Roles of Insulin Resistance and β-Cell Dysfunction in Dexamethasone-induced Diabetes

Atsushi Ogawa, John H. Johnson, Makoto Ohneda, Chris T. McAllister, Lindsey Inman, Tausif Alam, and Roger H. Unger

Center for Diabetes Research, Gifford Laboratories, Department of Internal Medicine and Pathology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235; and Department of Veterans Affairs Medical Center, Dallas, Texas 75216

Abstract

The roles of insulin resistance and β-cell dysfunction in glucocorticoid-induced diabetes were determined in Wistar and Zucker (*fa/fa*) rats. All Wistar rats treated with 5 mg/kg per d of dexamethasone for 24 d exhibited increased β-cell mass and basal and arginine-stimulated insulin secretion, indicating insulin resistance, but only 16% became diabetic. The insulin response to 20 mM glucose was normal in the perfused pancreas of all normoglycemic dexamethasone-treated rats but absent in every diabetic rat. Immunostainable high K_m β-cell transporter, GLUT-2, was present in $\sim$ 100% of β-cells of normoglycemic rats, but in only 25% of β cells of diabetic rats. GLUT-2 mRNA was not reduced. All Zucker (*fa/fa*) rats treated with 0.2–0.4 mg/kg per d of dexamethasone for 24 d became diabetic and glucose-stimulated insulin secretion was absent in all. High K_m glucose transport in islets was 50% below nondiabetic controls. Only 25% of β cells of diabetic rats were GLUT-2-positive compared with $\sim$ 100% in controls. Total pancreatic GLUT-2 mRNA was increased twofold suggesting a posttranscriptional abnormality. We conclude that dexamethasone induces insulin resistance, whether or not it induces hyperglycemia. Whenever hyperglycemia is present, GLUT-2-positive β cells are reduced, high K_m glucose transport into β cells is attenuated and the insulin response to glucose is absent. (*J. Clin. Invest.* 1992. 90:497–504.) Key words: dexamethasone • diabetes • GLUT-2 • insulin resistance • β-cell function

Introduction

The earliest demonstrable functional lesion of β cells in spontaneously occurring non–insulin-dependent diabetes mellitus (NIDDM)[1] of man and rodents is selective loss of glucose-stimulated insulin secretion (1–4). Although β cells of human diabetics have not been studied, in rats with NIDDM this functional deficit is associated with a profound reduction in the expression in pancreatic β cells of the high K_m glucose transporter, GLUT-2 (5, 6), and in high K_m glucose uptake by the islets (5). These observations have led to the suggestion that underexpression of GLUT-2 is the cause of the β-cell insensitivity to glucose and the associated inability to correct hyperglycemia (7).

However, it is widely believed that insulin resistance is the primary defect of NIDDM and can cause hyperglycemia without derangement of β cells. To assess the relative roles of insulin resistance and impairment of glucose-stimulated insulin secretion in the pathogenesis of diabetic hyperglycemia we studied glucocorticoid-induced NIDDM. In this model the hyperglycemia is generally attributed to insulin resistance rather than a β-cell defect and there is no reported evidence that glucose-stimulated insulin secretion is selectively compromised. Consequently, this form of diabetes seemed ideal to determine whether or not hyperglycemia can occur in the absence of β-cell dysfunction.

Methods

To induce glucocorticoid diabetes normal Wistar rats received daily intraperitoneal injections of 5 mg/kg of dexamethasone for 24 d (Azium®; Schering Corp., Kenilworth, NJ). 4 of 25 rats became diabetic by the fifth day. Food intake and body weight of the diabetic rats declined but did not differ from that of the 21 rats that remained nondiabetic throughout the 24 d of steroid administration. The pancreata of all 4 diabetic rats, 5 of the nondiabetic steroid-treated rats, and 4 untreated controls were then isolated and perfused by the method of Grodsky and Fanska (9) as modified by Tominaga et al. (10). Insulin was measured by radioimmunoassay (10).

Each pancreas was fixed in Bouin's solution immediately after perfusion and processed for immunofluorescence staining for insulin and GLUT-2, as previously described (11). GLUT-2 antibody 1092 was raised against a synthetic hexadecapeptide from the COOH-terminal portion of the predicted sequence of GLUT-2 (5, 12). Volume fraction of insulin-positive cells versus GLUT-2-positive cells was determined in 5-μm thick adjacent sections of pancreas using the stereologic method of Weibel (13) on 10 randomly selected islets per animal. The volume fraction of the endocrine pancreas was determined in hematoxylin-eosin–stained sections by measuring the cross-sectional area of all islets within the section and dividing by the total area of the pancreatic section.

We also treated Zucker fatty rats with dexamethasone for 24 d. Diabetes developed in every rat within 4 d of treatment with only 0.2–0.4 mg/kg, thus providing a sufficiently large pool of islets from diabetic animals to perform kinetic studies of glucose transport 24 d after the onset of diabetes. The low 16% incidence of diabetes in Wistar precluded such studies in Wistar rats with dexamethasone-induced diabetes. Only Zucker fatty rats were therefore employed for studies of the uptake of 3-*O*-methyl glucose into dispersed islet cells performed as described previously in detail (14).

For immunoblot analysis of GLUT-2 total membranes were prepared from islets isolated from each of five untreated Wistar rats, five untreated Zucker fatty female controls, and five dexamethasone-treated diabetic Zucker fatty female rats. 20 μg of total membrane protein from each rat was separated by SDS-PAGE as described (5) and blotted with anti–GLUT-2 antibody 1092. ^{125}I-labeled goat anti-rabbit Fab serum was applied and exposed to XAR-40 x-ray film for 19 h. Autoradioautograms were quantitated by densitometry.

Proinsulin and GLUT-2 mRNA were determined in pancreata excised from both Zucker fatty female and Wistar rats and frozen in

1. *Abbreviation used in this paper:* NIDDM, non–insulin-dependent diabetes mellitus.

Table I. Comparison of Parameters Reflecting of Compensation for Insulin Resistance in Dexamethasone-treated Rats

Rat groups with terminal blood glucose	Number of islets per mm² of pancreas	Volume fraction of endocrine pancreas	Basal IRI secretion
mg/dl		*%*	*μU/ml per min*
A. Normal Wistar rats			
Untreated controls 129±5 (n = 3)	0.27±0.05	0.36±0.05	10.9±3.8
Dex-treated nondiabetics 112±7 (n = 4)	0.90±0.10*	2.01±0.32*	372±50*
Dex-treated diabetics 349±47 (n = 3)	0.61±0.15	1.29±0.25‡	206±35*
B. Zucker rats			
Untreated controls 128±4 (n = 3)	0.78±0.02*	1.39±0.31‡	127±42‡
Dex-treated diabetics 442±12 (n = 3)	0.89±0.12‡	1.88±0.33‡	272±45‡

* $P < 0.005$. ‡ $P < 0.05$, vs. untreated Wistar rats.

liquid nitrogen. RNA was extracted by the method of McDonald et al. (15) using 4 M guanidinium isothiocyanate, 0.1 M citrate, pH 7.4, and 2% β-mercaptoethanol followed by phenol-chloroform extraction and alcohol precipitation. The poly A+ RNA fraction from total RNA was prepared using oligo(dT)-cellulose. From each animal 4 μg of poly A+ RNA was subjected to electrophoresis in 1.5% agarose gel containing formaldehyde. The RNA was transferred from the gel to a positively charged nylon membrane, UV–cross-linked, and sequentially probed with ³²P-labeled antisense RNA probes for rat GLUT-2, actin, and insulin (16). For quantitation of GLUT-2 and proinsulin, mRNA auto-radiograms were scanned with a Hoefer GS300 densitometer (GS300; Hoefer Scientific Instruments, San Francisco, CA) and normalized to the actin signal.

Statistical analyses were performed by Student's *t* test for two groups.

Results

Induction of diabetes by dexamethasone treatment in lean Wistar and obese Zucker rats. The daily administration of 5 mg/kg of dexamethasone by intraperitoneal injection induced diabetes in 4 of 25 Wistar rats within 5 d. In these four rats the mean of all morning glucose levels during the subsequent 3 wk was 381±19 mg/dl and the mean of daily urinary excretion of glucose was 275±94 mg per 24 h. Continued dexamethasone treatment for 3 wk failed to induce diabetes in any of the 21 rats that were still normoglycemic after 5 d of the steroid. Their morning glucose levels averaged 112±7 mg/dl and they were aglycosuric.

Obese Zucker (*fa*/fa) female rats were also treated with dexamethasone. All promptly developed diabetes on a dose of only 0.2–0.4 mg/kg, which was continued until the time of killing 24 d later. Their mean morning blood glucose level was 422±12 mg/dl, and their 24-h urinary glucose excretion averaged 365±30 mg. Blood glucose levels in untreated littermates averaged 128±4 mg/dl.

Effect of dexamethasone on β-cell mass and baseline insulin secretion of Wistar and Zucker rats. Since insulin resistance is associated with a compensatory increase in β-cell mass, we analyzed morphometrically the number and size of islets in all pancreatic specimens (Table I *A*). The volume fraction of the islets of nondiabetic Wistar rats treated with dexamethasone
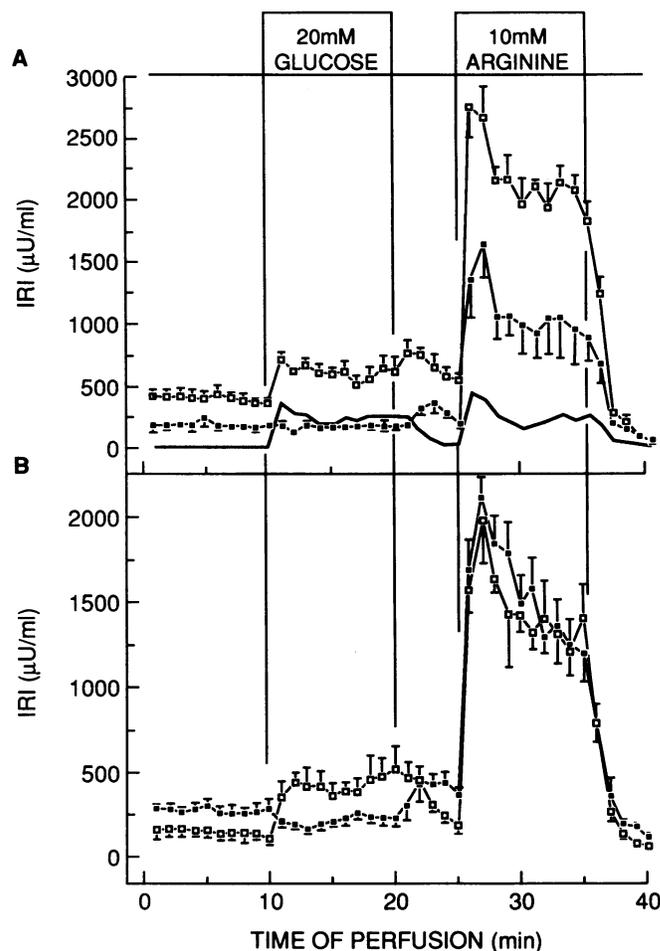

Figure 1. (*A*) Insulin response to 20 mM glucose and 10 mM arginine of the isolated perfused pancreata of dexamethasone-induced diabetic Wistar rats (■——■; n = 4), nondiabetic dexamethasone-treated Wistar rats (□——□; n = 4), and untreated nondiabetic control Wistar rats (——; n = 4). (*B*) Insulin response to 20 mM glucose and 10 mM arginine of isolated perfused pancreata of dexamethasone induced diabetic Zucker female rats (■——■; n = 4) and nondiabetic untreated Zucker female control rats (□——□; n = 3).
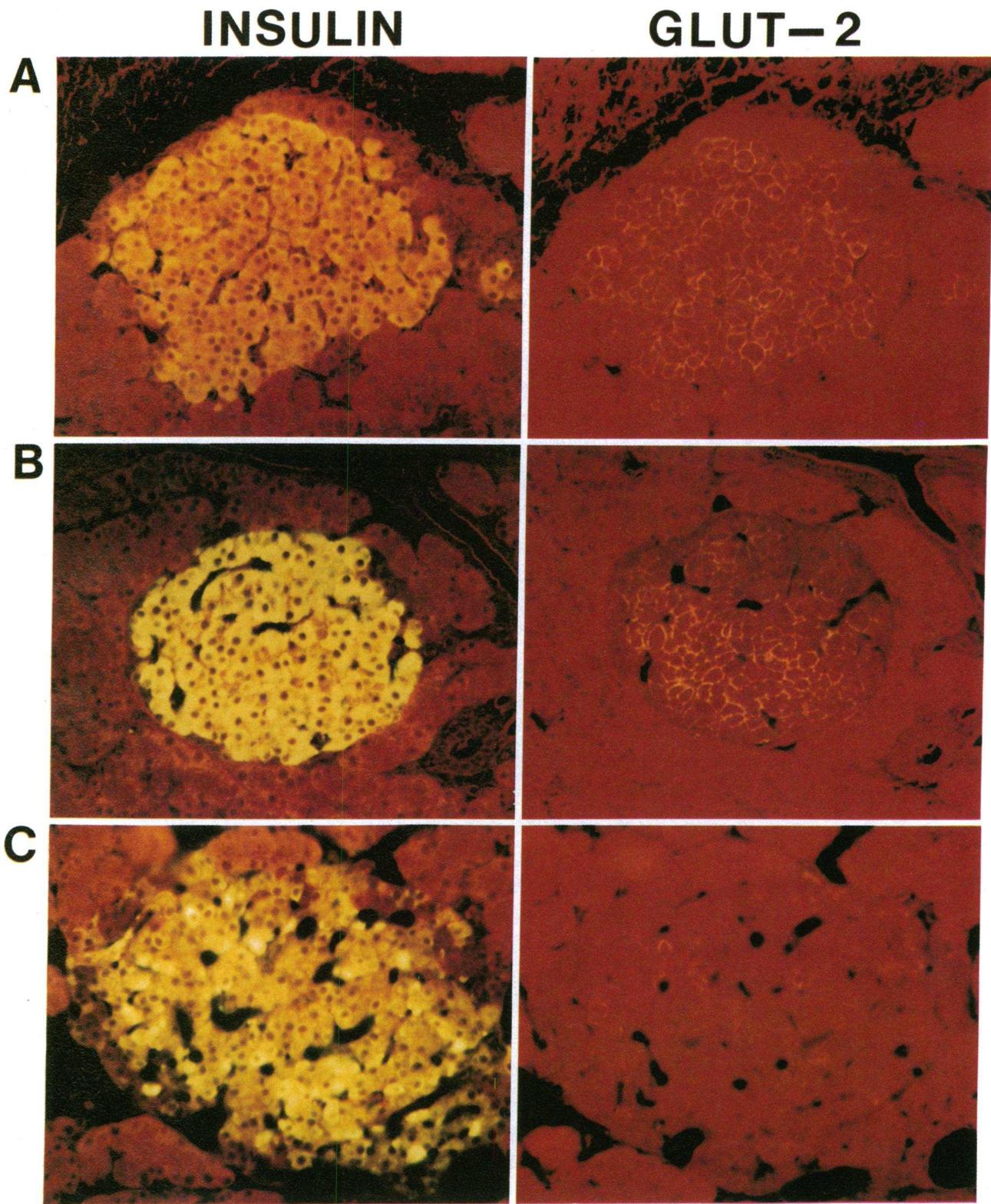
INSULIN

GLUT−2

Figure 2. Pairs of consecutive serial sections stained by immunofluorescence with antiinsulin (*left*) and anti-GLUT-2 (*right*) antibodies. (*A*) Islet of a nondiabetic Wistar control rat. Note that virtually all insulin-positive cells show positive staining for GLUT-2. (*B*) Islet of a nondiabetic dexamethasone-treated Wistar rat. GLUT-2 staining of β cells of the nondiabetic steroid-treated rat is completely normal. (*C*) The islet of a diabetic dexamethasone-treated rat showing a profound reduction of GLUT-2 in β cells, which are degranulated.

A. Ogawa, J. H. Johnson, M. Ohneda, C. T. McAllister, L. Inman, T. Alam, and R. H. Unger
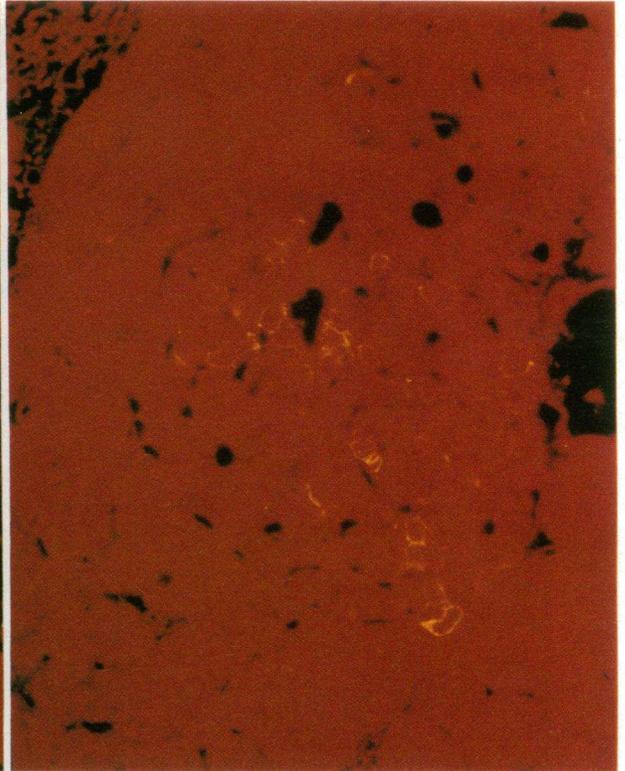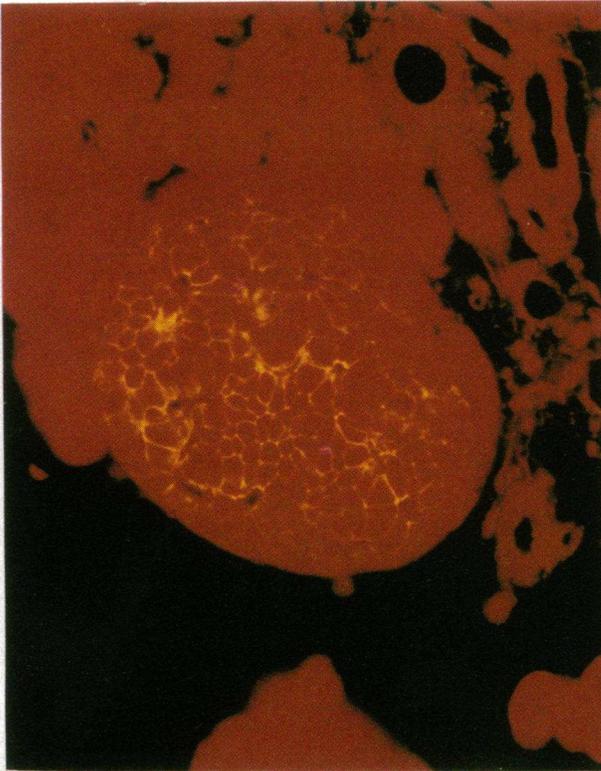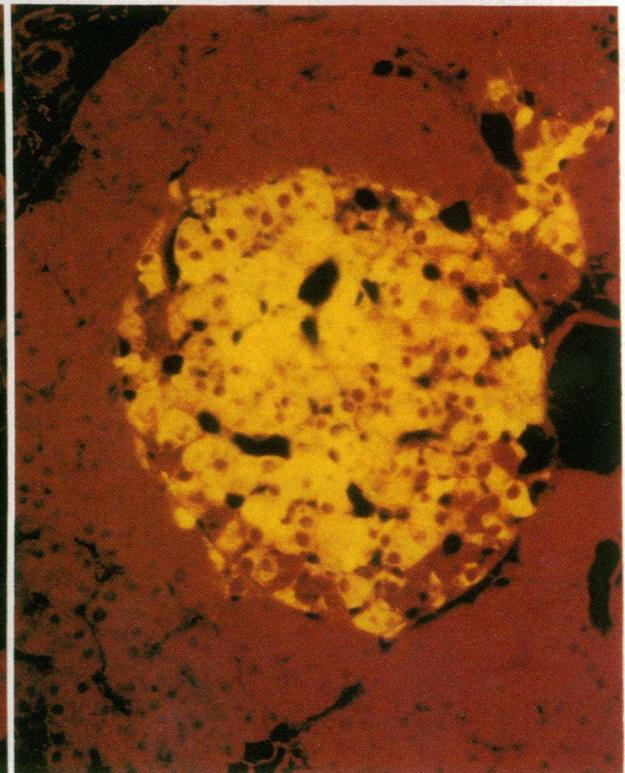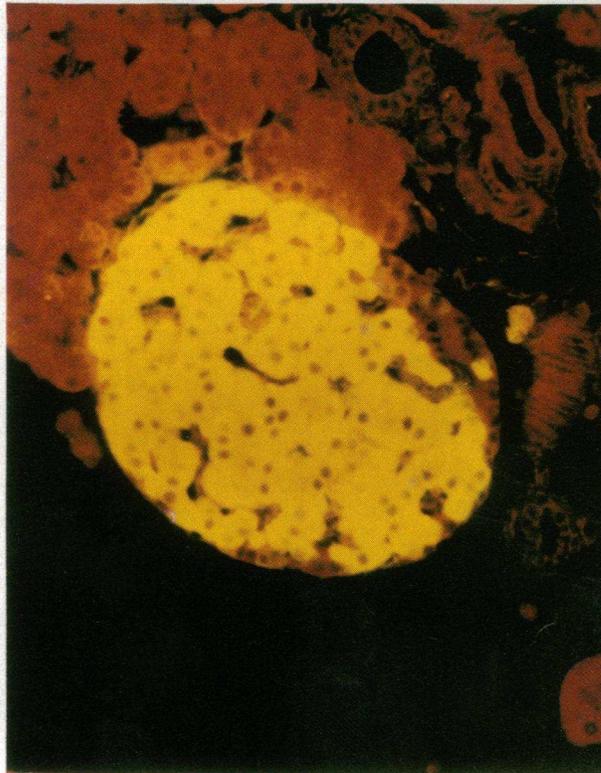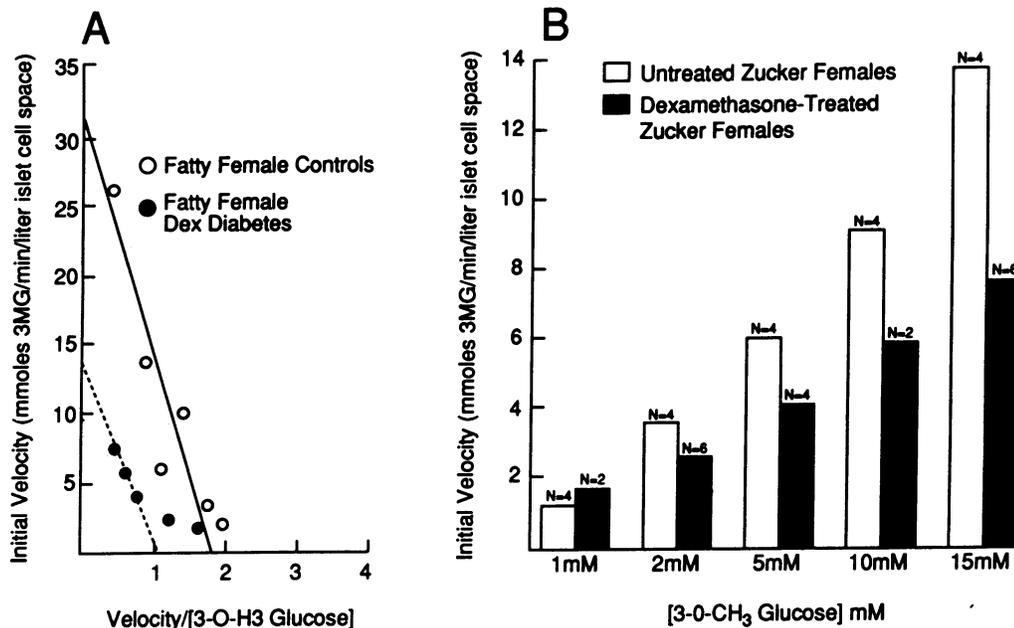
Figure 4. Glucose uptake by islets isolated from Zucker fatty female rats with dexamethasone-induced diabetes (●) or without dexamethasone-induced diabetes (○). Islets were isolated from six animals in each group and the uptake of 3-O-methyl glucose was measured on dispersed cells as described previously in detail (14). Initial rates of uptake were plotted as an Eadie-Hofstee transformation (A) based on data displayed in B. The closed bars and the closed circles represent the kinetics of glucose uptake by islets from normal Wistar rats (14). In A, the slope represents the K_m and the intercept with the y axis indicates the V_{max}. Although these experiments were designed to analyze only the high K_m function, the previously described (14, 17) low K_m function can be discerned in the diabetic group. In the control group, however, it is not apparent because of insufficient data points.

was 5.6 times greater than untreated Wistar controls, consistent with insulin resistance. In the three diabetic dexamethasone-treated rats studied the volume fraction of islets was 64% of the treated nondiabetics but this was still 3.6 times greater than the untreated controls.

In untreated Zucker rats the volume fraction of islets was more than three times that of lean Wistar controls, consistent with the preexisting obesity-related insulin resistance (Table I B). Dexamethasone-induced diabetes was not associated with a further increase.

Insulin resistance is also associated with an increase in insulin levels. In isolated perfused pancreata of nondiabetic dexamethasone-treated Wistar rats perfused with 5 mM glucose, baseline insulin levels averaged 20 times higher than in untreated Wistar controls; in the diabetic Wistar rats the average was 11 times greater than in normal (NS) (Fig. 1 A). Baseline insulin levels in dexamethasone-diabetic Zucker animals were also substantially higher than in untreated littermates (Fig. 1 B). Thus two indices of compensation by β cells for insulin resistance were present in all dexamethasone-treated rats whether or not they became diabetic.

Effects of dexamethasone on glucose-stimulated insulin secretion by isolated perfused pancreata. All forms of spontaneous diabetes thus far studied exhibit a selective loss of glucose-stimulated insulin secretion without parallel loss of the insulin response to nonglucose secretagogues (1–5, 10). The insulin response of diabetic dexamethasone-treated Wistar rats to 20 mM glucose was completely absent while the insulin response to 10 mM arginine in both diabetic and nondiabetic steroid-treated groups was greater than that of untreated controls (Fig. 1 A). The response to glucose was not reduced in any of the nondiabetic dexamethasone-treated animals (Fig. 1 A).

In the diabetic dexamethasone-treated Zucker rats the insulin response to glucose was also absent (Fig. 1 B), while the insulin response to arginine was virtually identical to untreated controls.

Effect of dexamethasone on immunodetectable GLUT-2 in nondiabetic and diabetic rats. In previous studies of spontaneously occurring NIDDM the percent of GLUT-2 positive β cells was found to be profoundly reduced (5, 6). A similar reduction in the percent of GLUT-2-positive β cells was observed in dexamethasone-induced diabetes. In the pancreata of normal untreated Wistar rats and nondiabetic dexamethasone-treated Wistar rats, virtually 100% of β cells were GLUT-2-positive (Fig. 2, A and B). In diabetic Wistar rats, however, only 24±3% of insulin-positive cells stained positively for GLUT-2 (Fig. 2 C). Identical results were obtained in Zucker rats. In untreated Zucker controls GLUT-2 was present in 99±4% of insulin-positive cells, compared to 25±1% in dexamethasone-diabetic animals (Fig. 3, A and B).

However, total islet GLUT-2 protein determined densitometrically from immunoblots (not shown) of total membranes of islets of five dexamethasone-diabetic Zucker rats was 69±6% of that of untreated controls (0.05 > P < 0.1).

Glucose transport kinetics in dexamethasone-diabetic Zucker rats. High K_m transport is impaired in rats with spontaneous NIDDM and this is thought to contribute to loss of glucose-stimulated insulin secretion (5). Because of the large number of islets required for kinetic analyses of glucose transport in dexamethasone-induced diabetes, the low incidence of the disorder in Wistar rats precluded studies of glucose transport kinetics (17). The high incidence of diabetes in Zucker rats made it easy to assemble pools of diabetic islets sufficient for measurement of initial rates of 3-O-methyl glucose uptake at varying

---

Figure 3. Pairs of consecutive serial sections of pancreas of untreated Zucker control rats (A) and Zucker rats with dexamethasone-induced diabetes (B) stained for insulin and GLUT-2.
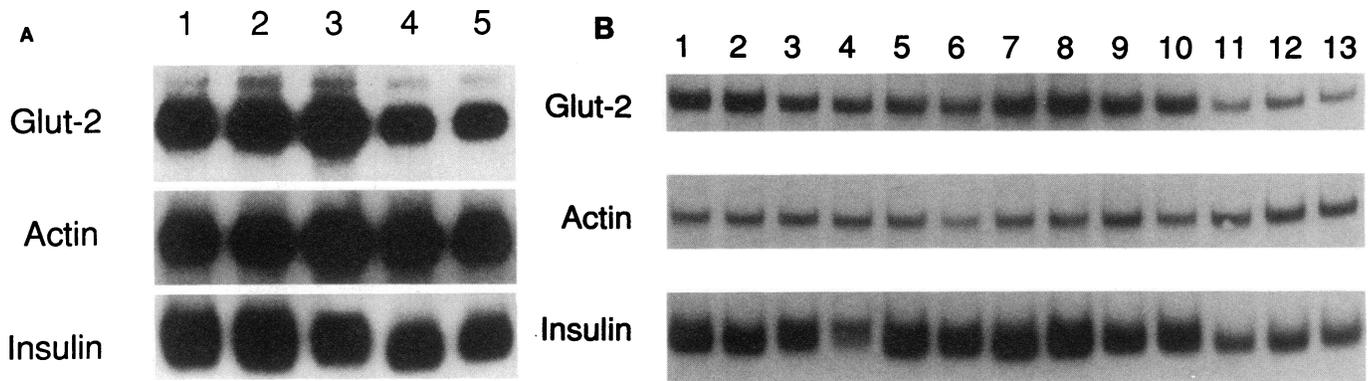
Figure 5. (A) RNA blot hybridization of diabetic dexamethasone-treated Zucker rats and untreated Zucker controls. Lanes *1*, *2*, and *3* represent 4 μg poly (A$^+$) pancreatic RNA from three Zucker rats treated with dexamethasone and lanes *4* and *5* are 4 μg RNA samples from two control Zucker rats. The bands marked as GLUT-2, actin, and insulin show the signal obtained in sequential probing of the same blot with the respective probes. (B) RNA blot hybridization of one diabetic dexamethasone-treated Wistar (lane *4*), nine nondiabetic dexamethasone-treated Wistar rats (lanes *1-3* and *5-10*) and three untreated Wistar rats (lanes *11-13*).

concentrations of 3-*O*-methyl glucose. The V_{max} of the high K_m component in islets isolated from Zucker rats with dexamethasone-induced diabetes was found to be reduced by 50% compared to untreated controls (Fig. 4), evidence of reduced transporter abundance.

GLUT-2 mRNA levels in diabetic and nondiabetic dexamethasone-treated rats. To determine if the reduction in GLUT-2-positive β cells of dexamethasone-induced diabetes, like that in spontaneously occurring NIDDM of rats, is pretranslationally mediated, pancreatic GLUT-2 mRNA was measured. Surprisingly, in dexamethasone-induced Zucker diabetic rats GLUT-2 mRNA was 2.4 times greater than in untreated control animals but there was no difference in the proinsulin mRNA (Fig. 5 *A* and Table II *A*).

In a diabetic Wistar rat with a mean blood glucose level of 276±12 mg/dl and nine other Wistar rats with blood glucose levels averaging 131±2 mg/dl, pancreatic GLUT-2 mRNA values were markedly elevated compared to untreated controls (Fig. 5 *B* and Table II *B*). However, when corrected for the increase in islet volume fraction observed in Table I there were no differences in the three groups of Wistar rats.

Discussion

Massive doses of dexamethasone for 24 days induced diabetes in < 20% of Wistar rats. The diabetes began within five days of treatment and all animals that had not developed diabetes by this time remained nondiabetic despite continued treatment. Whether or not they became diabetic, all dexamethasone-treated rats exhibited indirect evidence of insulin resistance, i.e., their islet mass and basal and arginine-stimulated insulin secretion were strikingly increased. These indices of insulin resistance were present but less marked in the diabetic dexamethasone-treated rats, which could indicate inadequate compensation. Thus dexamethasone-induced insulin resistance alone was not sufficient to cause diabetes in Wistar rats; as in spontaneous NIDDM (5–7), a concomitant β-cell abnormality was present without exception whenever diabetic hyperglycemia occurred.

Zucker (*f a/fa*) female rats were far more sensitive to dexamethasone; diabetes was induced in 100% of animals with a dose of dexamethasone only 4–8% of the dose used in Wistar rats. In this group the values for islet mass and basal and argi-

Table II. Pancreatic GLUT-2 and Proinsulin mRNA in Dexamethasone (Dex)-treated Rats and Untreated Controls (Mean±SEM)

	Total pancreatic mRNA*		mRNA per islet volume fraction‡		Lane No. (Fig. 5)
	GLUT-2	Proinsulin	GLUT-2	Proinsulin	
A. Zucker					
Dex-diabetic (n = 3)	1.9±0.2	2.7±0.7	1.0	1.4	*A, 1-3*
Untreated (*n* = 2)	0.6	1.9	0.4	1.3	*A, 4, 5*
B. Wistar Rats					
Dex-diabetic (*n* = 1)	0.95	1.3	0.74	0.98	*B, 4*
Dex-nondiabetic (*n* = 9)	1.6±0.4	3.5±0.98	0.78±0.22	1.73±0.49	*B, 1-3, 5-10*
Untreated (*n* = 3)	0.27±0.03	0.8±0.04	0.74±0.08	2.26±0.11	*B, 11-13*

* Densitometric reading of each mRNA divided by that of actin mRNA. ‡ Total pancreata mRNA value divided by the volume fraction (from Table I).

nine-stimulated insulin secretion before treatment were high compared to Wistar rats, evidence of underlying insulin resistance; dexamethasone caused only a modest additional augmentation of these parameters.

In dexamethasone-induced diabetes of both Wistar and Zucker rats, as in all other models of diabetes thus far studied, glucose-stimulated insulin secretion was absent and the percent of β cells displaying immunostainable GLUT-2 was markedly reduced. However, the reduction of islet GLUT-2 measured by immunoblotting of total membrane preparations of islets isolated from Zucker diabetic rats was reduced by only 31%, compared with a 75% reduction in GLUT-2–positive β cells. We speculate that this difference is either due to masking of the epitope in situ or to an increase in GLUT-2 expression in the 25% of β cells that displayed GLUT-2. The fact that the V_{max} of the initial velocity of high K_m glucose transport was also reduced by only 50% suggests that the decrease in total number of functional transporters was less than the decrease in the percent of β cells positive for GLUT-2. In other words, in those β cells that remained GLUT-2 positive, the abundance of high K_m transporters (and thus the V_{max}) may actually have increased. However, the nondiabetic Wistar rats treated with dexamethasone showed no such increase. A doubling of functioning high K_m transporters in the GLUT-2–positive β cells would reconcile the results of the immunocytochemical data, the immunoblots, and the kinetic studies.

In steroid-induced diabetic Zucker rats pancreatic GLUT-2 mRNA was substantially above untreated Zucker controls, while in Wistar rats treated with dexamethasone the increase in total pancreatic GLUT-2 mRNA could be accounted for by the increase in islet size. Thus in neither group could the reduction in β cells displaying immunostainable GLUT-2 be explained by a reduction in total pancreatic mRNA. Possible explanations for the normal or increased pancreatic GLUT-2 mRNA in association with a decrease in functional protein, include (a) a glucocorticoid-induced translation block such as has been described for tumor necrosis factor-α (18); (b) a high rate of GLUT-2 protein degradation with a compensatory increase in GLUT-2 mRNA; (c) epitope masking with impairment of high K_m transport function and a compensatory increase in GLUT-2 mRNA; or (d) a very marked compensatory increase in GLUT-2 mRNA limited to the 25% of β cells that still display GLUT-2. A less likely explanation is that variant splicing of mRNA resulted in a hybridizable message but a protein that is either unstable or is functionally defective and immunocytochemically undetectable.

We had previously presented evidence that in spontaneous NIDDM of Zucker diabetic fatty rats the reduction in GLUT-2 might be the cause rather than the consequence of hyperglycemia because prevention of hyperglycemia failed to prevent underexpression of GLUT-2 in their β cells (6). In these studies no such experiments were carried out, making it impossible to separate cause and effect.

Lest the complete absence of glucose-stimulated insulin secretion, despite the fact that 25% of β cells were GLUT-2–positive, be construed as evidence against a causal role of GLUT-2 underexpression, it should be recalled that in spontaneously diabetic Zucker diabetic fatty rats glucose-stimulated insulin secretion was absent whenever the percent of GLUT-2–positive β-cells was below 60% (5). This could mean that only a

subset of β cells are glucose responsive, or it may indicate other molecular lesions in the β-cell glucose metabolic pathway distal to the glucose transporter. It could also reflect the effects of the chronic hyperglycemia, averaging 24 mM, which may be maintaining a chronic state of maximal glucose-stimulated insulin secretion in the 25% of β cells expressing GLUT-2. They would therefore be incapable of an additional response to 20 mM glucose, while the β cells lacking GLUT-2 are rendered incapable of such response by the impaired high K_m glucose transport.

In contrast to insulin resistance which occurred in all rats treated with dexamethasone, sensitivity to the diabetogenic effects of dexamethasone varied greatly in two genetically distinct rat populations. Recalling that the "cortisone-glucose tolerance test" was once touted as a means of revealing a β-cell defect in otherwise undetectable prediabetic subjects (19, 20), one can wonder if this varying sensitivity may not be genetically determined and, if so, whether it is relevant to the genetics of NIDDM. Based on the findings in this study the phenotype of the putative genotype could be inability of β cells to compensate sufficiently for antecedent glucocorticoid-induced insulin resistance.

References

1. Cerasi, E., R. Luft, and S. Efendic. 1971. Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects: a glucose dose-response study. *Diabetes*. 21:224–234.

2. Palmer, J. P., J. W. Benson, R. M. Walter, and J. W. Ensinck. 1976. Arginine-stimulated acute phase of insulin and glucagon secretion in diabetic subjects. *J. Clin. Invest.* 58:565–570.

3. Aronoff, S. L., P. H. Bennett, and R. H. Unger. 1976. Immunoreactive glucagon (IRG) response to intravenous glucose in prediabetes and diabetes among Pima Indians and normal Caucasians. *J. Clin. Endocrinol. & Metab.* 44:968–972.

4. Pfeiffer, M. A., J. B. Halter, and D. Porte, Jr. 1981. Insulin secretion in diabetes mellitus. *Am. J. Med.* 70:579–588.

5. Johnson, J. H., A. Ogawa, L. Chen, L. Orci, C. B. Newgard, T. Alam, and R. H. Unger. 1990. Underexpression of β-cell high K_m glucose transporters in noninsulin-dependent diabetes. *Science (Wash. DC)*. 250:546–549.

6. Orci, L., M. Ravazzola, D. Baetens, L. Inman, M. Amherdt, R. G. Peterson, C. B. Newgard, J. H. Johnson, and R. H. Unger. 1990. Evidence that down-regulation of β-cell glucose transporters in non-insulin-dependent diabetes may be the cause of diabetic hyperglycemia. *Proc. Natl. Acad. Sci. USA*. 87:9953–9957.

7. Unger, R. H. 1991. Diabetic hyperglycemia—link to impaired high K_m glucose transport in pancreatic β-cells. *Science (Wash. DC)*. 251:1200–1205.

8. Flier, J. S., and A. C. Moses. 1989. Diabetes and Cushing's Syndrome. *In* Endocrinology. L. J. DeGroot, editor. W. B. Saunders, Philadelphia. 1392–1394.

9. Grodsky, G. M., and R. E. Fanska. 1975. The in vitro perfused pancreas. *Methods Enzymol.* 39:364–372.

10. Tominaga, M., I. Komiya, J. H. Johnson, L. Inman, T. Alam, J. Moltz, B. Crider, Y. Stefan, D. Baetens, K. McCorkle, L. Orci, and R. H. Unger. 1986. Loss of insulin response to glucose but not arginine during the development of autoimmune diabetes in BB/W rats: relationships to islet volume and glucose transport rate. *Proc. Natl. Acad. Sci. USA*. 83:9749–9753.

11. Coons, A. H., E. H. Leduc, and J. M. Connolly. 1955. Studies on antibody production: method for histochemical demonstration of specific antibody and its application to study of hyperimmune rabbit. *J. Exp. Med.* 102:49–60.

12. Thorens, B., G. C. Weir, J. L. Leahy, H. F. Lodish, and S. Bonner-Weir. 1990. Reduced expression of the liver/beta-cell glucose transporter isoform in glucose-insensitive pancreatic beta-cells of diabetic rats. *Proc. Natl. Acad. Sci. USA.* 87:6492–6496.

13. Weibel, E. R. 1979. Practical methods for biological morphometry. *In* Stereological Methods. Vol. 1. Academic Press Ltd., London. 101–161.

14. Johnson, J. H., B. P. Crider, K. McCorkle, M. Alford, and R. H. Unger. 1990. Inhibition of glucose transport into rat islet cells by immunoglobulins from patients with new-onset insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 322:653–659.

15. McDonald, R. J., G. H. Swift, A. E. Przybyla, and J. M. Chirgwin. 1987. Isolation of RNA using guanidinium salts. *Methods Enzymol.* 252:219–227.

16. Chen, L., T. Alam, J. H. Johnson, S. Hughes, C. B. Newgard, and R. H. Unger. 1990. Regulation of β-cell glucose transporter gene expression. *Proc. Natl. Acad. Sci. USA.* 87:4088–4092.

17. Johnson, J. H., C. B. Newgard, J. L. Milburn, H. F. Lodish, and B. Thorens. 1990. The high K_m glucose transporter of islets of Langerhans is functionally similar to the low affinity transporter of liver and has an identical primary sequence. *J. Biol. Chem.* 265:6548–6551.

18. Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science (Wash. DC).* 232:977–980.

19. Fajans, S. S., and J. W. Conn. 1954. An approach to the prediction of diabetes mellitus by a modification of glucose tolerance test with cortisone. *Diabetes.* 3:296–304.

20. Rull, J. A., J. W. Conn, J. C. Floyd, and S. S. Fajans. 1970. Levels of plasma insulin during cortisone glucose tolerance tests in "nondiabetic" relatives of diabetic patients. Implications of diminished insulin secretory reserve in subclinical diabetes. *Diabetes.* 19:1–10.