

GLUT-2 Function in Glucose-unresponsive β Cells of Dexamethasone-induced Diabetes in Rats

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Abstract

Spontaneous and dexamethasone-induced noninsulin-dependent diabetes mellitus (NIDDM) in rats is associated with loss of glucose-stimulated insulin secretion (GSIS) and a reduction in both GLUT-2-positive β cells and high K_m glucose transport. To determine if the chronology and correlation of these abnormalities is consistent with a causal relationship, Zucker (*fa/fa*) rats were studied longitudinally before and during 10 d of dexamethasone-induced (0.4 mg/kg per d i.p.) NIDDM. Within 24 h of dexamethasone treatment blood glucose rose and GSIS declined, becoming paradoxically negative (-87 ± 12 $\mu\text{U}/\text{ml}$ per min) on day 10. Blood glucose was negatively correlated with GSIS ($r = -0.92$; $P < 0.001$). 3-O-methyl-D-glucose (3MG) transport was unchanged at 12 h, 23% below normal on day 1, and declined further to a nadir 59% below normal. The GLUT-2-positive β cell area did not decline until 48 h, reaching a nadir of 35% of normal at 10 d. The area of GLUT-2-positive β cells was correlated with GSIS ($r = 0.77$; $P < 0.005$). We conclude that the chronology and correlation between GSIS loss and hyperglycemia is consistent with a cause-effect relationship, but that the subtotal impairment in glucose transport by itself cannot explain the total loss of GSIS if one assumes that normal β cells are functionally homogenous. (*J. Clin. Invest.* 1993. 92:1950-1956.) Key words: noninsulin-dependent diabetes mellitus • GLUT-2 • β cells • glucose-stimulated insulin secretion • islet glucose transport

Introduction

In humans noninsulin-dependent diabetes mellitus (NIDDM)¹ usually occurs on a background of insulin resistance (1, 2). However, the onset of hyperglycemia in both humans (3-6) and rodents (7-10) is heralded by the disappearance of glucose-stimulated insulin secretion (GSIS). Since the functional loss appears largely restricted to glucose, responses to various nonglucose secretagogues being well preserved (6-11), the possibility of a defect in one or more of the molecules in the glucose metabolic pathway of insulin secretion has been suggested (12). One such molecule, the high K_m glucose transporter of β

cells, GLUT-2 (13), has been shown to be profoundly reduced in all five of the animal models of NIDDM thus far studied, the Zucker Diabetic Fatty (ZDF/Drt-*fa* [10]) rat (8), the GK rat (9), the *db/db* mouse (10), the dexamethasone-induced diabetic rat (11), and the neonatal streptozotocin rat (14). Associated impairment of high K_m glucose transport into β cells could theoretically render them insensitive and unable to correct postprandial hyperglycemia.

It is not clear if underexpression of immunostainable GLUT-2 in β cells of rats with NIDDM is the cause or the consequence of the associated metabolic derangement. While there is compelling evidence based on islet transplantation in mice that the lesion is secondary to some metabolic disturbance associated with NIDDM (10), hyperglycemia itself is an unlikely candidate since it is an upregulator of β cell GLUT-2 in normal rats (15). Moreover, in ZDF rats prevention of hyperglycemia failed to prevent the downregulation of β cell GLUT-2 (16). Therefore, GLUT-2 loss seemed to be a proximal event that contributed to the hyperglycemia via the following sequence: \downarrow β cell GLUT-2 \rightarrow \downarrow high K_m glucose transport in β cells \rightarrow loss of GSIS \rightarrow uncorrected postprandial hyperglycemia \rightarrow fasting hyperglycemia (12). On the other hand, in a second model of spontaneous NIDDM, the GK rat, the reduction in glucose transport was insufficient to explain the reduction in GSIS, suggesting that molecular sites distal to GLUT-2 were involved in the functional derangement of the β cells and in the causation of hyperglycemia (9). This has recently been confirmed by our group (17).

In all of the previous studies of such abnormalities the animals were studied at arbitrarily selected times without regard to the sequence of the appearance of the abnormalities. This longitudinal study was intended to determine the chronologic relationships and correlations between the various β cell abnormalities in the hope of providing new insights concerning possible causal relationships. We have measured blood glucose levels, GSIS, immunostainable β cell GLUT-2, and glucose transport kinetics of isolated islets in a longitudinal fashion during the evolution of a model of NIDDM. We chose dexamethasone-induced diabetic female (*fa/fa*) Zucker rats so as to synchronize the onset of diabetes in groups of animals large enough to permit a longitudinal assessment of the foregoing parameters.

Methods

10-wk-old female Zucker fatty rats (*fa/fa*) from Charles River Breeding Laboratories, Inc. (Wilmington, MA), kept in individual metabolic cages and fed ad libitum, received daily intraperitoneal injections of 0.4 mg/kg per d of dexamethasone (Azium[®]; Schering Corporation, Kenilworth, NJ), a dose previously shown to cause diabetes in 100% (11). Blood was obtained each morning (0900-1000 h) by tail vein bleeding and glucose measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Inc., Fullerton, CA).

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1. Abbreviations used in this paper: GSIS, glucose-stimulated insulin secretion; IRI, immunoreactive insulin; NIDDM, noninsulin-dependent diabetes mellitus; 3MG, 3-O-methyl-D-glucose.

Pancreata were isolated from untreated rats and from rats treated with dexamethasone for 1, 2, 3, 7, or 10 d and perfused by a modification (18) of the method of Grodsky and Fanska (19). The baseline perfusate consisted of Krebs-Ringer bicarbonate buffer (pH 7.6) containing 5.6 mM glucose and 5 mM of pyruvate, fumarate, and glutamate. After a 20-min preincubation period, the pancreas was perfused for 10 min with the baseline perfusate. This was followed by a 10-min challenge with 20 mM glucose, a 5-min interval of baseline perfusate, a 10-min challenge with 10 mM arginine, and a final 5 min of baseline perfusate. The flow rate was maintained at 2.7 ml/min. Fractions were collected at 1-min intervals and stored at -20°C until assayed for immunoreactive insulin (IRI) by the method of Yalow and Berson (20) as modified by Herbert et al. (21).

After the perfusion, pancreata were fixed in Bouin's solution and embedded in paraffin for indirect immunofluorescence staining as previously described (22). Serial sections of $5\ \mu\text{m}$ thickness were stained with a pork insulin antibody (1:100; Miles Inc., Elkhart, IN) or an antibody to the COOH-terminal hexadecapeptide of GLUT-2 (No. 1092; 1:1,000) (19) for 16 h at 4°C . After washing with 0.01 M phosphate-buffered saline (pH 7.6) the sections were layered with either fluorescein-conjugated anti-guinea pig IgG for the insulin antibody or anti-rabbit IgG for the GLUT-2 antibody (1:20; Jackson ImmunoResearch Labs, Inc., West Grove, PA) and incubated for 1 h at room temperature. Sections were then rinsed, counterstained with 0.03% Evans blue, and examined under the fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY). For precise and objective morphometric quantitation of GLUT-2-expressing β cells, the ratio of the volume density of insulin-positive and GLUT-2-positive cells was determined for 10 randomly selected islets from 10 sections of each pancreas using a point counting method (23) as described in detail by Orci et al. (22).

Glucose transport was measured in islets from rats treated with dexamethasone for 12, 24, 48, and 72 h and 7 d. Islets were isolated by a modification of the collagenase digestion technic of Naber et al. (24) and purified on Ficoll gradients. Within 1 h of the isolation, the uptake of 3-O-methyl-D-glucose (3MG) was determined at concentrations of 2, 5, 10, and 20 mM after incubations of 3, 6, and 15 s at 15°C using a method described previously in complete detail (25). Dispersed cells from 250–300 islets ($\sim 5 \times 10^5$ cells) were used at each time point. Uptake of L-[1- ^3H] glucose was measured by the same procedure to correct for extracellular space.

All data are expressed as mean \pm SEM. Statistical significance was determined by the two-tailed Student's *t* test for unpaired differences. The insulin secretory response to 20 mM glucose or 10 mM arginine was expressed as the mean of the sum of the increase in IRI above the baseline value during the perfusion of 20 mM glucose or 10 mM arginine divided by 10 min ($\mu\text{U}/\text{ml}$ per min). Regression analysis was used to determine correlations.

Table 1. Time Course of Blood Glucose, Basal, 20 mM Glucose-, and 10 mM Arginine-stimulated Insulin Secretion, GLUT-2-positive β Cell Area and 3MG Uptake before and during Dexamethasone Administration to Zucker Female Rats (*fa/fa*)

Days of R_x	Blood glucose	Basal insulin secretion	20 mM glucose-stimulated insulin secretion	10 mM Arginine-stimulated insulin secretion	GLUT-2-positive β cell area*	3MG uptake [‡]
	mg/dl	$\mu\text{U}/\text{ml}$ per min	$\mu\text{U}/\text{ml}$ per min	$\mu\text{U}/\text{ml}$ per min	%	%
0	121 \pm 1	13 \pm 2	118 \pm 22	347 \pm 87	100 \pm 2	100
1	175 \pm 8 [§]	47 \pm 7 [§]	38 \pm 8	530 \pm 37	96 \pm 2	77
2	333 \pm 35 [¶]	122 \pm 11 [¶]	-27 \pm 7 [¶]	1,056 \pm 221	86 \pm 4	41
3	359 \pm 35 [¶]	132 \pm 14 [¶]	-30 \pm 8 [¶]	877 \pm 147	83 \pm 3 [¶]	30
7	485 \pm 19 [¶]	191 \pm 31 [¶]	-48 \pm 7 [¶]	1,068 \pm 196	63 \pm 3 [¶]	44
10	549 \pm 10 [¶]	247 \pm 24 [¶]	-87 \pm 12 [¶]	797 \pm 110	35 \pm 6 [¶]	ND**

* Pancreata from three rats were examined in each group. [‡] 3MG uptake was determined by the average uptake at the concentration of 10 and 20 mM 3MG with untreated group (day 0) as 100%. A single determination was made at each time point. [§] $P < 0.005$ vs. control. ^{||} $P < 0.01$ vs. control. [¶] $P < 0.001$ vs. control. ** ND, not done.

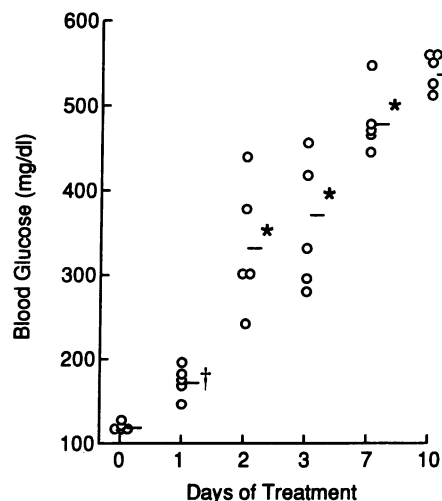


Figure 1. Individual (\circ) and mean ($—$) glucose levels during treatment of female Zucker (*fa/fa*) rats with 0.4 mg/kg per d of dexamethasone. ($n = 5$ from each group, * $P < 0.001$ and $\dagger P < 0.005$ vs. control.)

Results

Clinical findings. At 24 h after the start of dexamethasone administration, blood glucose levels averaged 175 ± 8 mg/dl, significantly above the pretreatment value of 121 ± 1 ($P < 0.005$) (Fig. 1). After 48 h all rats exhibited glucose levels > 200 mg/dl with an average of 333 ± 35 mg/dl. On day 10 of treatment glucose levels averaged 549 ± 10 mg/dl. Glycosuria was present after 24 h and reached a peak level of $1,150 \pm 120$ mg/24 h by the fourth day. Body weight declined from an average of 334 ± 6 g before treatment to a low of 289 ± 5 g on day 10 even though food intake remained constant.

Insulin response to glucose and arginine. We have reported previously that the insulin response to glucose is absent after 24 d of dexamethasone-induced diabetes (11). To determine if the hyperglycemia could be the consequence of the loss of GSIS, we perfused the pancreas of rats at various times after the start of dexamethasone treatment. After 24 h of dexamethasone treatment the insulin response to glucose was significantly

reduced from the pretreatment level of 118 ± 22 to 38 ± 8 $\mu\text{U}/\text{ml}$ per min ($P < 0.01$) (Fig. 2 and Table I). At 48 h and thereafter the positive insulin response to glucose became increasing negative, averaging -87 ± 12 $\mu\text{U}/\text{ml}$ per min on day 10. This paradoxical glucose-induced decline was followed by a paradoxical rise in insulin when the perfusion of 20 mM glucose was stopped and glucose concentration was lowered to 5.6 mM. Both the chronologic relationship and the strong negative correlation between the loss of GSIS and the appearance of

hyperglycemia is consistent with a role of this functional deficit in the pathogenesis of the hyperglycemia (Fig. 3).

Arginine-stimulated insulin secretion was increased during the administration of dexamethasone on all days examined but this was not statistically significant (Fig. 2 and Table I). The baseline insulin secretion rate increased progressively during dexamethasone administration from a pretreatment level of 13 ± 2 to 46 ± 7 $\mu\text{U}/\text{ml}$ per min after 24 h and 247 ± 24 $\mu\text{U}/\text{ml}$ per min on day 10 (Table I).

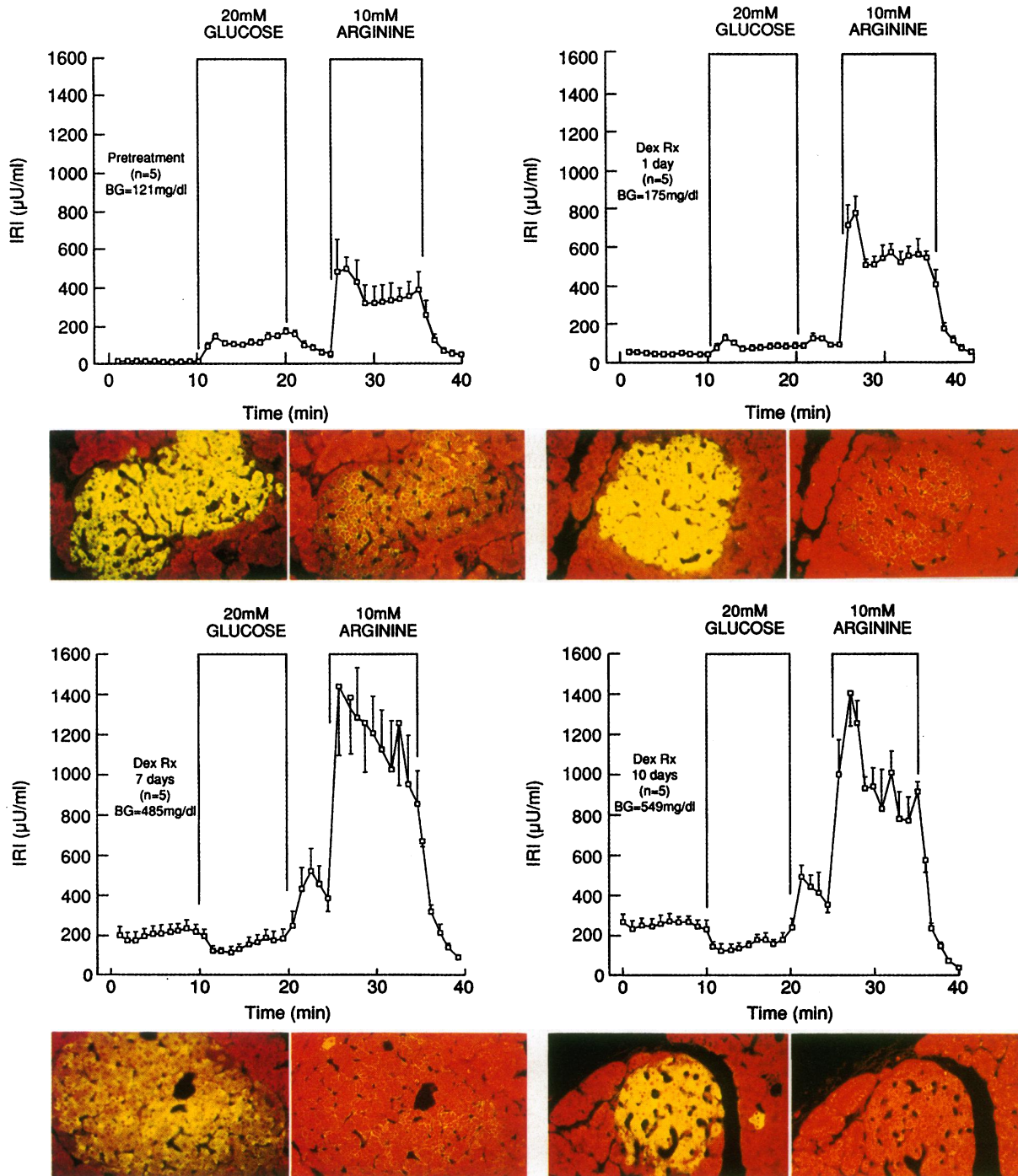


Figure 2. Insulin responses to 20 mM glucose and 10 mM arginine in pancreata isolated from female Zucker (*fa/fa*) rats before and at various times after the start of treatment with 0.4 mg/kg per day of dexamethasone. Representative serial sections obtained from the perfused pancreas of one member of each group of rats and immunostained for insulin (*left*) and GLUT-2 (*right*) are displayed under the insulin profiles ($\times 250$).

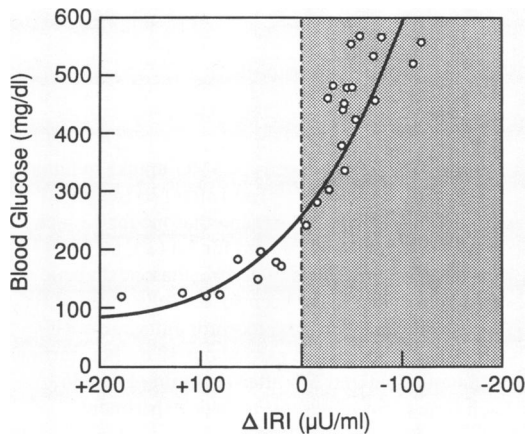


Figure 3. Relationship between the final blood glucose of each dexamethasone diabetic rat and the change in insulin secretion by its isolated perfused pancreas during challenge with 20 mM glucose ($r = -0.92$, $P < 0.001$).

Immunostainable GLUT-2. To determine if the previously reported reduction in β cell GLUT-2 (11) could explain the loss of glucose-stimulated insulin secretion and the appearance of hyperglycemia, the chronologic relationship between the percent of GLUT-2-positive β cells and the foregoing parameters was determined. Fig. 2 displays the perfusion data on various days during dexamethasone administration together with a representative islet from one of the animals; the full morphometric data from each group of rats are shown in Table 1. There was no reduction in immunostainable GLUT-2 24 h after the start of dexamethasone treatment to match the reduction in GSIS. On day 2 there was a significant reduction in the percent of the area of GLUT-2-positive β cells to 86% of the pretreatment level ($P < 0.01$). Thereafter a progressive decline was observed, and on day 10 of dexamethasone only 35% of the β cell area was GLUT-2 positive ($P < 0.001$). There was a

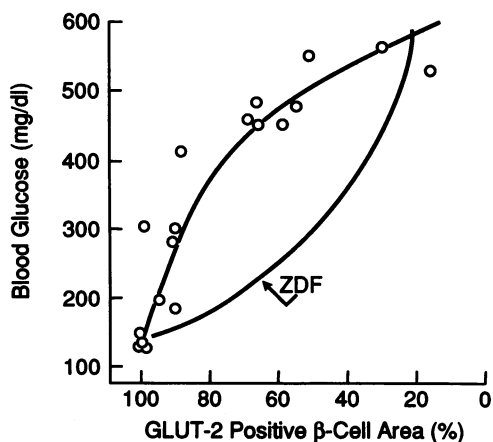


Figure 4. Relationship between the final blood glucose concentration of each dexamethasone-diabetic rat and the percentage of the area of insulin-positive cells that was positive for GLUT-2 by immunostaining ($r = -0.94$, $P < 0.001$) (cf Fig. 2). Relationship observed in the spontaneously occurring diabetes of male Zucker diabetic fatty (ZDF/Drt-*fa*[F10]) rats reported previously (reference 5) is also shown (ZDF).

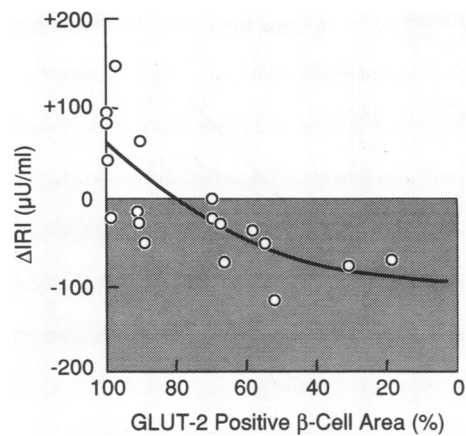


Figure 5. Relationship between the change in IRI induced by 20 mM glucose in perfused pancreata of dexamethasone-diabetic rats and the percent of insulin-positive cell area that was positive for GLUT-2 by immunofluorescent staining ($r = 0.77$, $P < 0.005$).

highly significant negative correlation ($r = -0.94$; $P < 0.001$) between the percent of the GLUT-2-positive β cell area and the final blood glucose level (Fig. 4); the correlation between loss of immunostainable GLUT-2 and loss of GSIS was also significant although less striking ($r = 0.77$; $P < 0.005$) (Fig. 5).

Glucose transport kinetics. The apparent lag in the loss of GLUT-2 immunostainability relative to the foregoing functional and clinical abnormalities could reflect dexamethasone-induced impairment of GLUT-2 function before reduction in the immunoreactivity of the transporter epitope. To determine if this was the case, the kinetics of 3MG uptake were studied in islets isolated from rats before and at 12, 24, 48, and 72 h and 7 d after dexamethasone treatment (Fig. 6 and Table I). The initial velocity of 3MG uptake was unchanged 12 h after the start of treatment but was 77% of the control value at 24 h (Fig. 6 B), at which time glucose-stimulated insulin secretion was only 32% of the control. It declined to 41% of the control on the second day, 30% on the third day, and 44% on the seventh day when the insulin response was a negative one (Fig. 6 B). Thus, a modest reduction in GLUT-2 function coincided temporally with a severe loss of glucose-stimulated insulin secretion, and it preceded the loss of GLUT-2 immunoreactivity. But the magnitude of the transport defect was small compared with the magnitude of the β cell dysfunction.

Effects of reversal of diabetes by the glucocorticoid receptor antagonist RU-486. To determine the effects of pharmacologic blockade of glucocorticoid action upon the changes in β cell function and GLUT-2 induced by dexamethasone, 40 mg/kg per d of RU-486 (Institut Roussel-Uclaf, Paris, France) suspended in sesame oil was injected subcutaneously in female Zucker rats beginning either on day 7 or 10 of dexamethasone administration. Sesame oil without RU-486 was used as the control. RU-486 induced a dramatic improvement in hyperglycemia and glycosuria, blood glucose declining progressively and reaching 100 ± 5 mg/dl within 6 d (Fig. 7). Surprisingly, the elevation in baseline insulin secretion by the perfused pancreata did not recede towards normal despite the amelioration of hyperglycemia. However, GSIS returned from its profoundly negative value to 86 ± 9 μ U/ml per min after 5 d of RU-486 treatment (Table II). During this time the relative

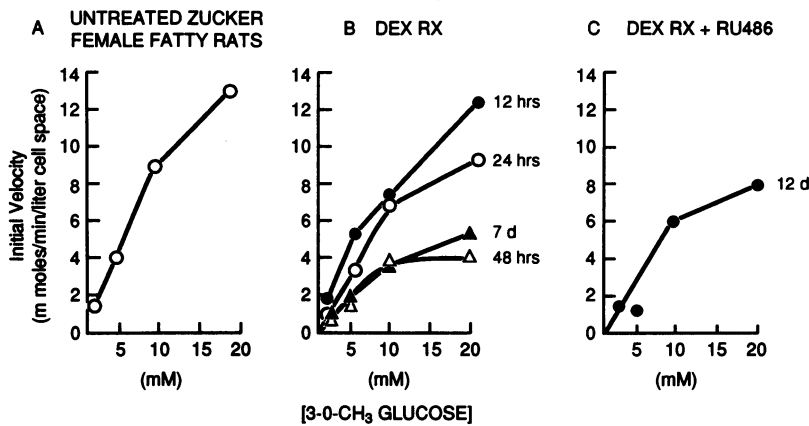


Figure 6. The initial velocity of 3MG uptake in female Zucker fatty rats before (A) and after (B) treatment with 0.4 mg/kg per d of dexamethasone for 12 h (●), for 24 h (○), for 48 h (△), and for 7 d (▲). (C) 3MG uptake by isolated islets of dexamethasone diabetic rats whose diabetes had been successfully reversed (cf Fig. 7) with glucocorticoid receptor antagonist, RU-486. These studies were carried out 12 d after starting dexamethasone and 5 d after beginning RU-486. Each time point represents a single experiment.

area of GLUT-2-positive β cells increased from 19 to 56%, and 3MG uptake rose to 64% of normal (Fig. 6 C).

Discussion

While insulin resistance is generally believed to be necessary in NIDDM (1, 2), hyperglycemia appears only when β cells become incapable of compensating for this peripheral abnormality. This study provides a chronicle of the β cell abnormalities associated with the onset of hyperglycemia in rats with dexamethasone-induced insulin NIDDM, namely, loss of GSIS, reduction in immunostainable GLUT-2 of β cells, and impaired high K_m glucose transport in islets of Langerhans (11). All of the foregoing β cell abnormalities were present in every dexamethasone-treated rat that became diabetic (both Wistar and Zucker rats) and were absent in every dexamethasone-treated rat (Wistar) that did not become diabetic (11). Since these strong associations did not indicate if these changes played a causative role in the pathogenesis of the metabolic derangement, we have tried to determine the sequence of appearance of these abnormalities in the hope of determining whether or not the following pathogenic sequence could be correct: $\downarrow \beta$ cell

GLUT-2 \rightarrow \downarrow high K_m β cell glucose transport \rightarrow loss of GSIS \rightarrow \uparrow glycemia. Previous work has already excluded the possibility that hyperglycemia downregulates GLUT-2 (15, 22).

We observed a remarkably strong temporal relationship between GSIS by the isolated perfused pancreas of the diabetic rats and their final blood glucose level. 24 h after the first dexamethasone injection GSIS was reduced by 68% in association with a 44% increase in blood glucose concentration. Subsequently, the positive response of insulin to glucose disappeared completely; by the second day of dexamethasone administration the 20 mM glucose challenge caused a significant inhibition of insulin secretion; this paradoxical glucose suppression of insulin secretion increased progressively. By day 10 insulin was suppressed to 87 μ U/ml per min below the unstimulated baseline value. This suppression was followed by a paradoxical 234- μ U/ml per min increase in insulin secretion when the perfusate glucose concentration was reduced to the 5.6-mM baseline level. Glucose-induced suppression of insulin secretion has been observed previously in models of spontaneous diabetes in rodents (7, 26) and in humans with NIDDM during intravenous glucose tolerance testing (27). The mechanism may be related to glycogenolysis within the islets (28, 29). It was reversed by treatment with the glucocorticoid receptor antagonist, RU-486, which abolished hyperglycemia. Surprisingly, the baseline level of unstimulated insulin secretion, which had risen progressively from the pretreatment level of 13 to 247 μ U/ml per min on day 10, was not reduced by RU-486 despite the probable improvement in insulin resistance. Although the mechanism of basal hyperinsulinemia is unknown, perhaps the reduction in GLUT-2-mediated high K_m glucose transport during dexamethasone therapy is associated with an increase in low K_m transport that renders β cells responsive to substimulatory levels of glycemia. RU486 may restore high K_m GLUT-2 transport without reducing low K_m transport.

The temporal relationships between the abundance of immunostainable GLUT-2, glucose transport, loss of GSIS, and the blood glucose concentration are depicted in Fig. 8. At 24 h a 68% loss of secretion coincided with only a 23% decline in 3MG uptake. At 48 h, when GSIS was negative, 3MG uptake had decreased by only 59%. Thus, if all β cells in normal islets participate to an equal degree in the response to glucose, the measured reduction in glucose uptake could not by itself account for the functional deficit (30). This implies that other

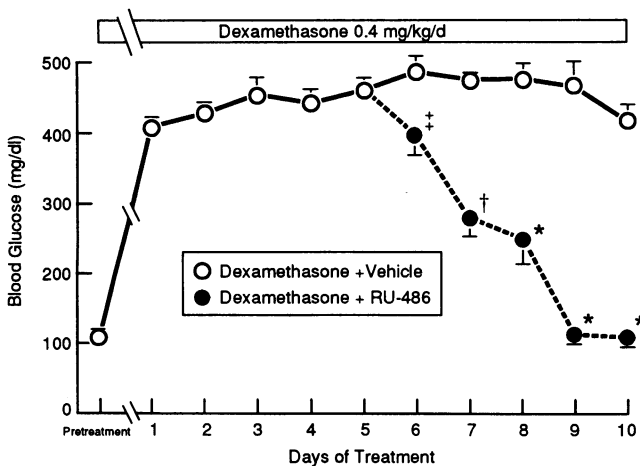


Figure 7. Effect of RU-486 treatment (●) on blood glucose levels of dexamethasone diabetic female Zucker rats ($n = 5$). (○) Injection of the sesame oil in vehicle without RU-486 ($n = 5$) (* $P < 0.001$, † $P < 0.005$, * $P < 0.05$ vs. dexamethasone + vehicle).

Table II. Blood Glucose, Basal, and 20 mM Glucose-stimulated Insulin Secretion, GLUT-2-positive β Cell Area before and during Dexamethasone Administration and Dexamethasone plus RU-486 in Zucker Female Rats (fa/fa)

Group	Blood glucose*	Basal insulin secretion	Glucose-stimulated insulin secretion	GLUT-2-positive β cell area [‡]
	mg/dl	μ U/ml per min	μ U/ml per min	%
Control (n = 5)	121 \pm 1	13 \pm 2	118 \pm 22	100 \pm 2
Dexamethasone (n = 5)	426 \pm 44	202 \pm 26	-48 \pm 15	19 \pm 2
Dexamethasone + RU-486 (n = 5)	100 \pm 5 [§]	223 \pm 41	86 \pm 9 [§]	56 \pm 7 [§]

* Blood glucose was determined the day of experiment. [‡] 10 randomly selected islets from each rat were examined for three rats in each group. [§] P < 0.001 vs. Dexamethasone + vehicle.

molecular defects or interactions must be involved in loss of GSIS in this form of diabetes as in other models of NIDDM (9, 31). In fact, preliminary evidence of impaired glyceraldehyde-stimulated insulin secretion has been obtained (Ohneda, M., and R. H. Unger, unpublished observations).

However, it would be premature to assume that this partial reduction in GLUT-2 function is necessarily irrelevant to the complete loss of GSIS. All normal β cells express GLUT-2 (22), but only a subset express glucokinase at high levels (32), a subset that responds to glucose (33–35); consequently, if GLUT-2 function were completely lost in the glucose-responsive subset of β cells, the partial reduction in GLUT-2 observed might account for the complete loss of GSIS. There is another scenario by which impairment of GLUT-2 function might completely explain the abnormality in GSIS; mounting evidence

suggests that GLUT-2 may have a role in GSIS in addition to glucose transport. For example, although glucose transport is almost identical in GLUT-1- and GLUT-2-transfected AtT-20_{ins} cells, only the latter exhibit GSIS (36); additionally, GLUT-2 transfection in RIN cells increases glucokinase activity (37). It is therefore possible that GLUT-2 loss may alter glucose metabolism and function of β cells via mechanisms other than the reduction in glucose transport.

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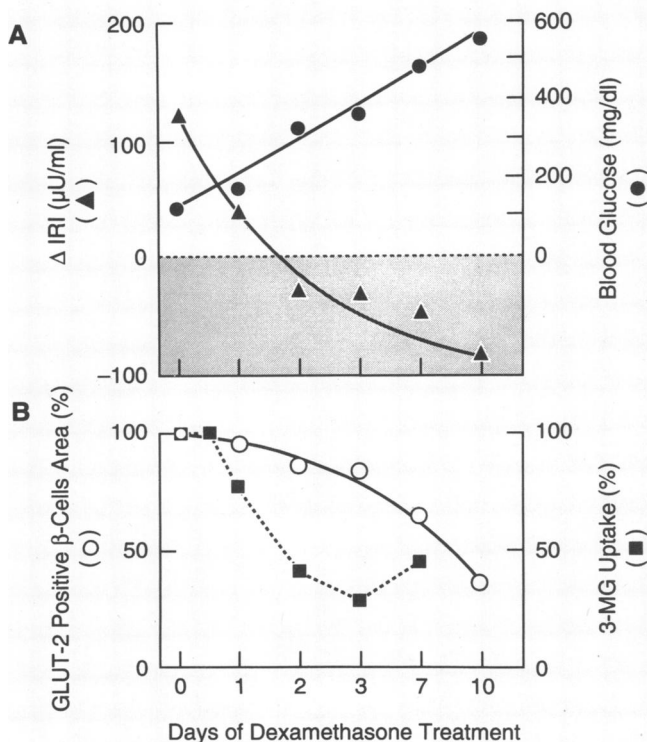


Figure 8. A chronology of changes in blood glucose, glucose-stimulated insulin secretion (IRI), the GLUT-2-positive β cell area (%) and 3-MG uptake during the induction of diabetes by dexamethasone.

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