

Aged Transgenic Mice With Increased Glucocorticoid Sensitivity in Pancreatic β -Cells Develop Diabetes

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Glucocorticoids are diabetogenic hormones because they decrease glucose uptake, increase hepatic glucose production, and inhibit insulin release. To study the long-term effects of increased glucocorticoid sensitivity in β -cells, we studied transgenic mice overexpressing the rat glucocorticoid receptor targeted to the β -cells using the rat insulin I promoter. Here we report that these mice developed hyperglycemia both in the fed and the overnight-fasted states at 12–15 months of age. Progression from impaired glucose tolerance, previously observed in the same colony at the age of 3 months, to manifest diabetes was not associated with morphological changes or increased apoptosis in the β -cells. Instead, our current results suggest that the development of diabetes is due to augmented inhibition of insulin secretion through α_2 -adrenergic receptors (α_2 -ARs). Thus, we found a significantly higher density of α_2 -ARs in the islets of transgenic mice compared with controls, based on binding studies with the α_2 -AR agonist UK 14304. Furthermore, incubation of islets with benextramine, a selective antagonist of the α_2 -AR, restored insulin secretion in response to glucose in isolated islets from transgenic mice, whereas it had no effect on control islets. These results indicate that the chronic enhancement of glucocorticoid signaling in pancreatic β -cells results in hyperglycemia and impaired glucose tolerance. This effect may involve signaling pathways that participate in the regulation of insulin secretion via the α_2 -AR. *Diabetes* 53 (Suppl. 1): S51–S59, 2004

Type 2 diabetes is characterized by hyperglycemia that develops because of peripheral insulin resistance, increased glucose production in the liver, and impaired β -cell function (1–3). It is generally accepted that the disease develops on the basis

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α_2 -AR, α_2 -adrenergic receptor; DAPI, 4'-6-diamino-2-phenylindole; GC, glucocorticoid; GR, GC receptor; HBSS, Hanks' balanced salt solution; IPGTT, intraperitoneal glucose tolerance test; IPITT, intraperitoneal insulin tolerance test; KRB, Krebs-bicarbonate buffer; TUNEL, TdT-mediated dUTP nick-end labeling.

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of interactions between heredity and environment. Among environmental factors, stress plays an important role (4,5). Glucocorticoids (GCs) are stress hormones that exert their diabetogenic effect by increasing glucose production, inducing insulin resistance in muscle, and decreasing insulin secretion (6,7). Although the effect of GCs on glucose production and insulin sensitivity is well studied, the mechanisms whereby glucocorticoids inhibit insulin secretion are poorly understood. Because in vivo treatment with GCs affects the metabolism of multiple organs (e.g., endocrine pancreas, liver, muscle, and adipose tissue), the significance of any direct effect of excess GCs on the β -cell in vivo is difficult to evaluate. Therefore, we have developed an animal model in which the GC sensitivity of the β -cells was selectively increased due to cell-specific overexpression of GC receptors (GRs). Using this transgenic model (TG mice), we have described impairments in glucose tolerance and insulin secretion both in vivo and in vitro (8,9).

Previous studies have suggested that the inhibitory effect of GCs on insulin secretion might be mediated by α -adrenergic receptors. Thus, GCs have been shown to upregulate α_2 -adrenergic receptor (α_2 -AR) expression and signaling in β -cell lines (10). This is associated with a profound inhibitory effect on insulin secretion. Similarly, overexpression of α_2 -AR attenuates basal and stimulated insulin secretion in insulinoma cells (11). α_2 -AR agonists also inhibit insulin release in vivo in humans and in experimental animals (12–15). Furthermore, α_2 -AR agonists, such as UK 14304, clonidine, and oxymethazoline, inhibit glucose-stimulated insulin release from isolated islets and insulin-producing cell lines (16–18). Moreover, transgenic mice overexpressing α_{2A} -AR in pancreatic β -cells showed decreased glucose-stimulated insulin release, resulting in glucose intolerance (19). The inhibitory effect of α_2 -AR agonists on insulin release is blocked by α_2 -AR antagonists (e.g., yohimbine or idazoxan) (20,21). In the present study, we investigated whether islet α_2 -ARs participate in the development of stress-induced diabetes. Therefore, we have studied whether the impairment of insulin secretion in TG mice is associated with an increase in expression and signaling by α_2 -ARs in the β -cells.

RESEARCH DESIGN AND METHODS

Animals. Control and transgenic mice, 12–15 months of age, were used. The characteristics and genetic background of these animals have been described previously (8). Mice were housed at 22°C with a daylight period of 12 h and fed diet and water ad libitum. Food was removed 15 h before fasting experiments. Experiments were performed according to the National Ethical Guidelines for the Care and Use of Laboratory Animals.

Intraperitoneal glucose tolerance test and acute insulin response.

Intraperitoneal glucose tolerance tests (IPGTTs) were performed in overnight fasted animals. Blood was collected before (0 min) and after intraperitoneal injection of glucose (2 g/kg body wt) at 15, 30, 60, 90, and 120 min. In a separate series of experiments, overnight fasted mice (both control and TG) were divided into two groups. One group of mice (six control and six TG) was killed and blood was collected (0 min). Another group of mice (six control and six TG) was injected with glucose (2 g/kg body wt i.p.), and blood was collected at decapitation after 10 min of glucose injection.

Intraperitoneal insulin tolerance test. An intraperitoneal insulin tolerance test (IPITT) was performed in overnight fasted animals. At -10 min, blood glucose levels were measured before injection of insulin (0.25 units/kg body wt; Actrapid, Novo Nordisk, Copenhagen, Denmark). Ten minutes after administration of insulin, blood glucose was measured (0 min) and a bolus of glucose (1 g/kg body wt i.p.) was given to the animals. Blood was then collected at intervals of 15, 30, 60, 90, and 120 min to determine glucose concentrations.

Experiments with isolated islets. Islets were isolated by collagenase digestion. In brief, the pancreas was digested for 10–12 min under continuous shaking (150 strokes/min) at 37°C in 2.5–3.0 ml Hanks' balanced salt solution (HBSS) containing 6–8 mg collagenase. After digestion, 15–20 ml of the cold HBSS was added into the incubation medium, and the suspension was allowed to settle. The sediment was washed several times with cold HBSS, and the islets from the sediment were collected by a pipette under stereomicroscopy.

For measurements of insulin release in islets, static incubations were used. Islets were always preincubated in Krebs-bicarbonate buffer (KRB) (pH 7.4) supplemented with 3.3 mmol/l glucose for 45 min at 37°C before being incubated at further concentrations or other stimuli. The incubation was stopped by chilling the samples in ice-cold water. The incubation medium was removed and stored at -20°C for insulin assay.

Insulin assay. Insulin was measured by radioimmunoassay with the addition of charcoal to separate free and bound antibody (22).

Ligand binding assay. Binding of [³H]UK 14304 to islets was performed as described previously (23). In brief, islets were preincubated for 60 min in KRB at 3.3 mmol/l glucose, after which triplicate batches of 25 islets in 100 μl KRB (without glucose) were placed into microfuge tubes containing 150 μl of a mixture of dibutylphthalate and dinonylphthalate (10:3), layered above 40 μl 6 mol/l urea. Then, 50 μl KRB containing 50 nCi [³H]UK 14304 (specific activity 75 Ci/mmol; NEN) and 50 nCi [¹⁴C]sucrose (specific activity 0.67 Ci/mmol; NEN) was added to the tubes. The final concentration of [³H]UK 14304 in the medium was 4.5×10^{-9} mol/l, except in experiments used for Scatchard analysis, in which serial dilutions (1:2) from 9×10^{-9} to 2.8×10^{-10} mol/l were used. The tubes were incubated for 40 min (unless indicated otherwise) at 37°C and then centrifuged for 15 s. The lower section of each tube containing the islets was cut off and placed in a liquid scintillation vial, 5 ml of scintillation fluid (Ultima Gold, Packard Instruments) was added, and radioactivity in the ³H and ¹⁴C peaks was determined by liquid spectrometry (Packard Tri-Carb 1900 TR Liquid Scintillation analyzer). Total binding was calculated as the radioactivity remaining in the islets after subtraction of radioactivity in the sucrose space. Specific binding was defined as the difference between total binding and nonspecific binding obtained in the presence of 3.3×10^{-6} mol/l unlabelled UK 14304 (Tocris Cookson, Bristol, U.K.).

For protein determination, islets were lysed in a buffer containing 80 mmol/l Tris, pH 6.8, 5 mmol/l EDTA, 5% SDS, 2 mmol/l *N*-ethylmaleimide, and 2 mmol/l phenyl methylsulfonyl fluoride and sonicated for 1.5 min. Total protein was quantified by BCA assay (Pierce) using BSA as a standard.

Tissue processing for immunocytochemistry. Pancreatic specimens were fixed by immersion overnight in Stefanini's fixative (2% formaldehyde and 0.2% picric acid in phosphate buffer, pH 7.2), rinsed repeatedly in Tyrode solution enriched with sucrose (10% wt/vol), and frozen on dry ice. Sections (10 μm thickness) were cut in a cryostat, collected on chrome alum-coated slides, and then processed for immunocytochemistry. The primary antibodies were as follows: for insulin detection, an anti-unconjugated human proinsulin antibody raised in guinea pig (code 9003, dilution 1:1,280; Euro-Diagnostica, Malmö, Sweden); for glucocorticoid receptor detection, a mAb against rat GR raised in mouse (8); for glucagon detection, an anti-protein-conjugated glucagon antibody raised in rabbit (code 7811, dilution 1:5,120; Euro-Diagnostica); and for detection of GLUT2, a rabbit anti-rat GLUT2 antiserum (dilution 1:200; Chemicon International, Temecula, CA). The sections were then incubated for 1 h at room temperature with a secondary antibody, coupled to fluorescein isothiocyanate with specificity for IgG (dilution 1:80; Euro-Diagnostica) of the primary antibody, and examined in a fluorescence microscope. **TdT-mediated dUTP nick-end labeling (TUNEL) assay for apoptosis.** TUNEL was performed on 4-μm thick paraffin sections of formaldehyde-fixed pancreatic tissue, using an *in situ* cell death detection kit, according to the

manufacturer's instructions (Roche Molecular Biochemicals, Mannheim, Germany) as described earlier (24). The sections were counterstained for 10 min with 4'-6-diamino-2-phenylindole (DAPI) solution (10 μmol DAPI in 800 mmol disodiumhydrogenphosphate) and embedded with Vectashield (H-1000; Vector Laboratories, Burlingame, CA). The stainings were analyzed with a fluorescence microscope (BX50; Olympus, Tokyo) equipped with a digital charge-coupled device camera (DKC5000, Sony, Tokyo).

cDNA synthesis and RT-PCR. Total RNA was extracted from isolated pancreatic islets using Trizol (Invitrogen-Life Technologies) according to the manufacturer's instructions. The RNA was DNase-treated with RQ1 DNase (Promega) for 30 min at 37°C in a buffer containing 40 mmol/l Tris-HCl, pH 8.0, 10 mmol/l MgSO₄, 1 mmol/l CaCl₂, and 40 units RNaseOut (Invitrogen-Life Technologies) and re-extracted with Trizol. Reverse transcription was carried out with SuperScript II (Invitrogen-Life Technologies) in a reaction containing 5 μg total RNA, 0.1 μg random hexamer primers (Amersham-Pharmacia), and 40 units RNaseOut. Appropriate negative controls for each RNA consisted of a reaction mix identical to that above, except that di-ethylpyrocarbonate-treated water was added instead of SuperScript II. Relative expression levels of the α₂AR were assessed with the following primers: 5'-AGC TCC CCA AAA CCT CTT CCT-3' (sense) and 5'-CCA GCG CCC TTC TTC TCT ATG-3' (antisense); PCRs (10 μl) contained 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1 mmol/l MgCl₂, 200 μmol/l of each dNTP, 5 pmol of sense and antisense primers, and 0.4 units *Taq* polymerase (Amersham-Pharmacia) and cycled (Master cycler, Eppendorf) as follows: 94°C for 3 min, 94°C for 30 s to 60°C for 30 s to 72°C for 30 s (for the number of cycles indicated in RESULTS), and 72°C for 5 min. Relative expression levels of ribosomal protein L30 were assessed with the following primers: 5'-AAG TGG GAA GTA CGT GCT GG-3' (sense) and 5'-CAC CAG TCT GTT CTG GCA TG-3' (antisense), and PCRs were assembled as above, except that 10 mmol/l Tris-HCl, pH 8.6, and 2.5 mmol/l MgCl₂ were used; cycling was as follows: 94°C for 3 min, 94°C for 30 s to 62°C for 30 s to 72°C for 30 s (for the number of cycles indicated in RESULTS), and 72°C for 5 min. PCRs were electrophoresed on 1% agarose/0.5× Tris-borate-EDTA gels, and products (339 bp for the α₂AR, 272 bp for ribosomal protein L30) were documented with a digital camera and the appropriate software (EDAS 290; Kodak). Amplification of reverse transcription-negative controls were included for both PCRs, and no product was obtained from these reactions in either case.

Statistical analyses. Data analysis was carried out using Sigma Plot for Windows. All results are expressed as means ± SE. The statistical significance of differences between means was assayed by the Student's *t* test for unpaired data.

RESULTS

Body weight and nonfasting blood glucose. Transgenic and control mice had similar body weights (40 ± 2 vs. 42 ± 2 g, respectively; $n = 32$), whereas nonfasting blood glucose concentrations were significantly higher in TG mice than in controls (7.7 ± 0.5 vs. 5.5 ± 0.5 mmol/l; $P < 0.001$; $n = 29$).

IPGTT and insulin response. Fasting blood glucose concentrations were significantly higher in TG mice than in controls ($P < 0.05$) (Fig. 1). In control mice, glucose levels peaked at 15 min after intraperitoneal glucose administration; thereafter, glucose returned to baseline levels after 120 min. In contrast, in TG mice, glucose continued to rise until 30 min after administration and remained significantly higher than controls throughout the experiment. The fasting plasma insulin concentration was significantly lower in TG mice compared with controls ($P < 0.001$) (Fig. 2). At 10 min after glucose administration, the insulin response was increased in both TG and control mice. This insulin response to intraperitoneal glucose was significantly lower in TG mice (23% increase in TG vs. 39% increase in controls, compared with basal levels, $P < 0.001$).

IPITT. In overnight fasted animals used for insulin tolerance tests, blood glucose concentrations were higher in TG mice than in control mice (Fig. 3). Ten minutes after the administration of insulin (0 min), blood glucose concentrations did not differ between TG and control mice.

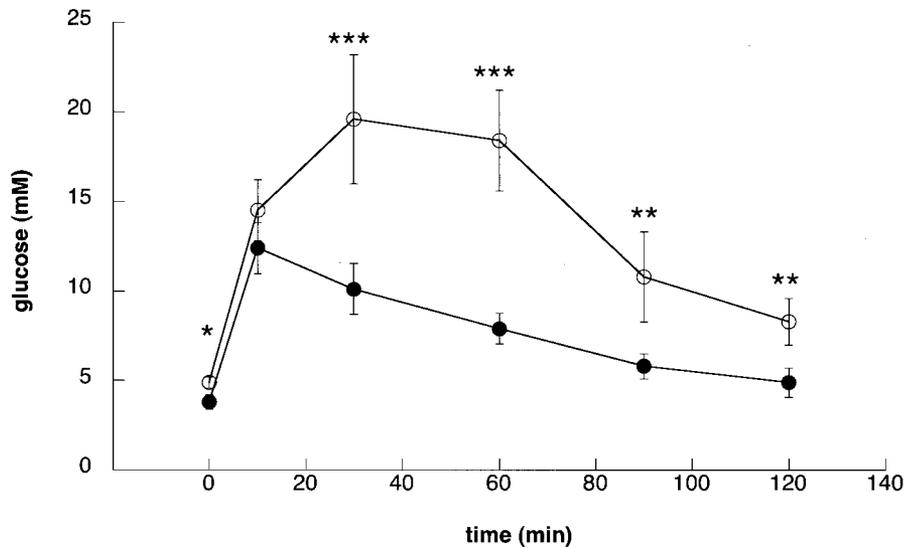


FIG. 1. IPGTT. Control (●) and TG (○) mice at 12–15 months of age were fasted overnight. Blood was collected before and after intraperitoneal injection of glucose (2 g/kg body wt) at the indicated times. Data are expressed as means \pm SE ($n = 12$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

There was a significant increase in blood glucose concentrations 15 min after the intraperitoneal administration of glucose in TG and control mice, and then glucose continued to fall during the whole period of the experiment (120 min). The increase in blood glucose concentration at 15 min and its subsequent fall was similar in TG and control animals.

Islet experiments. Islet insulin (198 ± 18.9 vs. 182 ± 21.4 μ U/islet; $n = 6$) and protein (1.56 ± 0.5 vs. 1.47 ± 0.7 mg/ml; $n = 6$) concentrations were similar in control and TG mice. In control mouse islets, glucose-induced insulin release was significantly higher at 16.7 mmol/l glucose than at 3.3 mmol/l glucose ($P < 0.001$) (Fig. 4). In TG

mouse islets, basal insulin release was similar to that from islets of control mice, but the insulin response to 16.7 mmol/l glucose was less in TG mouse islets than those of control mice. Insulin release at 3.3 mmol/l glucose in the presence of 20 mmol/l arginine was significantly higher than that at 3.3 mmol/l glucose alone, both in control and in TG islets, although TG islets secreted less insulin than control islets ($P < 0.001$). In TG islets, benextramine (10 μ mol), a selective α_2 -AR antagonist, had no effect on insulin release at 3.3 mmol/l glucose, whereas it completely normalized insulin release at 16.7 mmol/l (Fig. 5). Benextramine had no effect on insulin secretion in control islets at 3.3 and 16.7 mmol/l glucose.

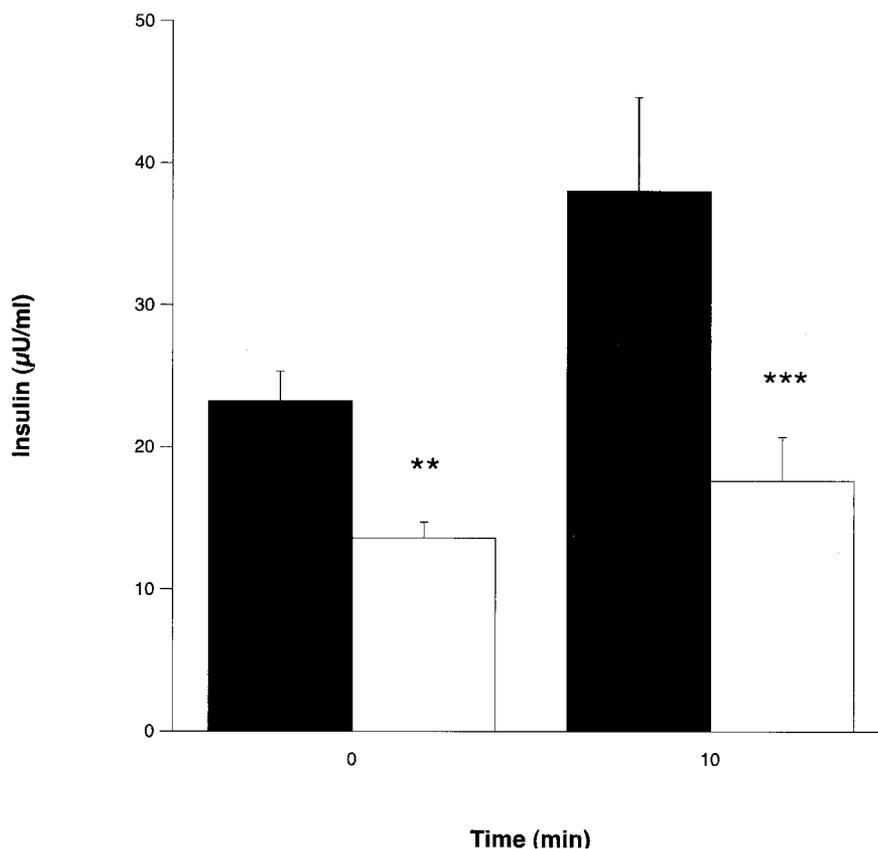


FIG. 2. Plasma insulin concentrations were measured in control (■) and TG (□) mice under basal conditions and 10 min after intraperitoneal glucose injection (2 g/kg body wt). Data are expressed as means \pm SE ($n = 6$). *** $P < 0.001$.

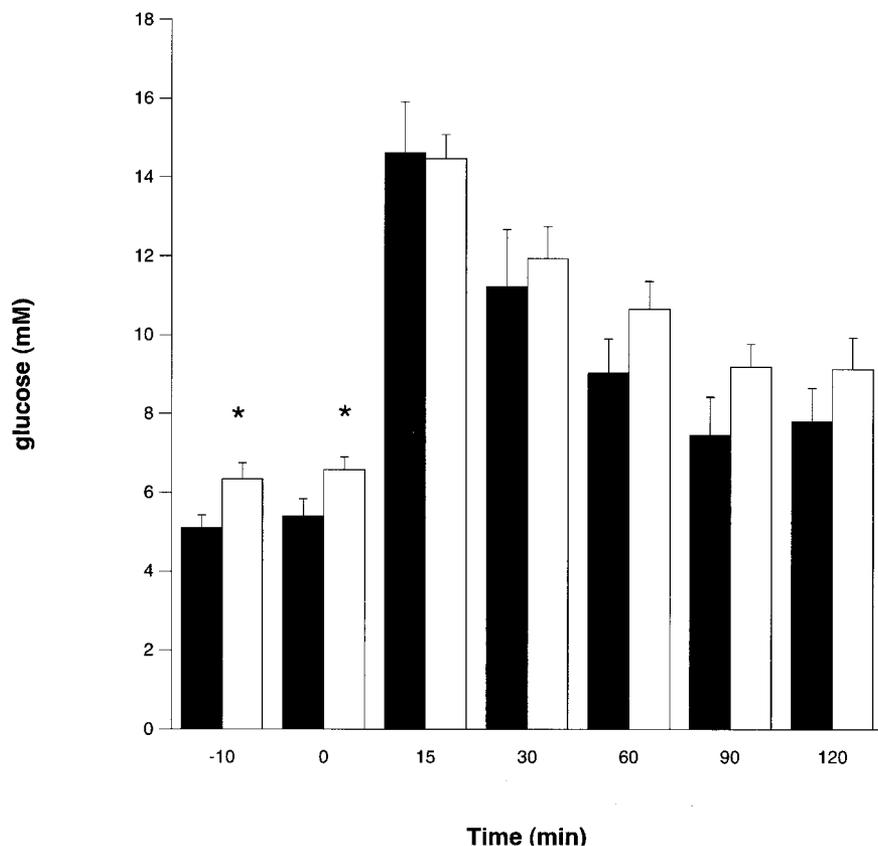


FIG. 3. IPITT. Control (■) and TG (□) mice at 12–15 months of age were fasted overnight. Blood glucose levels were measured before and after at the indicated times after challenge with insulin (0.25 units/kg body wt) and glucose (1 g/kg body wt) at the indicated times. Data are expressed as means ± SE (*n* = 6). **P* < 0.05.

α₂-AR determination by ligand binding assay. The specific binding of [³H]UK 14304 to both control and TG mouse islets was increased during 10–40 min of incuba-

tion, after which it reached equilibrium (data not shown). Hence, all further binding experiments were carried out using a 40-min incubation period. The maximum number

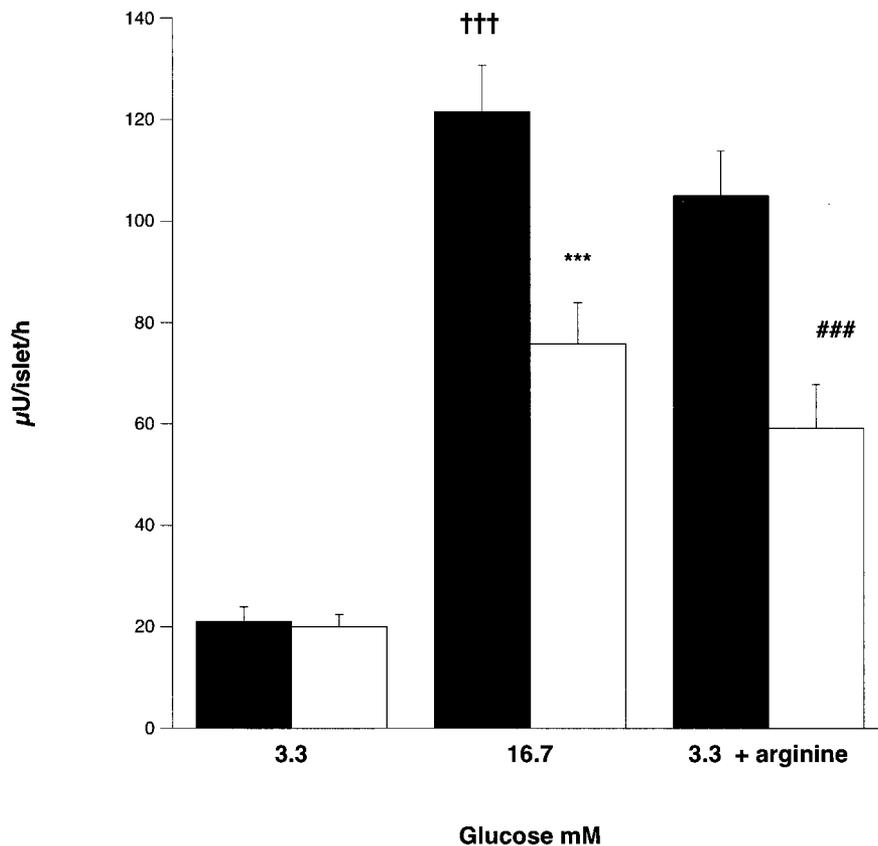


FIG. 4. Glucose-stimulated insulin secretion in isolated islets from control (■) and TG (□) mice. Batches of five islets were incubated in KRB containing glucose with or without 20 mmol/l arginine. Data are expressed as means ± SE (*n* = 5). †††*P* < 0.001 vs. 3.3 mmol/l glucose, ****P* < 0.001 vs. control, ###*P* < 0.001 vs. control.

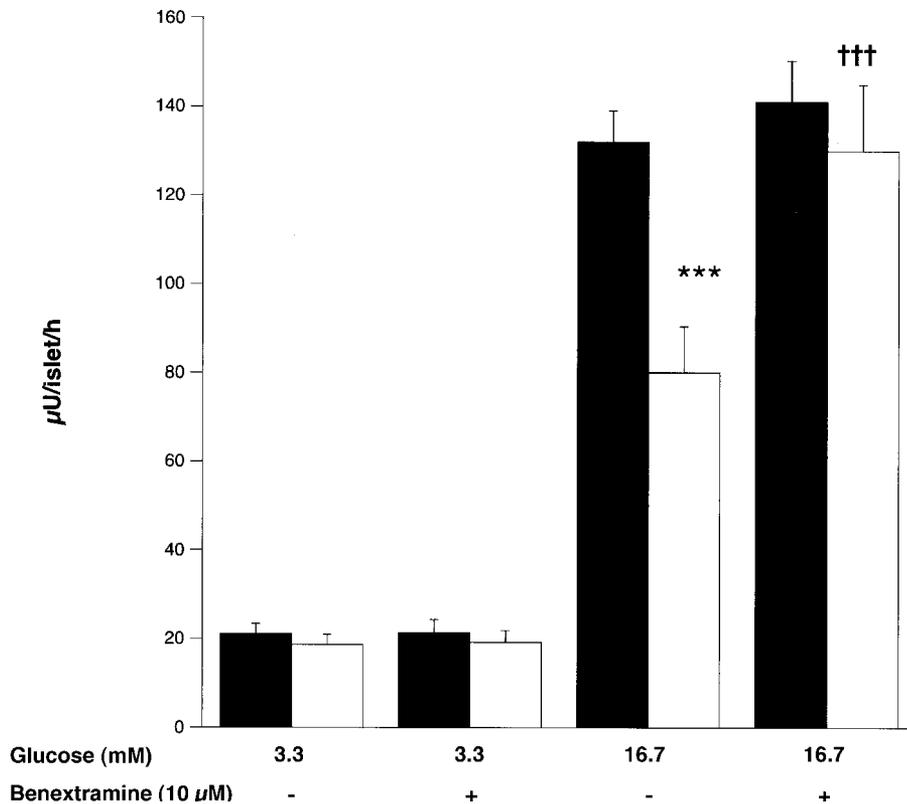


FIG. 5. Effects of the α_2 -AR antagonist, benextramine, on glucose-stimulated (16.7 mmol/l) insulin release from isolated islets of control (■) and TG (□) mice. Islets were incubated for 3 h in RPMI 1640 containing 3.3 or 16.7 mmol/l glucose with or without 10 μ mol/l benextramine. Data are expressed as means \pm SE ($n = 5$). *** $P < 0.001$ vs. control, ††† $P < 0.001$ vs. untreated TG.

of [3 H]UK 14304 binding sites (B_{max}) was estimated by Scatchard transformation of the binding curve (Fig. 6). Five independent experiments demonstrated that the [3 H]UK 14304 B_{max} values were 34 ± 5 and 23 ± 3 fmol/mg protein in TG and control islets, respectively ($P < 0.001$). No significant change in the dissociation constant (K_d) value was observed in TG versus control mice islets (1.6 ± 0.2 vs. 1.8 ± 0.3 nmol/l).

α_2 -AR determination by RT-PCR. The expression of the α_2 -AR mRNA in pancreatic islets isolated from control and TG mice was examined by semi-quantitative RT-PCR. The input of cDNA in each case was firstly normalized to give comparable amplification of an endogenous standard gene, ribosomal protein L30, for 28–35 cycles (Fig. 7A). Comparable amplification was obtained for ribosomal

protein L30 mRNA at the cDNA inputs used. This cDNA input was then used for amplification of the α_2 -AR mRNA. As shown in Fig. 7B, when compared with controls, expression of the α_2 -AR was increased in islets in which the GR was overexpressed.

Islet morphology. Immunocytochemistry revealed an increased expression of rat GR in the pancreatic β -cells of TG mice (data not shown), in accordance with our previous observation (8). Furthermore, in both control and TG mice, central regions of the islets consisted of insulin-producing cells, whereas glucagon-producing cells were located peripherally in the mantle zone. There were no differences regarding the groups in this sense; hence, islet cytoarchitecture did not seem to be affected by GR overexpression in the β -cells (Fig. 8). GLUT2 immunoreactivity

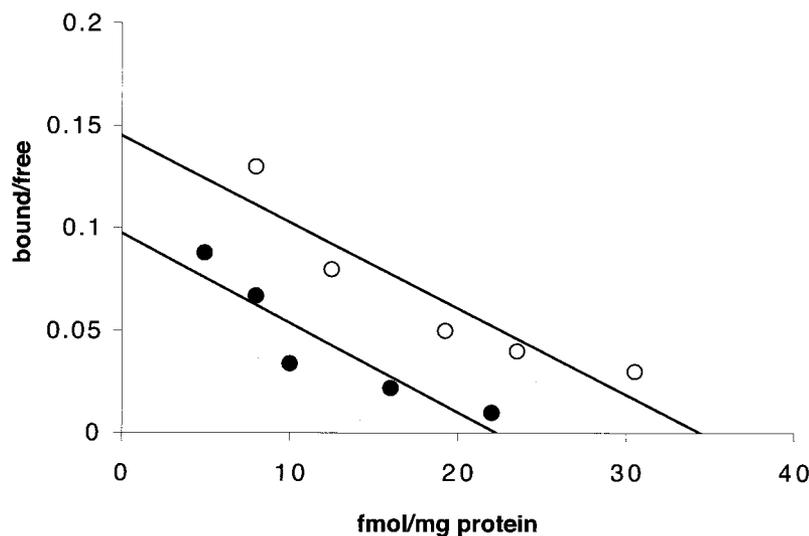


FIG. 6. Scatchard plots of [3 H]UK 14304 binding in islets of control (●) and TG (○) mice. The concentration of [3 H]UK 14304 varied from 9×10^{-9} to 2.8×10^{-10} mol/l. The amount of specific bound radioligand was quantified using 10^{-6} mol/l UK 14304 to estimate nonspecific binding. The maximum number of [3 H]UK 14304 binding sites (B_{max}) was 34 ± 5 and 23 ± 3 fmol/mg proteins in TG and control islets, respectively ($P < 0.001$). No significant change in the dissociation constant (K_d) was observed in TG versus control islets (1.6 ± 0.2 vs. 1.8 ± 0.3 nmol/l). Data are expressed as means \pm SE ($n = 5$).

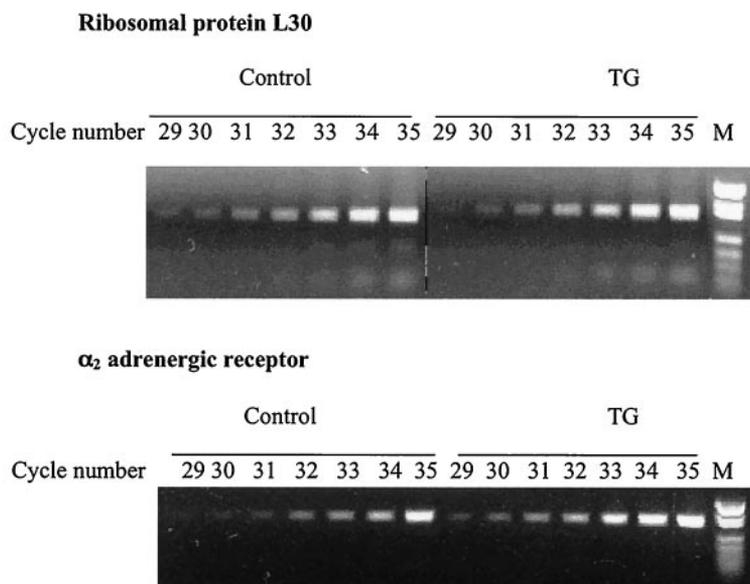


FIG. 7. Analysis of α_2 -AR by RT-PCR. Total RNA (5 μ g) from control and TG islets was used in RT-PCR using specific primers as indicated in RESEARCH DESIGN AND METHODS. Ribosomal protein L30 was used as an internal control.

was seen in the plasma membrane of centrally located islet cells in both types of animals. There was no difference regarding GLUT2 immunoreactivity between control and TG mice.

Apoptosis in islets. The TUNEL assay did not show any evidence of apoptosis in islets from control or TG mice. The weak green fluorescence observed in the islets after TUNEL staining corresponded exclusively to autofluorescent erythrocytes (Fig. 9).

DISCUSSION

We have found that TG mice overexpressing GR in the islet β -cells develop hyperglycemia both in the fed and overnight-fasted states by the age of 12–15 months. Using mice from the same colony, we previously demonstrated that at the age of 3 months, these animals developed impaired glucose tolerance and decreased insulin secretion, whereas the fed and fasting blood glucose concentrations were not elevated, compared with control mice (8). Hence, overexpression of GR in β -cells results in diabetes at old age.

The diabetogenic effects of glucocorticoids are well known. Long-term treatment with glucocorticoids leads to decreased glucose tolerance and even manifest diabetes, despite increased plasma insulin levels (6,7). Under these conditions, the diabetogenic effects of glucocorticoids are mainly accounted for by decreased insulin sensitivity in the liver, muscle, and adipose tissues. However, the compensatory increase in plasma insulin is attenuated by the direct inhibitory effect of glucocorticoids on insulin secretion, as suggested by *in vivo* and *in vitro* studies (8,9,25–27). Also in the present experiments, insulin secretion was significantly decreased in TG mice in both *in vivo* and *in vitro* conditions. Basal insulin levels were lower in TG mice than in control mice, whereas glucose elimination after intraperitoneal insulin administration did not differ between the groups, suggesting that insulin sensitivity was not reduced in TG mice. Therefore, it seems that the development of manifest diabetes in these animals is due to a direct inhibitory effect of GR overexpression on

insulin release, and is not related to reduction in insulin sensitivity.

The mechanisms by which GCs inhibit insulin secretion are not fully elucidated. It is well known that the sympathetic nervous system control of insulin secretion is mediated by catecholamines, which inhibit insulin release via the α_2 -ARs (12). The adrenergic nerves associated with islets contain a high concentration of catecholamines (28). Because β -cells express α_2 -ARs (29,30) and GCs upregulate α_2 -AR expression and signaling in β -cell lines (10), we investigated the possibility that inhibition of insulin secretion in TG mice involves a signaling pathway via the α_2 -AR. In the present study, we found a significantly higher density of α_2 -ARs in islets from TG mice, as revealed by binding studies using the α_2 -AR agonist [3 H]UK 14304 as a ligand. Furthermore, analysis by RT-PCR also showed increased expression of α_2 -AR in TG mouse islets. Moreover, the incubation of islets with benextramine, a selective antagonist of α_2 -AR, completely restored the insulin response to glucose in isolated islets from TG mice, whereas it had no effect on control islets. Similarly, in 3-month-old TG mice, benextramine normalized the islet insulin response to glucose (31). These above findings together suggest that increased expression and binding by α_2 -ARs is involved in the inhibition of insulin secretion in TG mice.

In insulin-secreting HIT cells, the activation of α_2 -AR by clonidine and oxymetazoline inhibited insulin secretion, whereas the pretreatment of the cells with pertussis toxin, which inhibits G-proteins, prevented the suppression of insulin secretion (17). This indicates that the mechanism behind the inhibition of insulin secretion by α_2 -AR is mediated through a pertussis toxin-sensitive G-protein. Further evidence for the involvement of G-proteins in α_2 signaling in the β -cell was provided by Lang et al. (32). Using HIT-T15 cells, they demonstrated that the activation of α_2 -ARs caused pertussis toxin-sensitive inhibition of insulin secretion. Thus, the activation of the heterometric G-proteins G_i (G_{α_i1} , G_{α_i2} , G_{α_i3}) and G_o (G_{α_o2}) by either a functionally important receptor or receptor-mimetic pep-

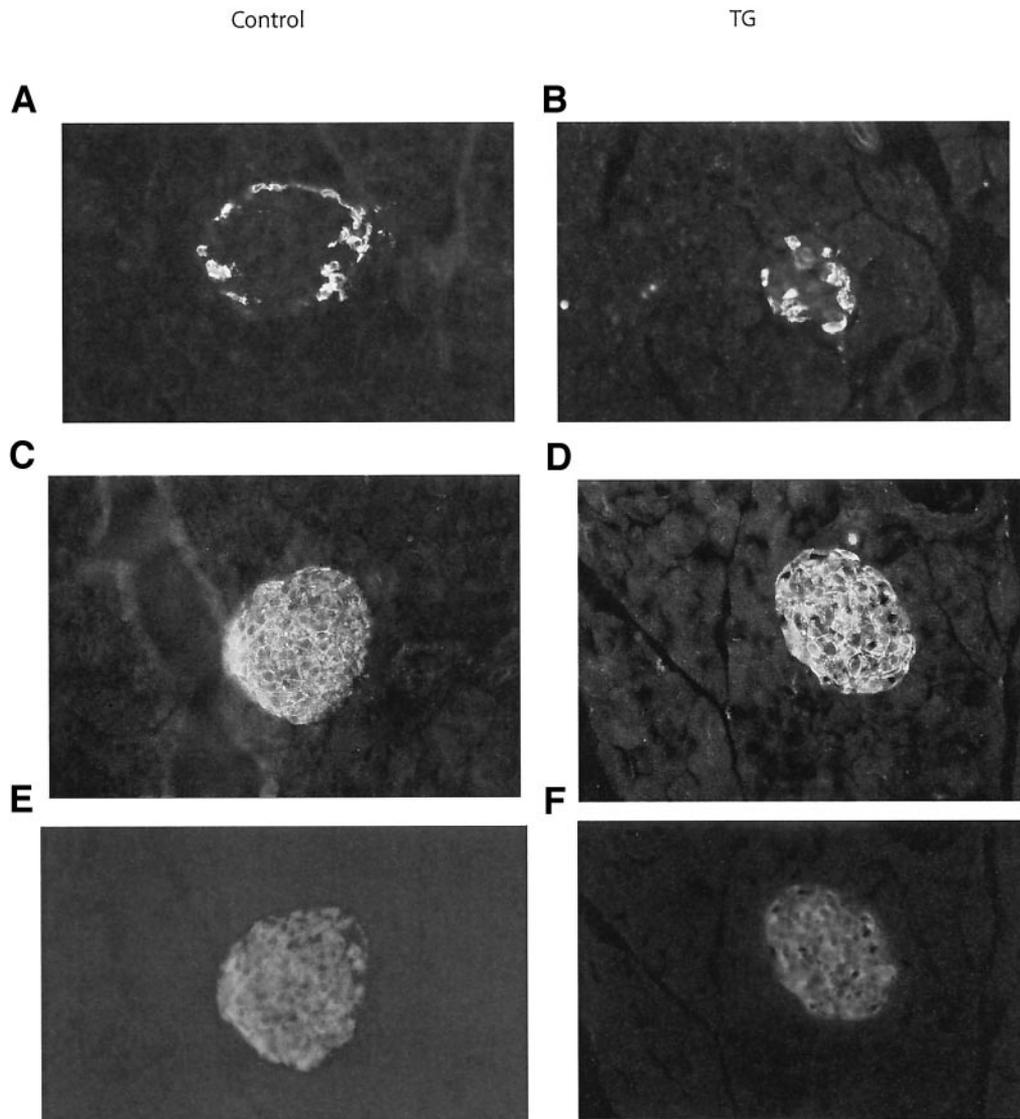


FIG. 8. Distribution of glucagon (*A* and *B*), GLUT2 (*C* and *D*), and insulin (*E* and *F*) in pancreatic sections from control (*A*, *C*, and *E*) and TG (*B*, *D*, and *F*) mice, as revealed by immunofluorescence. The results are representative figures after evaluation of three to five animals in each group (100 \times).

tides caused the inhibition of exocytosis. Because the inhibition of insulin secretion from islets by *in vitro* dexamethasone treatment was prevented by pertussis toxin treatment (26), it is possible that the decreased insulin secretion in our TG mouse islets with increased expression and binding of α_2 -ARs may involve signaling via G-proteins.

In the present study, not only glucose-induced, but also arginine-induced, insulin release was reduced in islets of TG mice. Because catecholamine also inhibits arginine-induced insulin release (33), this finding is compatible with the hypothesis that increased signaling by α_2 -AR is involved in the regulation of insulin secretion in the β -cells of TG mice.

Impaired insulin secretion from TG islets was not due to decreased insulin content, which was similar to controls. It has previously been demonstrated that ZDF rats with impaired glucose tolerance become frankly diabetic after dexamethasone treatment, in part due to an increase in

β -cell apoptosis (34). Our aged TG mice also become diabetic after 12 months, but the development of the diabetic state did not involve apoptosis of β -cells, because we did not detect any apoptotic nuclei in islets from these mice. In accord with our results, dexamethasone treatment of partially pancreatectomized hyperglycemic rats did not cause any apoptotic changes in the β -cell (35). Morphological studies of pancreatic islets revealed that the islet cytoarchitecture was intact in TG animals, as judged by the normal distributions of insulin- and glucagon-producing cells.

The novel finding in our study is that TG mice become diabetic over a period of 12–15 months. Hence, increased GR sensitivity in the pancreatic β -cells is sufficient for development of the diabetic state, and our results suggest that this is mainly due to overexpression of α_2 -ARs. This would support notions of a role for stress in the development of type 2 diabetes, which thus may be mediated both by the effects of increased GC signaling on islet function as

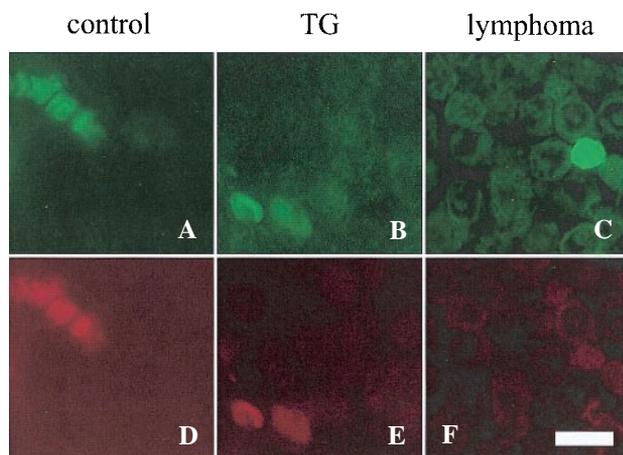


FIG. 9. TUNEL analysis of islets from control and TG mice as well as a positive control (lymphoma). A–C show green fluorescence analysis and D–F show red fluorescence analysis. No TUNEL-specific green fluorescein isothiocyanate (FITC) fluorescence was seen in either mouse sample (A and B). The weak green fluorescence in the islets exclusively corresponds to autofluorescent erythrocytes (A and B) that also show red fluorescence (D and E) and no DAPI staining (data not shown). The positive lymphoma control shows FITC-specific nuclear fluorescence in apoptotic cells (C) without red autofluorescence (F). Magnification bar: 10 μ m.

well as by increased β -cell α_2 -AR expression. Our results may also have implications for the use of islets for transplantation. Thus, Shapiro et al. (36) demonstrated a major progress in islet transplantation to type 1 diabetic patients, using a protocol that employed GC-free immunosuppressive therapy, compared with previous reports in which GC was used as an immunosuppressive agent (37). This would suggest that direct effects of GC on β -cells deteriorate insulin secretion from transplanted islets in humans and animals receiving immunosuppressive therapies that include GC.

In conclusion, we have demonstrated that prolonged and increased GR sensitivity of pancreatic β -cells to GC exerts a strong diabetogenic action due to decreases in insulin release. The inhibitory effect of GC on insulin release may involve increased signaling via α_2 -AR.

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REFERENCES

1. Efendic S, Luft R, Wajngot A: Aspects of the pathogenesis of type 2 diabetes. *Endocr Rev* 5:395–410, 1984
2. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–368, 1992
3. Kahn CR: New concepts in the pathogenesis of diabetes mellitus. *Adv Intern Med* 41:285–321, 1996
4. Surwit RS, Schneider MS, Feinglos MN: Stress and diabetes mellitus. *Diabetes Care* 15:1413–1422, 1992
5. Chrousos GP, Gold PW: The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. *JAMA* 267:1244–1252, 1992
6. McMahon M, Gerich J, Rizza R: Effects of glucocorticoids on carbohydrate metabolism. *Diabete Metab Rev* 4:17–30, 1988
7. Andrews RC, Walker BR: Glucocorticoids and insulin resistance: old hormones, new targets. *Clin Sci* 96:513–523, 1999

8. Delaunay F, Khan A, Cintra A, Davani B, Ling ZC, Andersson A, Östenson CG, Gustafsson J, Efendic S, Okret S: Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. *J Clin Invest* 100:2094–2098, 1997
9. Ling ZC, Khan A, Delaunay F, Davani B, Östenson CG, Gustafsson JA, Okret S, Landau BR, Efendic S: Increased glucocorticoid sensitivity in islet beta-cells: effects on glucose 6-phosphatase, glucose cycling and insulin release. *Diabetologia* 41:634–639, 1998
10. Hamamdžic D, Duzic E, Sherlock JD, Lanier SM: Regulation of alpha 2-adrenergic receptor expression and signaling in pancreatic beta-cells. *Am J Physiol* 269:E162–E171, 1995
11. Rodriguez-Pena MS, Collins R, Woodard C, Spiegel AM: Decreased insulin content and secretion in RIN 1046-38 cells overexpressing alpha 2-adrenergic receptors. *Endocrine* 7:255–260, 1997
12. Robertson RP, Halter JB, Porte D: A role for alpha-adrenergic receptors in abnormal insulin secretion in diabetes mellitus. *J Clin Invest* 57:791–795, 1976
13. Miller RE: Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the islets of Langerhans. *Endocr Rev* 2:471–494, 1981
14. Nakadate T, Nakaki T, Muraki T, Kato R: Adrenergic regulation of blood glucose levels: possible involvement of postsynaptic alpha-2 type adrenergic receptors regulating insulin release. *J Pharmacol Exp Ther* 215:226–230, 1980
15. Ahren B, Taborsky GJ, Porte D: Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. *Diabetologia* 29:827–836, 1986
16. Östenson CG, Cattaneo AG, Doxey JC, Efendic S: Alpha-adrenoceptors and insulin release from pancreatic islets of normal and diabetic rats. *Am J Physiol* 257:E439–E443, 1989
17. Hirose H, Seto Y, Maruyama H, Dan K, Nakamura K, Saruta T: Effects of alpha 2-adrenergic agonism, imidazolines, and G-protein on insulin secretion in beta cells. *Metabolism* 46:1146–1149, 1997
18. DiTullio NW, Cieslinski L, Matthews WD, Storer B: Mechanisms involved in the hyperglycemic response induced by clonidine and other alpha-2 adrenoceptor agonists. *J Pharmacol Exp Ther* 228:168–173, 1984
19. Devedjian JC, Pujol A, Cayla C, George M, Casellas A, Paris H, Bosch F: Transgenic mice overexpressing alpha2A-adrenoceptors in pancreatic beta-cells show altered regulation of glucose homeostasis. *Diabetologia* 43:899–906, 2000
20. Östenson CG, Pignon J, Doxey JC, Efendic S: Alpha 2-adrenoceptor blockade does not enhance glucose-induced insulin release in normal subjects or patients with noninsulin-dependent diabetes. *J Clin Endocrinol Metab* 67:1054–1059, 1988
21. Ito K, Hirose H, Kido K, Koyama K, Maruyama H, Saruta T: Adrenoceptor antagonists, but not guanethidine, reduce glucopenia-induced glucagon secretion from perfused rat pancreas. *Diabetes Res Clin Pract* 30:173–180, 1995
22. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
23. Östenson CG, Grill V: Evidence that hyperglycemia increases muscarinic binding in pancreatic islets of the rat. *Endocrinology* 121:1705–1710, 1987
24. Heiden T, Castanos-Velez E, Andersson LC, Biberfeld P: Combined analysis of DNA ploidy, proliferation, and apoptosis in paraffin-embedded cell material by flow cytometry. *Lab Invest* 80:1207–1213, 2000
25. Khan A, Östenson CG, Berggren PO, Efendic S: Glucocorticoid increases glucose cycling and inhibits insulin release in pancreatic islets of ob/ob mice. *Am J Physiol* 263:E663–E666, 1992
26. Lambillotte C, Gilon P, Henquin JC: Direct glucocorticoid inhibition of insulin secretion: an in vitro study of dexamethasone effects in mouse islets. *J Clin Invest* 99:414–423, 1997
27. Larsson H, Ahren B: Insulin resistant subjects lack islet adaptation to short-term dexamethasone-induced reduction in insulin sensitivity. *Diabetologia* 42:936–943, 1999
28. Lundquist I, Ahren B, Hansson C, Hakansson R: Monoamines in pancreatic islets of guinea pig, hamster, rat, and mouse determined by high performance liquid chromatography. *Pancreas* 4:662–667, 1989
29. Chan SL, Perrett CW, Morgan NG: Differential expression of alpha 2-adrenoceptor subtypes in purified rat pancreatic islet A- and B-cells. *Cell Signal* 9:71–78, 1997
30. Niddam R, Angel I, Bidet S, Langer SZ: Pharmacological characterization of alpha-2 adrenergic receptor subtype involved in the release of insulin from isolated rat pancreatic islets. *J Pharmacol Exp Ther* 254:883–887, 1990
31. Ling ZC, Davani B, Östenson CG, Okret S, Efendic S, Khan A: Involvement of adrenergic receptors in the regulation of insulin release in transgenic mice with overexpressed β -cell glucocorticoid receptor (Abstract). *Diabetologia* 41 (Suppl. 1):A-153, 1998
32. Lang J, Nishimoto I, Okamoto T, Regazzi R, Kiraly C, Weller U, Wollheim CB: Direct control of exocytosis by receptor-mediated activation of the

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- heterotrimeric GTPases Gi and G (o) or by the expression of their active G alpha subunits. *EMBO J* 14:3635–3644, 1995
33. Efendic S, Luft R, Cerasi E: Quantitative determination of the interaction between epinephrine and various insulin releasers in man. *Diabetes* 27:319–326, 1978
 34. Pick A, Clark J, Kubstrup C, Levisetti M, Pugh W, Bonner-Weir S, Polonsky KS: Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes* 47:358–364, 1998
 35. Laybutt DR, Kaneto H, Hasenkamp W, Grey S, Jonas JC, Sgroi DC, Groff A, Ferran C, Bonner-Weir S, Sharma A, Weir GC: Increased expression of antioxidant and antiapoptotic genes in islets that may contribute to beta-cell survival during chronic hyperglycemia. *Diabetes* 51:413–423, 2002
 36. Shapiro AM, Lakey JR, Ryan EA, Korbitt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
 37. Warnock GL, Kneteman NM, Ryan EA, Rabinovitch A, Rajotte RV: Long-term follow-up after transplantation of insulin-producing pancreatic islets into patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 35:89–95, 1992