Pancreatic β Cells Are Important Targets for the Diabetogenic Effects of Glucocorticoids

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Abstract

Abnormalities contributing to the pathogenesis of non–insulin-dependent diabetes mellitus include impaired β cell function, peripheral insulin resistance, and increased hepatic glucose production. Glucocorticoids are diabetogenic hormones because they decrease glucose uptake and increase hepatic glucose production. In addition, they may directly inhibit insulin release. To evaluate that possible role of glucocorticoids in β cell function independent of their other effects, transgenic mice with an increased glucocorticoid sensitivity restricted to their β cells were generated by overexpressing the glucocorticoid receptor (GR) under the control of the insulin promoter. Intravenous glucose tolerance tests showed that the GR transgenic mice had normal fasting and postabsorptive blood glucose levels but exhibited a reduced glucose tolerance compared with their control littermates. Measurement of plasma insulin levels 5 min after intravenous glucose load demonstrated a dramatic decrease in acute insulin response in the GR transgenic mice. These results show that glucocorticoids directly inhibit insulin release in vivo and identify the pancreatic β cell as an important target for the diabetogenic action of glucocorticoids. (*J. Clin. Invest.* 1997. 100:2094–2098.) Key words: glucocorticoid receptor • glucocorticoid sensitivity • insulin release • non–insulin-dependent diabetes mellitus • transgenic mice

Introduction

Non–insulin-dependent diabetes mellitus (NIDDM)[1] is a complex and heterogeneous disease resulting from interactions among multiple defects of both genetic and environmental origin. The major abnormalities contributing to its pathogenesis are impaired β cell function, peripheral insulin resistance, and increased hepatic glucose production (1, 2). Manifest diabetes develops when β cells no longer can secrete adequate insulin in response to glucose to overcome decreased insulin sensitivity and hyperglycemia. Stress hormones such as glucocorticoids, growth hormone, and catecholamines induce insulin resistance and can precipitate glucose intolerance in situations where they are in excess (3, 4). Glucocorticoids also increase hepatic glucose production by stimulating gluconeogenesis (5). Insulin resistance and increased hepatic glucose production induced by glucocorticoids result in increased plasma insulin levels. However, this increase in plasma insulin is probably attenuated by a direct inhibitory effect of glucocorticoids on insulin release from β cells as suggested by in vitro (6–10) and in vivo (11, 12) studies.

Glucocorticoids mediate their effects through a specific intracellular receptor present in almost all cell types, including pancreatic β cells (13). The glucocorticoid receptor (GR) belongs to the superfamily of nuclear hormone receptors, which function as ligand activatable transcription factors (14, 15). Previous experiments have demonstrated that GR expression level is a major factor determining cellular sensitivity towards glucocorticoids both in vitro (16, 17) and in vivo (18).

To evaluate the significance of glucocorticoids in β cell function independent of their effects on glucose uptake and hepatic glucose production, we have generated transgenic mice with an increased β cell sensitivity to glucocorticoids. This was achieved by overexpressing the GR under the control of the insulin promoter.

Methods

Transgene construction. All recombinant DNA work was performed according to standard procedures (19). A 2.8-kb BamHI fragment containing the full-length coding region of the rat GR cDNA (20) was isolated from the expression plasmid pSVGR1 and blunted. A reporter plasmid containing the fragment −410/+1 of the rat insulin promoter I (RIP1) and an SV40 polyadenylation signal (21, 22) was digested with HindIII and HpaI to remove the chloramphenicol-acetyltransferase sequence and blunted. The GR fragment was then ligated to the insulin promoter and SV40 sequences to create a β cell–specific expression plasmid termed pRIP1-GR. The control plasmid pRIP1 used in transfection experiments contains only the insulin promoter.

Cell culture and transfection. Hamster HIT-15 β cells (23) were grown in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37°C and 5% CO_2. Cells were plated in 6-well multidishes at a density of 4×10^5 cells/well 24 h before transfection. Cells were transiently transfected with 6 μg plasmid DNA for 5–6 h by the calcium phosphate coprecipitation technique. The mixture contained 1 μg mouse mammary tumor virus–alkaline phosphatase (pMMTV-AP) reporter

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1. *Abbreviations used in this paper:* AP, alkaline phosphatase; GR, glucocorticoid receptor; LIR, low insulin response; MMTV, mouse mammary tumor virus; NIDDM, non–insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test; RIP1, rat insulin promoter I.

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plasmid (24), 0–2.5 μg pRIP1-GR expression plasmid, and 2.5 μg pGEM plasmid as carrier DNA. The amount of insulin promoter was kept to 2.5 μg by cotransfecting the pRIP1 plasmid. After transfection, cells were grown in the absence or presence of 1 μM dexamethasone for 48 h. Secreted AP activity was measured as described (24).

Transgenic mice. A 3.6-kb PvuII linear fragment containing the rat GR fused to the insulin promoter and SV40 polyadenylation signal was isolated from the pRIP1-GR expression plasmid and introduced into male pronuclei of zygotes obtained from F1 (C57BL/6J × CBA/J) mice using standard microinjection techniques (25). Embryos were reimplanted into pseudopregnant female mice, and transgenic animals were identified by PCR on DNA from tail biopsies (26). The sense and antisense primers used for the PCR screening were 5′-TGATTGTGCTGTGAACTGCTT-3′ and 5′-CTCCTCCCCTCAGGCTTTTAT-3′, respectively. Copy number was determined by Southern blot analysis using a fragment from the rat GR cDNA as a probe (20). Animals were housed at 22°C with a daylight period of 12 h and fed diet and water ad libitum.

Immunocytochemistry. Animals were killed by cervical dislocation, and the heart was perfused first with 50 ml saline solution and then with 50 ml fixative (4% paraformaldehyde, 0.2% picric acid, 0.1 M phosphate buffer). Organs were dissected, postfixed overnight, and washed in 0.05 M PBS containing 10% sucrose for 72 h. 14-μm-thick sections were incubated with a mouse mAb against the rat GR N°7 (27) and a guinea pig anti–human insulin polyclonal antibody or a rabbit anti–human glucagon polyclonal antibody (DAKO Corp., Santa Barbara, CA) at 4°C overnight. Immunoreactivity was revealed by incubation with FITC-conjugated donkey anti–mouse and CY-3–conjugated donkey anti–rabbit antisera at 37°C for 2 h.

Intravenous glucose tolerance tests and acute insulin response. Nontransgenic control (body wt = 26.0±0.8 g) and transgenic mice (body wt = 25.2±0.8 g) at 3–4 mo of age were fasted overnight. Blood was collected from the orbital plexus before and after intravenous injection of a glucose solution (0.5 or 2 g/kg body wt) at the indicated times. Blood glucose was measured using a glucose oxidase method (model 23A glucose analyzer; Yellow Springs Instruments Co., Yellow Springs, OH). Insulin was measured by RIA with the addition of charcoal to separate free and bound antibody (28). Corticosterone was measured by RIA (Biotrak® kit; Amersham Corp., Arlington Heights, IL).

Results

The effect of GR overexpression on β cell sensitivity to glucocorticoids was analyzed first in transiently transfected HIT-15 β cells using the β cell–specific GR expression plasmid pRIP1-GR (Fig. 1) and the AP reporter gene under the control of the MMTV promoter. Increasing amounts of transfected GR resulted in a dose-dependent activation of the cotransfected glucocorticoid–responsive reporter gene when the cells were treated with dexamethasone (Fig. 2). This result demonstrates that GR concentration determines the sensitivity of β cells to glucocorticoids. This is in accordance with observations made in other cell types (16). In addition, this experiment also showed that HIT-15 β cells contain some endogenous GR, since dexamethasone-induced AP activity was observed in the absence of pRIP1-GR expression plasmid.

The RIP1-GR transgene was microinjected into mouse embryos, four founder animals harboring 2–10 copies of the transgene were identified, and lines were established. The transgene was transmitted over generations in a Mendelian fashion. Immunocytochemical analysis using a specific anti–rat GR mAb revealed a high expression of the transgene in the pancreatic β cells of two transgenic mouse lineages. Expression was restricted exclusively to the β cells of transgenic mice (Fig. 3). This was confirmed in double staining experiments using the anti–rat GR antibody together with antiinsulin or antiglucagon antibodies (Fig. 3, *A–D*). No rat GR staining was observed in control islets (Fig. 3 *E*), or in the brain (Fig. 3 *G*) or liver (Fig. 3 *H*) of transgenic mice. The expression pattern was heterogeneous among β cells, probably reflecting variable activity of the insulin promoter in different β cell subpopulations (29). Islets from transgenic mice showed normal morphology and insulin immunoreactivity compared with control islets (Fig. 3, *B* and *F*). Transgenic mice were bred with nontransgenic mice to obtain age-matched RIP1-GR heterozygous transgenic and nontransgenic control animals for phenotypic analysis. RIP1-GR heterozygous mice had normal growth rate

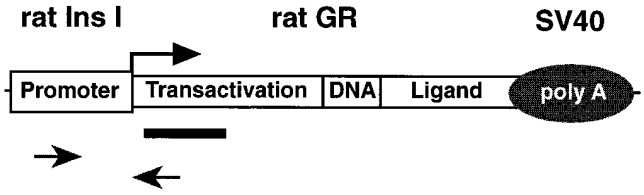

Figure 1. Structure of the RIP1-GR transgene used to generate transgenic mice. The full-length coding region of the rat GR cDNA was fused to the (−410/+1) promoter region of the rat insulin gene I and an SV40 polyadenylation signal. Functional domains of the glucocorticoid receptor for transactivation, DNA binding, and ligand binding are indicated. *Arrows* and *thick bar*, Position of the primers and the probe used for the PCR and Southern blot genotyping of transgenic mice, respectively.

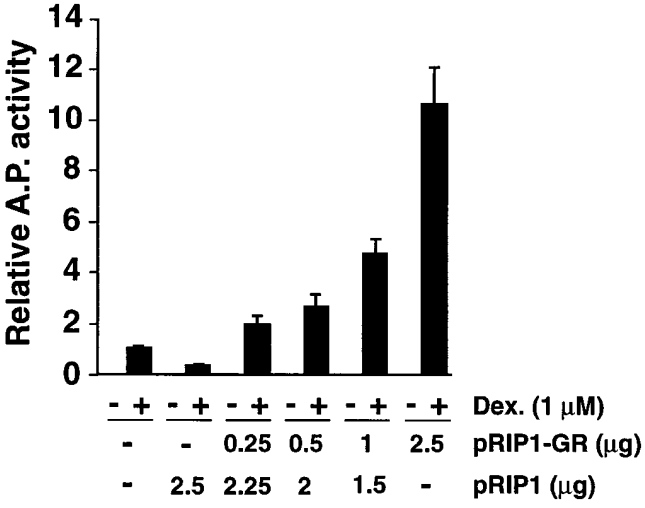

Figure 2. GR overexpression enhances the sensitivity of pancreatic β cells to glucocorticoids. HIT-15 cells were transiently transfected by the calcium phosphate coprecipitation technique with 1 μg pMMTV-AP reporter plasmid, 0–2.5 μg pRIP1-GR expression plasmid, and 2.5 μg pGEM plasmid as carrier DNA. The amount of insulin promoter was kept to 2.5 μg by cotransfecting the pRIP1 plasmid. After transfection, cells were grown in the absence (−) or presence (+) of 1 μM dexamethasone (*Dex.*) for 48 h, and AP activity was determined. The dexamethasone-induced AP activity in the absence of expression plasmid was given the arbitrary value of 1, and the other AP activities were expressed relative to this nominal value. Data are mean±SEM from five transfections.

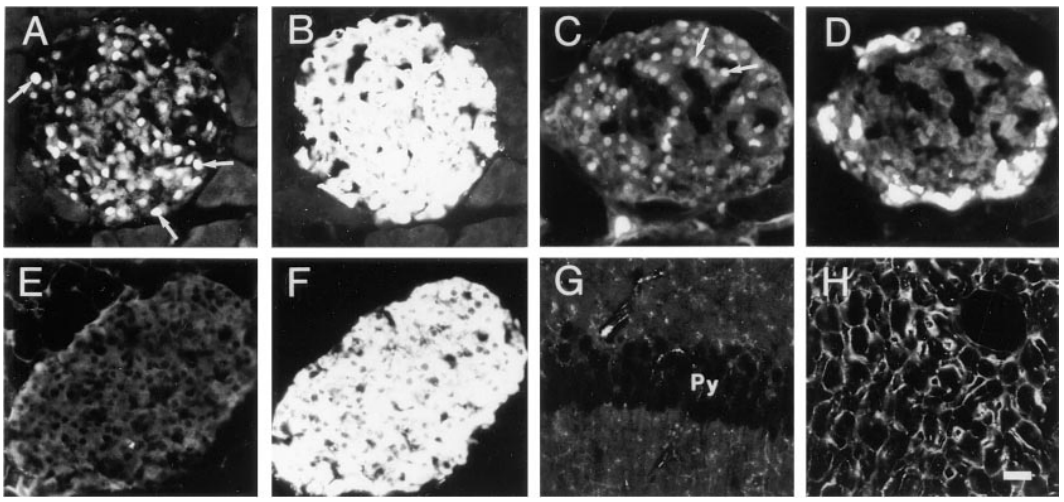

Figure 3. Immunocytochemical analysis of RIP1-GR transgenic mice. (*A* and *C*) Two different transgenic islets stained for rat GR. *Arrows*, Nuclear staining of expressing β cells. (*B*) Same islet as in *A*, stained for insulin. (*D*) Same islet as in *C*, stained for glucagon. (*E*) Control islet stained for rat GR. (*F*) Control islet stained for insulin. (*G*) Pyramidal neurons (*Py*) of CA1 area from a transgenic mouse brain stained for rat GR. (*H*) Hepatocytes from a transgenic mouse liver stained for rat GR. Magnification bar, 25 μm.

and reproductive capacity compared with nontransgenic littermates.

Consequences of GR overexpression on glucose homeostasis and β cell function were next investigated in vivo in 3–4-mo-old mice. Overnight-fasted mice were subjected to intravenous glucose tolerance tests using a dose of 0.5 or 2 g glucose/kg body wt. Transgenic and nontransgenic control mice had identical fasting blood glucose concentrations (Table I). Blood glucose levels measured at 5 or 10 min after intravenous injection were proportional to the glucose dose and similar in both transgenic and control groups (Table I). In contrast, transgenic mice exhibited significantly higher blood glucose concentrations than control mice at 60 min after intravenous injection of 0.5 g glucose/kg body wt ($P < 0.05$) (Table I). Similarly, when 2 g glucose/kg body wt was administered intravenously, glucose tolerance was decreased ($P < 0.001$) (Table I). The transgenic mice had slightly lower fasting plasma insulin concentrations compared with control mice (Table II). Plasma insulin concentrations were reduced dramatically in transgenic mice at 5 min after injection of 0.5 g glucose/kg body wt, compared with control mice, indicating that insulin release was impaired in the transgenic mice ($P < 0.05$-0.001) (Table II). After 60 min, plasma insulin concentrations were not significantly higher in the transgenic mice (Table II). This is additional evidence for a defect in insulin release in the transgenic mice, since they had higher blood glucose levels at the 60-min time point after glucose injection. No sex differences were observed in blood glucose and plasma insulin concentrations. Plasma corticosterone levels did not differ significantly between transgenic and control mice (340±27 vs. 294±21 ng/ml, $n = 5$).

Table I. Intravenous Glucose Tolerance Test in RIP1-GR Transgenic Mice after Intravenous Injection of 0.5 and 2 g Glucose/kg Body Wt

	Blood glucose (mM)			
	Controls	*n*	Transgenics	*n*
0.5 g/kg body wt				
0 min	3.5±0.1	25	3.4±0.2	26
5 min	18.0±0.6	26	18.9±0.6	23
60 min	8.2±0.3	26	10.1±0.7*	26
2 g/kg body wt				
0 min	4.3±0.4	6	4.2±0.6	6
10 min	44.0±1.9	6	45.9±3.9	6
120 min	6.2±0.3	6	8.6±0.5‡	6

Nontransgenic (*Controls*) and RIP1-GR transgenic mice at 3–4 mo of age were fasted overnight. Blood was collected before and after intravenous injection of a glucose solution (0.5 or 2 g/kg body wt) at the indicated times. Data are expressed as mean±SEM, and differences between control and transgenic groups were tested for significance using Student's *t* test for unpaired data. *$P < 0.05$. ‡$P < 0.001$.

Table II. Acute Insulin Response in RIP1-GR Transgenic Mice after Intravenous Injection of 0.5 g Glucose/kg Body Wt

	Plasma insulin (μU/ml)			
	Controls	*n*	Transgenics	*n*
Experiment 1				
0 min	9.3±0.7	16	7.2±0.8‡	27
5 min	21.0±1.6	26	9.7±0.9§	28
60 min	ND		ND	
Experiment 2				
0 min	6.3±0.5	11	5.5±0.5	9
5 min	16.7±2.7	6	8.6±1.4*	4
60 min	6.4±0.8	5	8.1±1.4	5

Nontransgenic (*Controls*) and RIP1-GR transgenic mice at 3–4 mo of age were fasted overnight. Blood was collected before and after intravenous injection of a glucose solution (0.5 g/kg body wt) at the indicated times. Data are expressed as mean±SEM, and differences between control and transgenic groups were tested for significance using Student's *t* test for unpaired data. *$P < 0.05$. ‡$P < 0.005$. §$P < 0.001$. ND, not determined.

Discussion

Glucocorticoids are referred to as diabetogenic hormones primarily because they stimulate hepatic glucose production and induce insulin resistance, two key abnormalities involved in the pathogenesis of NIDDM. Although glucocorticoid-induced hyperglycemia results in stimulation of insulin secretion, there are in vitro data suggesting a direct inhibitory effect of glucocorticoids on insulin release (6–10). To investigate whether glucocorticoids play a significant role in β cell function independent of their effects in other tissues, we have generated transgenic mice with an increased β cell glucocorticoid sensitivity by overexpressing the rat GR in β cells.

We demonstrate that GR overexpression in β cells results in decreased glucose tolerance due to impaired insulin release. This indicates that glucocorticoids have a direct inhibitory effect on glucose-induced insulin release in vivo. The mechanism by which glucocorticoids directly inhibit insulin release in β cells is not known but is likely to involve up- or downregulation of genes important for the glucose-sensing–insulin secretion coupling. It is generally accepted that glucose is transported into the β cell via the glucose transporter GLUT-2 and enhances insulin release by generating ATP, which in turn increases the ATP/ADP ratio, leading to the closure of ATP-sensitive K^+-channels, depolarization of the plasma membrane, opening of voltage-activated Ca^{2+}-channels, entry of extracellular Ca^{2+}, and, finally, exocytosis of insulin-secretory granules (30). It has been shown previously in in vitro studies that the decreased insulin release caused by dexamethasone in pancreatic islets is associated with a decreased GLUT-2 protein stability (10) and an increase in glucose-6-phosphatase activity (31), glucose cycling (32), neuropeptide Y expression (33), and α_2-adrenergic receptor expression (34). Thus, there are multiple genes involved in glucose signaling in the β cell that could mediate the inhibitory effect of glucocorticoids on insulin release in vivo.

The physiological role of the direct inhibition of glucose-induced insulin release by glucocorticoids is not clear. However, it could be an important regulatory mechanism during stress, allowing a transient attenuation of the insulin response to hyperglycemia in order to ensure sufficient glucose for the needs of the brain. This β cell–directed action of glucocorticoids thus operates in concert with other well-established effects of these steroid hormones, such as decreased glucose uptake and increased hepatic glucose production.

The acute insulin responses to intravenous glucose load were abolished in the transgenic animals in vivo, while glucose tolerance was decreased only moderately. This mild glucose intolerance could be the result of an increased insulin sensitivity in the peripheral tissues, as suggested by the lower fasting plasma insulin levels in some transgenic mice. Interestingly, $\sim$ 20% of healthy humans also exhibit a markedly decreased insulin response to glucose, but maintain almost normal glucose tolerance due to enhanced insulin sensitivity (35). Rull et al. and we have demonstrated that many individuals with low insulin response (LIR) develop impaired glucose tolerance after a short treatment with glucocorticoids, due to their inability to increase insulin release in response to hyperglycemia (36, 37). Follow-up studies have shown that these LIR subjects have an increased risk of developing NIDDM and are therefore considered prediabetics (38). Furthermore, it has been shown that 26% of the healthy offspring of NIDDM patients, but with an impaired cortisol oral glucose tolerance test (OGTT), develop impaired glucose tolerance within 1–7 yr. In contrast, only 3% of the individuals with normal cortisol OGTT develop impaired glucose tolerance during the same time period (39). Extending our results with the RIP1-GR transgenic mice, it may be suggested that impaired cortisol OGTT in LIR humans possibly reflects increased glucocorticoid sensitivity in the β cells. Factors that could alter glucocorticoid sensitivity of β cells include intracellular hormone availability, GR expression level or transcriptional activity, structure of response elements within GR target genes, and interaction of GR with other transcription factors (40). Our data demonstrating a direct inhibitory effect of glucocorticoids on insulin release in vivo also provide an explanation for the relative insulinopenia observed in patients with Cushing syndrome (41).

In conclusion, we have demonstrated using a transgenic approach that glucocorticoids directly inhibit insulin release in vivo, revealing the pancreatic β cell as a novel and critical target for the diabetogenic action of glucocorticoids.

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