (58.0%), $P < 0.05$) and this reduction was significantly attenuated by aldosterone (322 out of 490 cells (65.7%), $P < 0.05$; Fig. 9).

Discussion

We found that semi-chronic *in vitro* exposure of rat β -cells to pathophysiological concentrations of corticosterone for 3 days suppressed the $[\text{Ca}^{2+}]_i$ response to glucose and this suppression was mediated by activation of GR. Furthermore, simultaneous stimulation of MR by corticosterone partially counteracted the GR-mediated suppression by corticosterone and thereby protected the β -cell responsiveness to glucose.

Corticosterone administered in culture at a high concentration of 500 ng/ml significantly inhibited both the incidence and amplitude of $[\text{Ca}^{2+}]_i$ responses to glucose. Corticosterone at lower concentrations of 10 and 50 ng/ml also significantly decreased the incidence of $[\text{Ca}^{2+}]_i$ responses and tended to decrease the amplitude of $[\text{Ca}^{2+}]_i$ responses albeit not significantly. Thus, corticosterone at 500 ng/ml markedly and at 10–50 ng/ml partially suppressed $[\text{Ca}^{2+}]_i$ responses to glucose. These concentration-dependent effects of corticosterone on islet β -cells virtually fit with those reported previously (Lambillotte *et al.* 1997, Jeong *et al.* 2001). A GR antagonist RU-486, when co-administered with corticosterone, counteracted the corticosterone suppression of the incidence and amplitude of $[\text{Ca}^{2+}]_i$ responses to glucose. Conversely, when β -cells were cultured with a GR agonist dexamethasone, the incidence and amplitude of $[\text{Ca}^{2+}]_i$ responses to glucose were strongly depressed in which the magnitude of depression was somewhat greater than that obtained with corticosterone at the highest concentration. Dexamethasone is ~ 100 times a more potent agonist for GR than corticosterone, and has little MR agonist activity (Delaunay *et al.* 1997). These results indicate that impairment of β -cell responses to glucose by 3 days corticosterone treatment is mediated primarily by activation of GR. We also found that RU-486 was effective in the absence of exogenous corticosterone: treatment with RU-486 increased the incidence and amplitude of $[\text{Ca}^{2+}]_i$ responses to glucose in β -cells. This finding suggests that corticosterone present in the serum-containing culture medium exerts certain suppressive effects via GR in β -cells and this effect is blocked

Figure 4 Effects of administration of RU-486 with/without corticosterone on $[\text{Ca}^{2+}]_i$ responses to glucose in β -cells. $[\text{Ca}^{2+}]_i$ responses to 8.3 mM glucose in β -cells following culture for 3 days under (A) control conditions and those added with (B) a GR antagonist RU-486 at 500 ng/ml, (C) corticosterone at 500 ng/ml, and (D) corticosterone plus RU-486. $[\text{Ca}^{2+}]_i$ responses to 8.3 mM glucose were impaired in β -cells after culture with corticosterone, and this impairment was counteracted by co-administration of RU-486. Treatment with RU-486 by itself increased the amplitude of $[\text{Ca}^{2+}]_i$ responses to 8.3 mM glucose.

Figure 5 Effects of administration of RU-486 with/without corticosterone on incidence and amplitude of $[Ca^{2+}]_i$ responses to glucose in β -cells. (A) Incidence and (B) amplitude of $[Ca^{2+}]_i$ responses to glucose were suppressed by 500 ng/ml corticosterone and restored by 500 ng/ml RU-486 co-administered with corticosterone. When added to control culture without corticosterone, RU-486 significantly enhanced both the incidence and amplitude of $[Ca^{2+}]_i$ responses to glucose. * $P < 0.05$ versus control. # $P < 0.05$ between 500 ng/ml corticosterone plus 500 ng/ml RU-486 and 500 ng/ml corticosterone.

by RU-486. Hence, glucocorticoids at physiological levels could negatively regulate β -cell functions and insulin release via GR. In support of this notion, the β -cell-specific GR transgenic mice display a marked decrease in acute insulin response in glucose tolerance tests (Delaunay *et al.* 1997, Davani *et al.* 2004).

When spironolactone, an antagonist of MR, was administered simultaneously with high concentrations of corticosterone in culture, both the incidence and amplitude of $[Ca^{2+}]_i$ responses to glucose in β -cells were further reduced compared with those in the group with corticosterone alone. This result suggests that MR stimulation by high concentrations of corticosterone counteracts the GR-mediated suppressive effect of corticosterone and this action is blocked by spironolactone. This function of MR was further supported by the result that aldosterone, the natural ligand for MR, significantly attenuated the corticosterone suppression of $[Ca^{2+}]_i$ responses to glucose. On the other hand, spironolactone by itself affected neither the incidence nor the amplitude of $[Ca^{2+}]_i$ responses to glucose in

Figure 6 $[Ca^{2+}]_i$ responses to glucose in single β -cells after treatment with dexamethasone for 3 days in culture. $[Ca^{2+}]_i$ responses to 8.3 mM glucose in β -cells following culture for 3 days under (A) control conditions and those added with (B) dexamethasone (1000 ng/ml). $[Ca^{2+}]_i$ responses to 8.3 mM glucose were impaired in β -cells after culture with dexamethasone. (C) Incidence and (D) amplitude of $[Ca^{2+}]_i$ responses to glucose were suppressed by dexamethasone at 1000 ng/ml but not at 10 ng/ml. * $P < 0.05$ between 1000 ng/ml dexamethasone and control.

β -cells, suggesting that MR does not function in the regular culture. It could be due to this, that the corticosterone concentration in the culture medium containing 10% serum is within physiological levels and that corticosterone is a less potent ligand for MR than for GR. The results indicate a

novel function of MR to counteract the deteriorating effect of excessive GR activation by high levels of corticosterone in β -cells. In contrast, MR may not function under physiological conditions, since neither spironolactone nor aldosterone, the natural ligand for MR, affected glucose-induced $[Ca^{2+}]_i$ increases in β -cells.

In this study, treatment with corticosterone or dexamethasone for 3 days suppressed the first-phase $[Ca^{2+}]_i$ responses to glucose. It was reported that treatment with dexamethasone for 18 h altered the $[Ca^{2+}]_i$ oscillations during the second-phase $[Ca^{2+}]_i$ responses to glucose, while the first-phase $[Ca^{2+}]_i$ responses were intact in mouse islets (Henquin *et al.* 2006). The selective alteration of $[Ca^{2+}]_i$ oscillations by shorter term exposure to dexamethasone fits with the notion that the $[Ca^{2+}]_i$ oscillation is a fragile process that is easily influenced by diabetogenic factors (Hellman *et al.* 1990). Collectively, though $[Ca^{2+}]_i$ oscillations can be altered by short-term exposure to glucocorticoids, suppression of the first-phase $[Ca^{2+}]_i$ responses requires exposure for longer periods.

It was reported that dexamethasone suppression of insulin secretion was blunted in mouse islets treated with pertussis toxin (PTX; Lambillotte *et al.* 1997), an inhibitor of Gi/Go subtypes of trimetric G-proteins (Katada & Ui 1979) that play a crucial role in inhibition of insulin secretion (Sharp 1996). A PTX-sensitive Gi subtype is linked to activation of voltage-dependent K^+ (Kv) channels and reduction of glucose-induced first-phase $[Ca^{2+}]_i$ increases in rat islet β -cells (Dezaki *et al.* 2007). Dexamethasone increases the expression of Kv1.5 via upregulation of serum- and glucocorticoid-inducible kinase 1 and thereby reduces glucose-induced $[Ca^{2+}]_i$ increases in mouse islets and INS-1 cells (Ullrich *et al.* 2005). Collectively, PTX-sensitive Gi/Go proteins and K^+ channels could be involved in the corticosterone suppression of first-phase $[Ca^{2+}]_i$ increases in β -cells, though the precise mechanisms remain to be elucidated. Corticosterone could additionally affect Ca^{2+} channels and/or pumps in different internal stores that have been implicated in the glucose-induced $[Ca^{2+}]_i$ signaling in β -cells (Hamakawa & Yada 1995, Roe *et al.* 1998).

The novel and important finding of this study is that stimulation of MR counteracts the GR-mediated suppression of glucose-induced $[Ca^{2+}]_i$ increases in β -cells. This suggests that MR could serve as an attenuator of the GR-mediated deteriorating action of excessive glucocorticoid on islet β -cells, which leads to progression of type 2 diabetes.

Figure 7 Effects of administration of spironolactone with/without corticosterone on $[Ca^{2+}]_i$ responses to glucose in β -cells. $[Ca^{2+}]_i$ responses to 8.3 mM glucose in β -cells following culture for 3 days under (A) control conditions and those added with (B) an MR antagonist spironolactone at 5000 ng/ml, (C) corticosterone at 500 ng/ml, and (D) corticosterone and spironolactone. $[Ca^{2+}]_i$ responses to 8.3 mM glucose were impaired in β -cells after culture with corticosterone, and this impairment was enhanced by co-administration of spironolactone. Treatment with spironolactone by itself had little effect on $[Ca^{2+}]_i$ responses to 8.3 mM glucose.

Figure 8 Effects of administration of spironolactone with/without corticosterone on incidence and amplitude of $[Ca^{2+}]_i$ responses to glucose in β -cells. (A) Incidence and (B) amplitude of $[Ca^{2+}]_i$ responses to 8.3 mM glucose were decreased by corticosterone (500 ng/ml) and further inhibited by spironolactone (5000 ng/ml) co-administered with corticosterone. When added to control culture without corticosterone, spironolactone affected neither incidence nor amplitude of $[Ca^{2+}]_i$ responses to glucose. * $P < 0.05$ between 500 ng/ml corticosterone versus control. # $P < 0.05$ between 500 ng/ml corticosterone versus 5000 ng/ml spironolactone and 500 ng/ml corticosterone.

Figure 9 Effects of administration of aldosterone with/without corticosterone on incidence of $[Ca^{2+}]_i$ responses to glucose in β -cells. Incidence of $[Ca^{2+}]_i$ responses to 8.3 mM glucose in β -cells following culture for 3 days in control conditions and those added with aldosterone at 5 ng/ml. $[Ca^{2+}]_i$ responses to 8.3 mM glucose were impaired in β -cells after culture with 500 ng/ml corticosterone and restored by 5 ng/ml aldosterone co-administered with corticosterone. * $P < 0.05$ between 500 ng/ml corticosterone and control. # $P < 0.05$ between 500 ng/ml corticosterone plus 5 ng/ml aldosterone and 500 ng/ml corticosterone.

Blockade of GR action and upregulation of MR action in combination could be an effective tool to protect islet β -cells against stress and metabolic syndrome, and thereby prevent type 2 diabetes. Further studies are definitely required to clarify the roles and mechanisms for the novel protective action of MR in pancreatic β -cells.

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