Impaired Insulin Biosynthetic Capacity in a Rat Model for Non-insulin-dependent Diabetes

Studies with Dexamethasone

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SUMMARY

These studies of a rat model for non-insulin-dependent diabetes mellitus (NIDDM) were performed to determine whether hyperglycemia occurs when capacity to synthesize insulin is exceeded. The neonatal streptozocin (STZ)-treated rat has acute hyperglycemia with marked destruction of pancreatic β-cells, followed by gradual regeneration to 50–70% normal β-cell number. At age 4 wk, fed serum glucose concentration is only mildly elevated relative to controls. With age, the rats become progressively hyperglycemic, and by 12 wk they have marked impairment of glucose-stimulated insulin release. In these studies, dexamethasone (0.125 mg/kg/day for 4 days) was administered to control and to STZ-treated animals to produce insulin resistance. The relationship between insulin biosynthesis and serum glucose concentrations was assessed.

In control rats, response to dexamethasone was similar at both 4 and 12 wk. Serum glucose levels and pancreatic insulin concentration remained unchanged. Both insulin biosynthetic rates (as measured by ^{3}H-leucine incorporation into proinsulin) and proinsulin mRNA levels increased twofold.

STZ-treated rats at age 4 wk demonstrated mild hyperglycemia. Dexamethasone injection resulted in an increase in insulin biosynthesis and proinsulin mRNA in these animals, while serum glucose did not increase. STZ-treated rats at 12 wk showed more profound hyperglycemia (serum glucose 315 ± 38 mg/dl versus control, 187 ± 12 mg/dl). A marked rise in serum glucose (to 519 ± 42 mg/dl) was observed after 4 days of dexamethasone injection. Pancreatic insulin content became severely depleted relative to saline-injected, STZ-treated animals, and there was no response of levels of proinsulin mRNA.

These studies demonstrate a significant correlation between mean insulin biosynthetic rates and mean proinsulin mRNA levels in 4-wk-old rats. In these rats with mild glucose intolerance related to a decrease in insulin synthesis, the rate of biosynthesis can still increase if demand for insulin is acutely increased by induction of an insulin-resistant state. If the correlation between proinsulin mRNA and biosynthesis exists in 12-wk-old animals, the data suggest that older, more hyperglycemic animals lose the capacity to increase rates of insulin biosynthesis and secretion; severe hyperglycemia ensues when capacity to synthesize insulin is exceeded. DIABETES 1985; 34:235–40.

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A model for non-insulin-dependent diabetes mellitus (NIDDM) has been characterized in the rat.[1-4] Briefly, rats are injected 2 days after birth with streptozocin (STZ). An acute decrease in pancreatic β-cell number, to approximately 20% of normal values, is associated with a period of hyperglycemia. By 7–10 days of age there is a marked regeneration of β-cells, restoring the number to about two-thirds of control values. A period of nearly normal glucose tolerance ensues, which persists until approximately 6 wk of age, at which point frank hyperglycemia becomes evident. This model bears several striking phenotypic similarities to NIDDM in humans. Pancreatic insulin content is decreased variably from 30–90%, while the content of glucagon and somatostatin remains normal. There is a marked decrease in the ability of the pancreas to release insulin in response to glucose, while insulin release in response to other secretagogues is less dramatically impaired.[2,3,5] While the etiology of diabetes in this rat model is clearly different from NIDDM in man, insights into the pathogenesis of hyperglycemia may be obtained by studying these animals.

Previous studies have shown that insulin biosynthetic rates, as determined by incorporation of ^{3}H-leucine into proinsulin, and proinsulin mRNA levels were diminished in 4- and 7-wk-old animals.[4] At age 7 wk, the animals were hyperglycemic, and insulin biosynthesis seemed to be inadequate to meet demands. The deficit in proinsulin mRNA

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(30% of control) appeared to be more severe than the observed decrease in β-cell number (50–70% of control). This suggested that there might be a functional defect in the remaining islets, limiting the production of proinsulin mRNA and impairing the ability of the islets to make insulin.

To study further the relationship of proinsulin mRNA levels and insulin biosynthetic rates to glucose homeostasis, rats treated with STZ as neonates and normal controls were subjected to pharmacologic doses of glucocorticoids for 4 days, imposing a severe diabetogenic stress. The response to dexamethasone was compared with that in saline-injected animals from both normal and STZ-treated groups at 4 wk and at 12 wk of age. In 4-wk animals, plasma glucose was higher than in controls, but was unchanged in response to dexamethasone. Proinsulin mRNA levels and rates of insulin biosynthesis increased relative to STZ-treated animals not given dexamethasone. At age 12 wk, when fed hyperglycemia was overt, there was no longer any apparent compensatory biosynthetic response to dexamethasone administration. The increase in proinsulin mRNA levels seen at age 4 wk did not occur, and there was a further increase in hyperglycemia.

MATERIALS AND METHODS

Male Sprague-Dawley rats were made diabetic as previously described.[1] Two-day-old rat pups were injected with STZ, 90 mg/kg, i.p. (Dr. William E. Dulin, Upjohn Company, Kalamazoo, Michigan) in 0.1 M citrate buffer, pH 4.5. Controls were injected with an equivalent volume of citrate buffer. Animals were weaned at age 24 days. At 4 wk and 12 wk, one-half of the STZ-treated and normal rats were injected with dexamethasone (0.125 mg/kg, i.m.) daily for 4 days; the remainder were injected daily with saline and served as controls.

Blood was obtained from the animals fed ad libitum (Purina Rat Chow) until the time of killing (8–10 a.m.), allowed to clot at room temperature for 30 min, and serum was obtained. Aliquots were analyzed for glucose with a standard glucose-oxidase assay. Similar aliquots were assayed for insulin by a modification of a standard radioimmunoassay technique using purified rat insulin (Novo, Copenhagen, Denmark) as standard.[6]

At the time of killing, pancreatic tissue was excised and divided. Each portion was weighed separately. One of the two portions was placed in acid-ethanol and homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, New Jersey); aliquots were diluted into phosphate-buffered saline with sodium dodecyl sulfate (SDS) 1% and assayed for insulin as described above. Pancreatic insulin was expressed as μg/g of pancreas (Table 1). There was no difference in pancreatic weights of control and STZ-treated animals at either 4 or 12 wk of age. The other portion was used to measure proinsulin mRNA concentration.

Isolation of mRNA. Portions of pancreas were weighed and homogenized immediately at highest speed with the Polytron homogenizer in 10 ml of 4 M guanidine thiocyanate (Fluka, AG, Basel, Switzerland), 25 mM sodium citrate, pH 7.0, 0.1 M 2-mercaptoethanol (Eastman Kodak, Rochester, New York), 0.5% sodium lauroyl sarcosine, and 0.33% Anti-foam A (Sigma, St. Louis, Missouri), according to the method of Chirgwin et al.[7] Samples were purified and diluted to approximately 5 mg/ml and stored at −70°C until further use. RNA concentration was estimated by measuring sample absorbance at 260 nm.

Estimation of proinsulin mRNA concentration. Pancreatic RNA (20 μg) from each rat was reacted with glyoxal according to the method of McMaster and Carmichael,[8] electrophoresed on 2% agarose gels, transferred to diazophenylthio (DPT) paper, and hybridized as previously described.[9,10] The 450 base-pair insert from the recombinant plasmid pCRI354, containing 354 bases of DNA complementary to rat proinsulin I mRNA,[11] was removed from the parent plasmid pBR322 by digestion with Hind III, and purified by agarose gel electrophoresis. The insert was labeled with ^{32}P-deoxyribonucleotides by nick translation[12] to approximately 5–9 × 10^8 counts/min/μg and was used as a hybridization probe. Autoradiography was performed using preflashed Kodak XAR film and Dupont Chronex intensifying screens.

In 4-wk animals, proinsulin mRNA was quantified from densitometric tracings of the autoradiographs.[4] Three densitometric tracings of each autoradiograph were obtained, and results were averaged. A reference sample was electrophoresed at each end lane as an internal standard. Data from each autoradiograph were normalized using the internal standards. Data are expressed relative to proinsulin mRNA levels in controls treated with saline.

In the 12-wk rats, because the proinsulin mRNA from each of the experimental groups varied over a large range, autoradiographic signals exceeded the linearity of the film. Therefore, individual bands on the diazotized papers were located by autoradiography, equal-sized squares were cut, and the squares were counted in a liquid scintillation counter as described previously.[9] Background was estimated with equal-sized squares cut from elsewhere on the paper. Internal standards were included and used as described above.

In vivo measurement of insulin biosynthesis. Insulin biosynthesis was measured in vivo as described previously.[4] Four-week rats weighing approximately 100 g were injected i.p. with [3,4,5]^{3}H-l-leucine (New England Nuclear, Boston, Massachusetts; sp. act. 140.8 Ci/mmol, 1 mCi/100 g body wt). Forty-five minutes later, pancreata were removed and extracted with acid-ethanol. Forty-five minutes was chosen as the labeling period to allow only minimal conversion of proinsulin to insulin.[13] ^{3}H-Leucine incorporation into total pancreatic protein was determined on the acid-ethanol–soluble fraction by precipitation of an aliquot with trichloroacetic acid. Extracts were lyophilized, dissolved in 400–500 μl of 1 M acetic acid, and chromatographed on a Biogel P-30 column as previously described.[4] Fractions of labeled proinsulin were separated from insulin to facilitate immunoprecipitation, and results were expressed as percentage of total counts present in immunoprecipitated material. Total pancreatic protein synthesis was found to be equal in control and STZ-treated rats at 4 wk.[4] Insulin biosynthesis was not measured in 12-wk animals because pilot experiments indicated that animals this large would require at least 3 mCi of isotope for adequate labeling of proinsulin.

Data from the four groups were compared at each age using a nonpaired *t*-test. P-values are reported as measures of statistical significance.

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TABLE 1
Effect of dexamethasone (Dex) administration on serum glucose, pancreatic insulin concentration, proinsulin mRNA, and insulin biosynthetic rates in streptozocin-treated (STZRx) and control rats

Age	Group	Treatment	Body wt (g)	Serum glucose (mg/dl)	Pancreatic insulin concentration (μg/g)	Insulin biosynthetic rate*	Pancreatic RNA (mg)	Proinsuln mRNA(%)†
4 Wk	Control	Saline	100 ± 5	140 ± 4 (6)	40.3 ± 7.1 (3)	0.31 ± 0.02 (3)	5.0 ± 0.4	100 ± 7 (5)
		Dex	92 ± 4	129 ± 7 (5)	44.6 ± 5.7 (4)	0.68 ± 0.03 (4)†††	4.6 ± 0.3	189 ± 16 (6)‡‡
	STZRx	Saline	124 ± 6	171 ± 9 (6)‡	11.7 ± 1.7 (3)¶	0.19 ± 0.2 (3)‡‡‡	6.0 ± 0.3	52 ± 4 (6)§§
		Dex	112 ± 4	157 ± 15 (6)	5.6 ± 1.8 (4)#	0.26 ± 0.02 (5)§§§	3.7 ± 0.3‖‖‖	98 ± 12 (6)‖‖
12 Wk	Control	Saline	368 ± 37	187 ± 12 (5)	87.4 ± 19.1 (5)		7.6 ± 1.2	100 ± 16 (4)
		Dex	415 ± 34	200 ± 18 (6)	69.9 ± 14.2 (5)		10.9 ± 2.9	190 ± 25 (5)¶¶
	STZRx	Saline	393 ± 14	315 ± 38 (6)§	32.0 ± 6.7 (6)**		9.8 ± 0.5	33 ± 13 (6)##
		Dex	396 ± 13	519 ± 42 (6)‖	5.8 ± 2.6 (6)††,§§§§		9.2 ± 0.9	34 ± 9 (6)***

*[3]H-Leucine incorporated into immunoprecipitated proinsulin/incorporation into total acid-soluble pancreatic protein, as described in the text. Total pancreatic protein synthetic rates were equal in control and STZRx animals.[4]
†Proinsulin mRNA per 20 μg of total pancreatic RNA was determined as described in the text. Proinsulin mRNA is relative to controls separately for 4 and 12 wk.
‡P = 0.011 versus 4-wk saline control; §P = 0.025 versus 12-wk saline control; ‖P = 0.0005 versus 12-wk saline STZRx; ¶P = 0.018 versus 4-wk saline control; #P = 0.006 versus 4-wk saline control; **P = 0.04 versus 12-wk saline control; ††P = 0.002 versus 12-wk saline control; §§§§P = 0.004 versus 12-wk saline STZRx; ‡‡P = 0.004 versus 4-wk saline control; §§P = 0.0003 versus 4-wk saline control; ‖‖P = 0.003 versus 4-wk saline STZRx; ¶¶P = 0.037 versus 12-wk saline control; ##P = 0.012 versus 12-wk saline control; ***P = 0.005 versus 12-wk saline control; †††P = 0.001 versus 4-wk saline control; ‡‡‡P = 0.009 versus 4-wk saline control; §§§P = 0.028 versus 4-wk saline STZRx; and ‖‖‖P = 0.0001 versus 4-wk saline STZRx.

RESULTS

RESULTS IN 4-WK ANIMALS

Control animals. The effects of 4 days of dexamethasone treatment are presented in Table 1 and Figure 1A. Dexamethasone administration to control 4-wk animals had no effect on body weight or serum glucose. Pancreatic insulin concentration was maintained despite increased insulin demand produced by dexamethasone-induced insulin resistance.[3] ^{3}H-Leucine incorporation into immunoprecipitable proinsulin was used as an index of insulin biosynthesis in vivo in the 4-wk animals. ^{3}H-Leucine in proinsulin represented 0.31 ± 0.02% of that incorporated into total acid-soluble pancreatic protein. This proportion increased twofold with dexamethasone administration to 0.68 ± 0.03% (P = 0.001). The concentration of proinsulin mRNA in pancreas also increased approximately twofold (P = 0.004).

STZ-treated animals. In 4-wk rats treated with STZ as neonates, serum glucose was mildly but significantly higher than that in the control animals (171 ± 9 mg/dl versus 140 ± 4 mg/dl, P = 0.011). Dexamethasone administration at this age did not result in change in body weight or any further increase in serum glucose, despite the presence of mild fed hyperglycemia. Pancreatic insulin concentration in STZ-treated rats was 29% of that in control animals (P = 0.018). When given dexamethasone, pancreatic insulin concentration decreased further, from 29% to 14% of control values (P = 0.0006). Although rates of insulin biosynthesis were less in STZ-treated rats than in control rats (0.19 ± 0.02 versus 0.31 ± 0.02, P = 0.009), the relative rates of insulin biosynthesis in these animals increased after dexamethasone (to 0.26 ± 0.02, P = 0.028). Proinsulin mRNA in the STZ-treated rats was 52 ± 4% of that in control rats (P = 0.0003). After dexamethasone administration, proinsulin mRNA doubled (P = 0.0033) despite the lower levels of proinsulin mRNA, diminished pancreatic insulin stores, and elevated serum glucose.

Correlation of proinsulin mRNA levels with insulin biosynthetic rates. To determine the relationship between proinsulin mRNA concentration in total pancreatic RNA with insulin biosynthetic rates under the conditions of these experiments, mean levels of proinsulin mRNA for each experimental group of 4-wk animals were compared with the relative rates of insulin biosynthesis (Figure 2). The changes in mRNA levels correlated directly with changes in rates of insulin biosynthesis. The correlation coefficient was determined; r = 0.977, t = 11.2, and P = 0.0001. Total pancreatic RNA was not different in control and STZ-treated animals at either 4 or 12 wk (Table 1).

RESULTS IN 12-WK ANIMALS

Control animals. Dexamethasone had no effect on body weight, serum glucose, or pancreatic insulin concentration of control 12-wk animals (Table 1 and Figure 1B). As in the 4-wk animals, control animals at 12 wk were able to increase proinsulin mRNA twofold in response to the dexamethasone treatment.

STZ-treated animals. Dexamethasone given to 12-wk rats treated with STZ as neonates raised mean glucose levels substantially (from 315 ± 38 mg/dl to 519 ± 42 mg/dl, P = 0.0002). Pancreatic insulin concentration decreased fivefold, from 36% to 7% of control (P = 0.0004). Levels of proinsulin mRNA in rats treated with STZ as neonates were 33% of controls at 12 wk (P = 0.012). Response of proinsulin mRNA levels no longer occurred when 12-wk STZ-treated rats were challenged with dexamethasone. Values without versus with dexamethasone administration were 33% versus 34% of untreated control values (P = 0.946).

DISCUSSION

These studies were performed to determine whether impairment in the ability of pancreatic β-cells to make insulin correlates with development or severity of glucose intolerance. Insulin resistance was induced with pharmacologic

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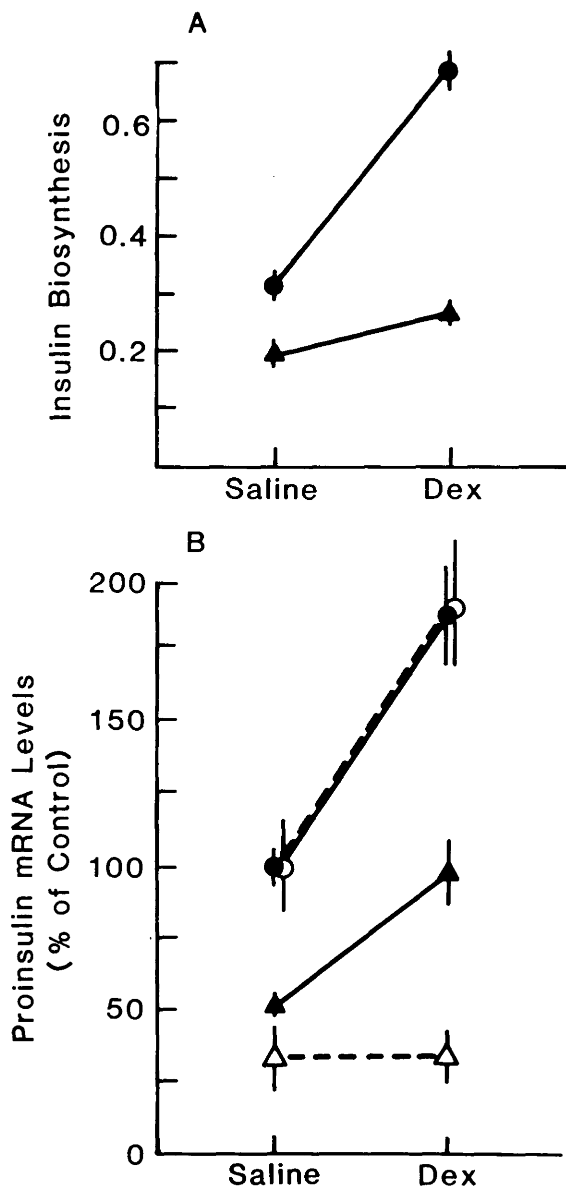

FIGURE 1. Effect of dexamethasone (Dex) administration (0.125 mg/kg/day for 4 days) versus saline on relative rates of insulin biosynthesis and proinsulin mRNA levels in 4- and 12-wk control rats and STZ-treated rats. (A) Incorporation of ^{3}H-l-leucine into immunoprecipitable proinsulin relative to incorporation into total acid-soluble pancreatic protein as described in the text. (B) Effect of dexamethasone on pancreatic proinsulin mRNA concentration in control rats and STZ-treated rats. The data from 4-wk and 12-wk rats were obtained by electrophoresing pancreatic RNA (20 μg) on 2% agarose gels as described in the text. RNA was transferred to diazotized paper, hybridized with a ^{32}P-labeled, 450-base-pair–cloned cDNA homologous to rat proinsulin I mRNA, and hybridizing bands were compared by densitometric analysis of the autoradiograph or counting as described in the text. (●——●), Four-week control animals; (▲——▲), 4-wk STZ-treated animals; (○- - -○), 12-wk control animals; and (△- - -△), 12-wk STZ-treated animals.

doses of glucocorticoids to test the ability of the pancreas to increase insulin production. Dexamethasone was administered at 4 days, since this was the earliest time at which a consistent increase in pancreatic proinsulin mRNA could be measured (manuscript in preparation). Animals were treated for the shortest period of time possible so that the changes measured might reflect increased insulin production in existent β-cells, rather than that from new cell formation.[14–16] The effect of glucocorticoids on β-cell replication has been assessed in this model. After 13 days of dexamethasone, normal 10-wk-old rats demonstrated a <15% increase in β-cell number; β-cell number in STZ-treated rats did not change.[3] In the current experiments, the diabetogenic stress of dexamethasone was tolerated quite well by control animals at both 4 and 12 wk of age. The increased insulin requirement resulted in no change of serum glucose or pancreatic insulin concentration. Insulin biosynthetic rates apparently increased commensurate with demand.

Previous studies[4] had shown that in vivo rates of insulin biosynthesis and proinsulin mRNA levels were diminished in the STZ rat model for NIDDM. The hypothesis to be tested in the current experiments was that the animals become hyperglycemic only after the capacity to synthesize insulin is exceeded. From the results of previous studies,[4] we had predicted that animals with any degree of glucose intolerance (i.e., 4-wk-old rats treated with STZ as neonates) would not increase insulin biosynthesis in response to the stress of dexamethasone, and that glucose levels would rise. Results in 4-wk rats were not as predicted; the data (Table 1) show that β-cells in 4-wk rats treated with STZ as neonates still have in reserve the capacity to increase insulin biosynthetic rates in response to increased demand. This is demonstrated by parallel increases of insulin biosynthetic rates

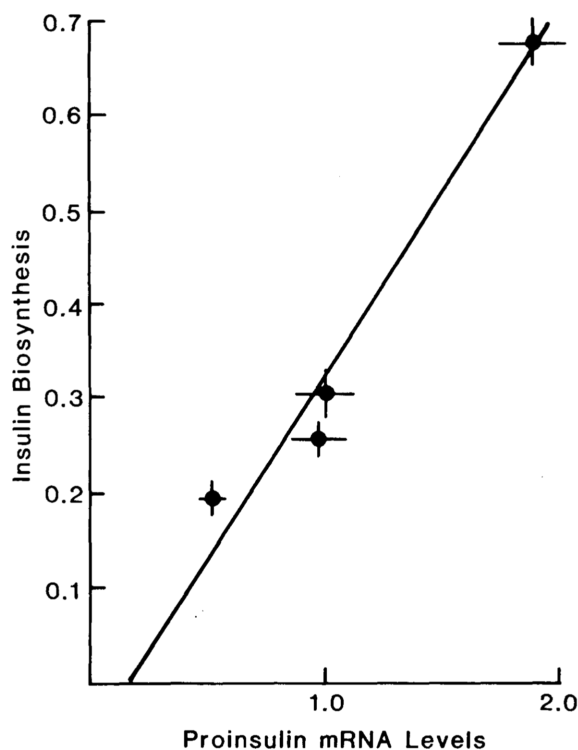

FIGURE 2. Correlation of rates of insulin biosynthesis with levels of proinsulin mRNA in 4-wk rats. Mean relative rates of insulin biosynthesis in 4-wk rats were plotted as a function of mean proinsulin mRNA levels. Mean values were plotted, since insulin biosynthesis and proinsulin mRNA determinations were done on separate animals. Standard errors are shown. A value of 1.0 for saline controls corresponds to 100% in Table 1. Insulin biosynthesis (y) was linearly related to proinsulin mRNA levels (x) by the regression equation $y = 0.37x - 0.04$. The correlation coefficient was 0.977 with a standard error of 0.080 and a *t*-value of 11.2, P = 0.0001.

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and proinsuln mRNA levels after 4 days of dexamethasone administration. No worsening of hyperglycemia occurred in these animals.

By 12 wk of age, in rats treated with STZ as neonates, levels of proinsulin mRNA no longer increased in response to dexamethasone injection. Insulin biosynthetic rates were not measured directly at 12 wk because very large quantities of labeled amino acid would be required for labeling proinsulin. In smaller, 4-wk animals, 1 mCi of ^{3}H-leucine was required for adequate labeling. Levels of proinsulin mRNA appeared to correlate very well with changes in rates of insulin biosynthesis in the 4-wk animals (Figure 2). If the same relationship exists in 12-wk animals, levels of proinsulin mRNA may be a reasonable measure of insulin biosynthetic capacity. Lack of mRNA response to dexamethasone in the 12-wk STZ-treated animals implies that insulin biosynthetic capacity is severely limited. The data suggest that marked hyperglycemia results when insulin resistance is superimposed on this more severely limited capacity to synthesize insulin.

The use of proinsuln mRNA concentration as a measure of insulin biosynthetic capacity is not precise. Nevertheless, there is considerable evidence for a strong correlation between proinsulin mRNA levels and insulin biosynthetic rates in rodents. In fasted rats, pancreatic insulin and plasma insulin concentrations are markedly diminished, along with an 80% drop in proinsulin mRNA concentration.[10] Twenty-four hours after refeeding or glucose injection, pancreatic and plasma insulin concentrations rise along with proinsulin mRNA.[9,10] In the STZ-diabetic rat model, there is approximately one-half as much proinsulin mRNA and one-half as much insulin biosynthesis in the diabetic animals compared with control rats (ref. 4 and Table 1). In control rats injected with dexamethasone for 4 days, proinsulin mRNA doubles along with the doubling of ^{3}H-leucine incorporation into proinsulin (Table 1). Recently, we have measured proinsulin mRNA in partially pancreatectomized rats. After 50% pancreatectomy, proinsulin mRNA concentration doubled in the remnant 14 wk after surgery.[21] In a genetic mouse model for diabetes,[17] these animals demonstrate early hyperphagia, hyperinsulinemia, and marked increases in pancreatic insulin content. During this hyperinsulinemic phase, when plasma glucose concentrations are only mildly elevated, a fourfold increase in proinsulin mRNA concentration was noted. Later, when the animals become severely hypoinsulinemic and diabetic, proinsulin mRNA markedly diminished.[21] Thus, in all of these conditions, there was a strong correlation between insulin production and proinsulin mRNA levels. Whether proinsulin mRNA represents biosynthetic capacity depends on the contribution of translational modulation superimposed on proinsulin mRNA. Translational control seems to be important in isolated islets in vitro during short-term incubations.[18,19] In vivo experiments to date, however, suggest a very tight relationship between mRNA levels and insulin biosynthesis. Thus, the contribution of the translational component in vivo under physiologic and pharmacologic conditions has not been assessed.

These studies offer no explanation for differences in ability to increase insulin biosynthesis observed in 4- versus 12-wk animals. The effects of age, of physical development, of increasing body mass, and of exposure of β-cells to continued hyperglycemia have been considered. Age alone does not appear to affect biosynthetic responsiveness, as normal animals show similar adaptation to dexamethasone at both ages studied; age alone may have very different effects on control and STZ rats, however. The effects of pubertal development have recently been evaluated in this model,[5] and do not appear to be important at least for development of hyperglycemia. Evaluation of the insulin biosynthetic response of older rats treated with STZ as neonates, which have had their glucose levels normalized with insulin therapy, or have had restricted diet from onset of glucose intolerance, may test the importance of prolonged hyperglycemia and of body weight on insulin biosynthetic capacity in this model. The data presented in the present study continue to suggest that impairment of insulin synthesis and secretion from a reduced β-cell mass is fundamental to the inability to maintain euglycemia in the face of increased insulin requirements that occur with age.[20]

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