Insulin Treatment Improves Glucose-Induced Insulin Release in Rats With NIDDM Induced by Streptozocin

MICHELINE KERGOAT, DANIELLE BAILBE, AND BERNARD PORTHA

SUMMARY

Insulin-deficient diabetes in humans, as well as in the neonatal streptozocin-induced rat model of non-insulin-dependent diabetes mellitus (NIDDM), are associated with islet β-cell insensitivity to glucose. We hypothesized that the chronic hyperglycemia-hypoinsulinemia pattern causes this impairment of the glucose influence on insulin secretion. This study was designed to determine whether the glucose defect could be counteracted by normalizing the diabetic state in rats with NIDDM after insulin therapy. Mixte lente insulin (5 U · kg^{-1} · day^{-1}) was given daily at 1700 h over 24 h or 5 consecutive days. Insulin secretion was studied the morning after the last insulin injection with the isolated perfused pancreas preparation. Fed basal plasma glucose levels decreased in diabetic rats from 183 ± 8 to 136 ± 10 mg/dl after the 1-day insulin treatment and to 135 ± 5 mg/dl after the 5-day insulin treatment (vs. 116 ± 3 mg/dl in control rats). Pancreatic insulin stores were not affected by insulin therapy.

Although the 1-day insulin treatment did not modify the lack of glucose response in the diabetic rats, the 5-day insulin treatment improved their glucose-induced insulin secretion. Moreover, insulin therapy improved the priming effect of glucose on a second stimulation with glucose. The return of this glucose effect was hardly detectable after the 1-day insulin therapy but was clearly present after the 5-day treatment. The hyperresponse to arginine, characteristic of the untreated diabetic rat, returned similar to that in controls after a 1-day insulin therapy, and it was again amplified at high glucose levels, although amplification remained lower than that of control rats. This indicates that the potentiating effect of glucose on the response to arginine was regained more precociously than the acute insulin response to glucose after insulin therapy. These data agree with the hypothesis that the chronic hyperglycemia-hypoinsulinemia in the NIDDM rat causes abnormal glucose influence on glucose- and arginine-stimulated insulin release. *Diabetes* 36:971–77, 1987

Neonatal rats treated with steptozocin (STZ) at birth exhibit an insulin-deficient, acute diabetes (STZ-D) 3–5 days after birth that is characterized by a rapid spontaneous remission (1,2). We have previously shown that this remission is accompanied by β-cell regeneration and accumulation of pancreatic insulin stores (1,3–5) starting 3–5 days after birth. Moreover, immunocytochemical and ultrastructural studies of pancreatic tissue from STZ-D rats indicate that, although a few β-cells survive the toxic effect of STZ and remain in scarred islets, most of the β-cells found 3–5 days after birth may originate from undifferentiated duct cells (4,5). In this context, note that adult rats treated with STZ at birth exhibited non-insulin-dependent diabetes mellitus (NIDDM) with specific failure of insulin release in response to glucose, as characterized in vitro with the perfused pancreas technique (6,7). This pattern of glucose unresponsiveness, coexisting with a relative preservation of the response to other secretagogues, resembles that shown in some studies of human NIDDM (8,9) and is also characteristic of insulin-dependent diabetes in the early stage of the disease (10) and of NIDDM of the young (11). The mechanism responsible for the inability of β-cells to respond to glucose in the neonatal STZ-D rat is unknown thus far.

In a previous study, we excluded the possibility that pathogenesis of this lesion can be explained by a permanent toxic action of STZ on the glucose-sensitive insulin-secretion mechanism (12). Alternatively, this lesion may result from a chronic stimulation by hyperglycemia of the 50%-reduced β-cell mass in this model of NIDDM (B.P. and R. McEvoy, unpublished observations). Such a hypothesis is supported

From the Laboratory of Developmental Physiology, University of Paris, Paris, France.
Address correspondence and reprint requests to Bernard Portha, MD, Laboratoire de Physiologie du Développement, CNRS UA 307, Université Paris 7, Tour 54, 2 Place Jussieu, F-75251 Paris Cedex 05, France.
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by the finding that in this model the derangement of islet function is not solely intrinsic to the islets but depends to a great extent on the environment in vivo (14). This study was designed to determine if a lowered plasma glucose level for 1 or 5 days restores glucose-induced insulin release, reverses the hyperresponse found to arginine, and restores the glucose-potentiating influence on arginine- or glucose-stimulated insulin release.

MATERIALS AND METHODS

Animals. Albino Wistar rats bred in our colony were fed ad libitum with commercial pelleted chow (UAR, Villemoisson, France). At birth the rats received 100 μg/g STZ in 25 μl of 0.05 M citrate buffer, pH 4.5, through the saphenous vein, which was made directly accessible by transcutaneous puncture as previously described (1). The litters were limited to eight rats. In the control litter, newborns received only citrate buffer. Four days after birth, all experimental neonates exhibited glycosuria reaching the 3+ value on Clinistix (Ames, Miles, Slough, UK). Spontaneous evolution of neonatal diabetes led to an NIDDM state in the adult that was stable and chronic, as previously described (2). All the animals were weaned 21 days after birth.

Insulin therapy. At 4 mo of age the diabetic rats were given 5 U/kg body wt s.c. Mixte lente monocomponent pork insulin (Novo, Bagsvaerd, Denmark) in one daily injection at 1700 h. The first injection was given 36 h (acute treatment) or 4.5 days (chronic treatment) before the isolation of the pancreas. The last injection was given at 1700 h on the day preceding the isolation procedure. Blood for plasma glucose measurements was obtained by tail snipping before the first injection at 0900 and 1700 h on each day of the insulin treatment, and at 0900 h on the morning of the experiment. This was done in both treated and untreated diabetic rats and in control rats. Insulin secretion was studied with the in vitro isolated perfused pancreas.

Isolated-pancreas perfusion technique. The animals were anesthetized with pentobarbital sodium (4 mg/100 g body wt i.p.). Isolation and perfusion of the pancreas were performed as previously described (7). The perfusate was a Krebs-Ringer bicarbonate buffer with the following components: 118 mM NaCl, 4 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.25 g/L fatty acid–free bovine serum albumin (Sigma, St. Louis, MO), and 40 g/L dextran T 70 (Pharmacia, Uppsala, Sweden). When needed, L-arginine (Sigma) or D-glucose (Merck, Darmstadt, FRG) was administered through a sidearm syringe. The complete effluent (3 ml/min) was collected from the cannula in the portal vein at 1-min intervals in chilled tubes and frozen for storage at −20°C until assay.

Analytical techniques and calculations. Plasma glucose was determined with a glucose analyzer (Beckman, Fullerton, CA). Immunoreactive insulin in the perfusate was estimated with purified rat insulin as standard (Novo) and antibodies to mixed (porcine and bovine) insulin and porcine [^{125}I]monoiodinated insulin (15). Charcoal was used to separate free from bound hormone. The method allows the determination of 6 μU/ml (0.25 ng/ml), with a coefficient of variation within and between assays of 10%.

Insulin secretion rate per total pancreas was calculated by multiplying the insulin concentration in the samples by the flow rate and was expressed as microunits per minute. Total insulin response to glucose and arginine was obtained by planimetry of the individual perfusion profiles and expressed as the difference in hormonal secretion rate (units insulin/min) relative to the mean hormonal output recorded

TABLE 1
Basal plasma glucose concentrations, pancreatic insulin stores, and body weight during insulin treatment (5 U · kg^{-1} · day^{-1}) in diabetic rats

	Untreated diabetics	*n*	Treated diabetics	*n*	Controls (*n* = 5)
			404 ± 35*	8	
Body weight (g)	412 ± 7	10	390 ± 32†	19	410 ± 13
Fed plasma glucose (mg/dl)					
Day 1					
1700 h	190 ± 6	5	183 ± 8	27	115 ± 3
Day 2					
0900 h			140 ± 7	27	
1700 h	185 ± 10	5	160 ± 6	27	118 ± 5
Day 3					
0900 h			136 ± 10	27	
1700 h	195 ± 8	5	154 ± 4‡	27	123 ± 2
Day 4					
0900 h			125 ± 10	27	
1700 h	175 ± 4	5	151 ± 4§	27	124 ± 4
Day 5					
0900 h			121 ± 6	27	
1700 h	182 ± 12	5	154 ± 5‖	27	121 ± 2
Day 6					
0900 h	183 ± 6	5	135 ± 5‡	17	116 ± 3
Pancreatic insulin (U/pancreas)	2.56 ± 0.21	10	2.28 ± 0.36*	9	5.12 ± 0.24
			2.08 ± 0.17†	23	

Values are means ± SE.
*One-day insulin therapy.
†Five-day insulin therapy.
‡$P < .001$, §$P < .02$, and ‖$P < .05$ vs. untreated diabetics.

TABLE 2
Insulin secretory rates from perfused pancreases of diabetic rats untreated or treated with insulin (5 U · kg^{-1} · day^{-1}) for 1 or 5 days

Insulin output (μU/min)	Basal	Stimulated release			
		19 mM arginine	19 mM arginine plus 16 mM glucose	16 mM glucose (1st challenge)	16 mM glucose (2nd challenge)
Controls	21 ± 6 (8)	219 ± 28 (8)	2020 ± 290 (5)	41 ± 6 (9)	80 ± 12 (9)
Diabetics					
Untreated	13 ± 8 (9)	547 ± 71* (5)	680 ± 153§ (5)	4.1 ± 1.3 (5)	4.3 ± 0.9* (5)
1-day insulin treated	24 ± 3 (5)	224 ± 75† (5)	624 ± 15§ (5)	3.0 ± 0.8 (4)	9.9 ± 4.0* (4)
5-day insulin treated	26 ± 7 (8)	108 ± 18*† (8)	361 ± 82* (8)	28 ± 7 (7)	45 ± 10‖¶ (7)

Basal release values were expressed as absolute values. Stimulated release values were calculated as mean increase above basal release. Values are means ± SE. Number of observations is shown in parentheses.
*$P < .001$, §$P < .01$, and ‖$P < .05$ vs. controls.
†$P < .02$, ‡$P < .001$, and ¶$P < .01$ vs. untreated diabetics.

at the end of the prestimulation period. Results are given as means ± SE. Statistical analyses were performed by Student's unpaired *t* test.

RESULTS

Effect of insulin therapy on basal plasma glucose concentrations in diabetic rats. The plasma glucose values of the different groups, measured at 0900 and 1700 h each day during a 5-day insulin treatment (5 U · kg^{-1} · day^{-1}), are shown in Table 1. Before insulin therapy the basal plasma glucose levels of the two diabetic groups averaged 183–190 mg/dl, values significantly higher than the 115 mg/dl value observed in the control group. After 16 h the values of the insulin-treated group had fallen to 140 mg/dl, remaining low (121–160 mg/dl) during the 5-day insulin therapy and averaging 135 mg/dl before study with the perfused

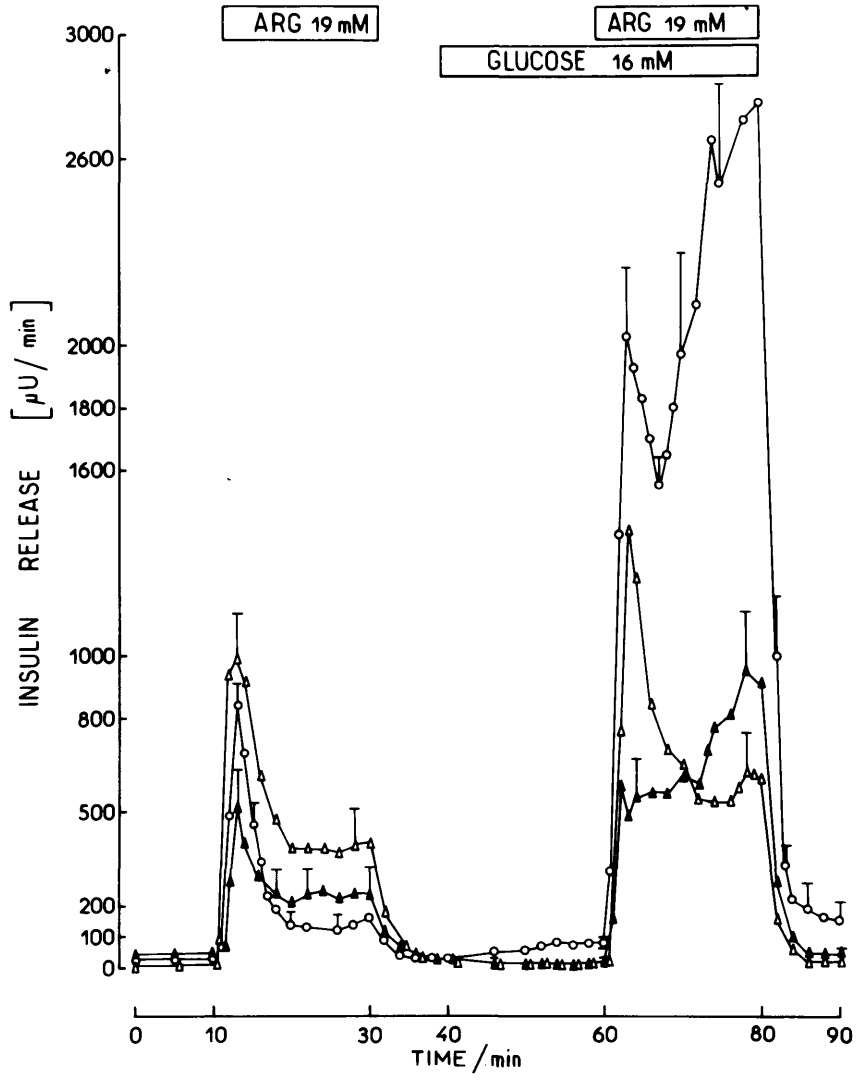

FIG. 1. Effect of 19 mM arginine and of 19 mM arginine plus 16 mM glucose on insulin release from perfused pancreases of control rats (○), untreated diabetic rats (△), and diabetic rats that received 1-day insulin therapy (▲). Experiments were carried out in absence of glucose during pre- and poststimulatory periods. Each *point* is mean ± SE of 5–9 observations in each group.

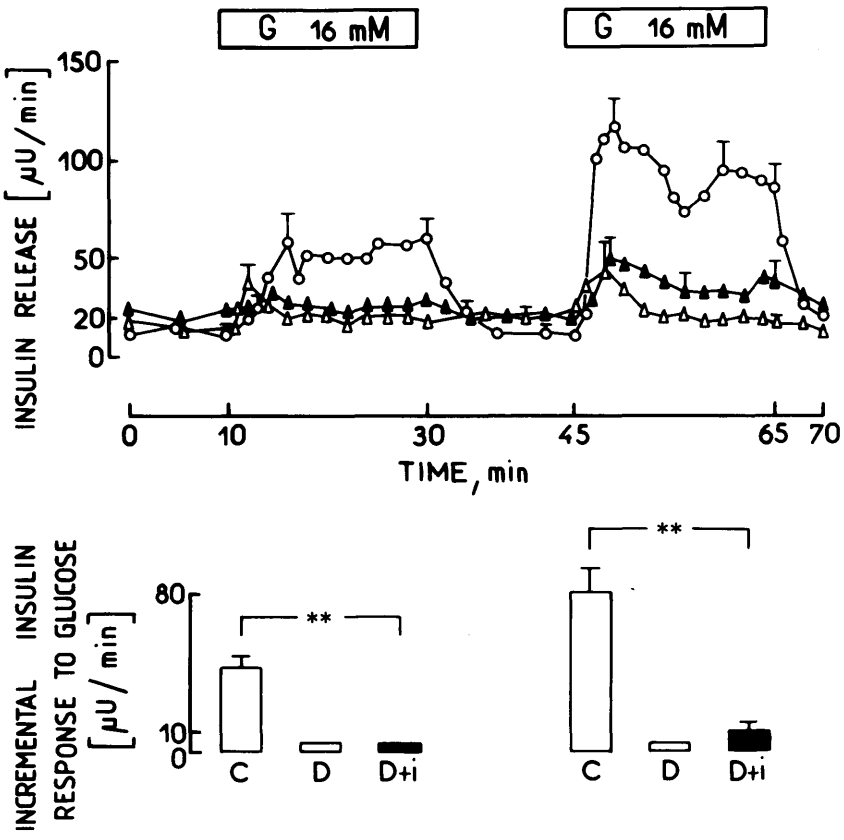

FIG. 2. Effect of 2 successive 16-mM glucose stimulations on insulin release from perfused pancreases of control rats (○), untreated diabetic rats (△), and diabetic rats that received 1-day insulin therapy (▲). Experiments were carried out in absence of glucose during pre- and poststimulatory periods. Each *point* is mean ± SE of 4–9 observations in each group. Data are also expressed as mean incremental response to glucose above basal release in each group. **$P < .001$.

pancreas (vs. 183 mg/dl in untreated diabetic group). The insulin-treatment protocol did not achieve a complete normalization of basal plasma glucose levels.

Insulin therapy did not affect growth of the animals, because body weight of the treated diabetic rats was similar to that in the untreated diabetic and control rats. Mean pancreatic insulin content was reduced by half in the diabetic groups compared with that in control rats. Insulin therapy did not significantly modify the pancreatic insulin content of the diabetic rats (Table 1).

Effect of 1-day insulin treatment on insulin responses to glucose and arginine. Basal rates of insulin secretion from the perfused pancreases were similar in the three experimental groups (Table 2; Figs. 1 and 2). Exposure to 16 mM glucose for 20 min induced the biphasic pattern of insulin release in the control pancreases. In contrast, 16 mM glucose did not elicit a clear-cut increase of insulin output in the untreated diabetic pancreases, and previous insulin therapy did not modify this lack of glucose response in the diabetic rats. In the control group, a second stimulation period with 16 mM glucose elicited twofold more insulin release than the first stimulation period (Table 2; Fig. 2). This priming effect of glucose was not observed in pancreases of untreated diabetic rats, in which the total response during the second glucose stimulation was not significantly different from that obtained during the initial stimulation. Previous insulin therapy in the diabetic rats did not significantly modify the response of the pancreases to the second glucose stimulation, despite a doubling of the total insulin response (Table 2).

In the absence of glucose, 19 mM arginine caused a significant insulin release from the control pancreases. When 16 mM glucose was added, a moderate insulin release was observed, and 19 mM arginine given simultaneously stimulated a marked insulin response (Table 2; Fig. 1). Insulin response to 19 mM arginine in the absence of glucose was significantly higher in the untreated diabetic group than in the control group. The addition of 16 mM glucose caused no increase in the insulin secretory rate compared with basal, but again arginine caused a marked response similar to that obtained in the same pancreases in the absence of glucose (Table 2; Fig. 1). Therefore, the potentiating effect of glucose normally seen on arginine-stimulated insulin secretion was lacking in untreated diabetic rats. In contrast, after a 1-day insulin therapy, response to arginine, in the absence of glucose was now similar to that in the control group, and the glucose influence on arginine-stimulated insulin release reappeared, because 16 mM glucose was now able to potentiate (by 3-fold, compared with the response in absence of glucose) the response to 19 mM arginine (Table 2; Fig. 1). Nevertheless, the glucose-potentiating effect did not return completely, because under these experimental conditions, glucose amplified the response to arginine by 10-fold in the control pancreases but by only 3-fold in the pancreases of the insulin-treated rats.

Effect of 5-day insulin treatment on insulin response to glucose and arginine. In 3 out of 10 insulin-treated diabetic animals, lack of response to glucose persisted; the individual values for insulin release in response to the first glucose challenge were 3, 4, and 2 μU/min and were in the range of those obtained in untreated diabetic rats (4.1 ± 1.3, n = 5; Table 2). In contrast, in the remaining 7 insulin-treated rats an insulin response to glucose could be easily detected;

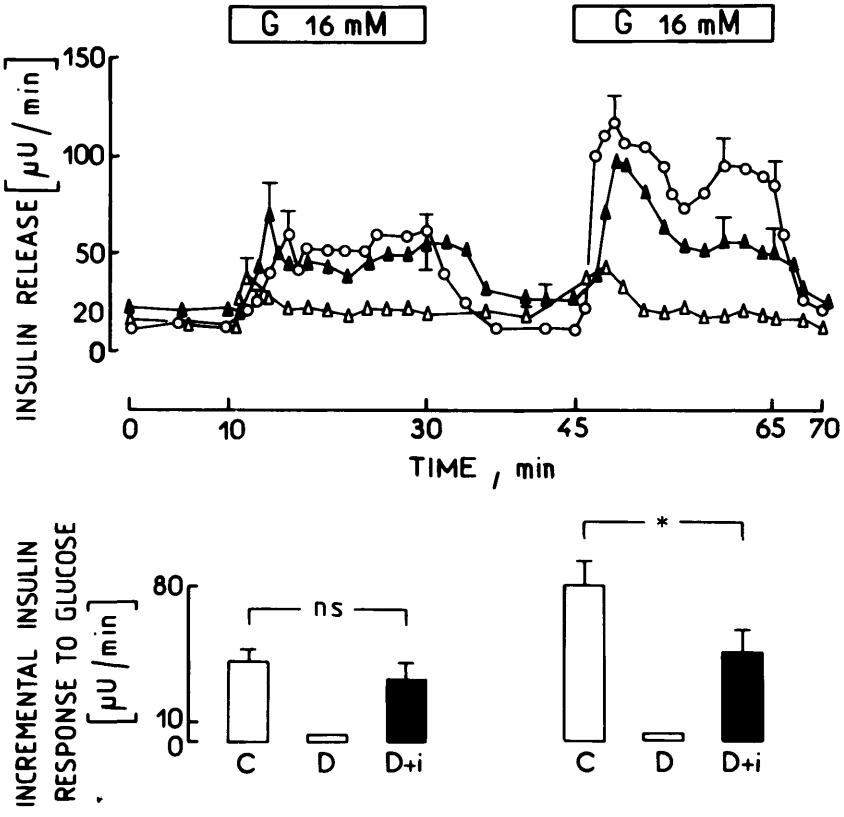

FIG. 3. Effect of 19 mM arginine and of 19 mM arginine plus 16 mM glucose on insulin release from perfused pancreases of control rats (○), untreated diabetic rats (△), and diabetic rats that received 5-day insulin therapy (▲). Experiments were carried out in absence of glucose during the pre- and poststimulatory periods. Each *point* is mean ± SE of 5–9 observations in each group. Data are also expressed as mean incremental response to glucose above basal release in each group. *$P < .05$.

the stimulated release values for these rats were 57, 26, 56, 12, 9, 21, and 15 μU/min.

Thus, in the 10 insulin-treated diabetic rats, two groups can be defined, a nonresponding group (3 rats) and a responding group (7 rats). There was no overlap between the two groups. One reason for the persistence of glucose insensitivity in the 3 rats may be that the lowering of plasma glucose did not enable glucose to reach normal values, as were probably reached in the 7 other rats. This assumption is supported by the fact that the mean plasma glucose level during the 5-day insulin therapy remained significantly higher in the 3 nonresponding rats (175 ± 8 mg/dl, $P < .02$) compared with the 7 others (150 ± 4 mg/dl). Furthermore, the mean pretreatment plasma glucose level was significantly higher ($P < .001$) in the 3 nonresponding rats (283 ± 17 mg/dl) than in the 7 others (184 ± 6 mg/dl). This could explain why 3 animals were less responsive to the insulin treatment, because the posology (5 U insulin · kg^{-1} · day^{-1}) was the same for all animals. The results reported in Table 2 are from the 7 rats that were responsive to the insulin treatment. Basal rates of insulin secretion from the perfused pancreases were similar in the three experimental groups (Table 2; Fig. 3). Exposure to 16 mM glucose for 20 min elicited a biphasic pattern of total insulin response not significantly different from that obtained in control rats (Table 2; Fig. 3). A second stimulation period with 16 mM glucose elicited 1.6-fold greater insulin release than the first stimulation period in the insulin-treated rats. Total insulin response in that group remained significantly lower ($P < .05$) than that obtained in the control pancreases during the second glucose stimulation (Table 2; Fig. 3).

In the insulin-treated diabetics the response to 19 mM arginine in the absence of glucose was lower than that in the untreated diabetics, and the glucose influence on arginine-stimulated insulin reappeared, because 16 mM glucose was now able to potentiate (by 3-fold) the response to 19 mM arginine (Table 2). On the other hand, compared to the 1-day treatment, the 5-day insulin treatment markedly reduced the insulin release in response to arginine in the diabetics (in presence or absence of glucose; Table 2).

DISCUSSION

The data herein confirm previous reports by us and others (6,12,16–18) that the neonatal STZ-D rat model of NIDDM is characterized by a lack of β-cell response to glucose. Glucose-induced insulin secretion and the potentiating effect of glucose on this response to other insulin secretagogues, e.g., arginine or IBMX, were lost (16,17,19). Our results also indicate that the induction of the priming effect of glucose on a subsequent stimulation with glucose did not exist in the diabetic rats, in accord with reports by others (19). The concern of our study was to determine if lowering the plasma glucose level with insulin treatment for short (24 h) or chronic (5 days) duration would *1*) restore the response to glucose, *2*) reverse the hyperresponse found with arginine, and *3*) restore the glucose-modulating effect on arginine- or glucose-stimulated insulin release.

The major finding of this study is that a 5-day decrease of plasma glucose levels in diabetic rats improved their glucose-induced insulin secretion. Indeed, recovery of both first- and second-phase insulin release was observed, with a total insulin release during glucose stimulation no longer significantly different from that in controls, in 7 out of 10 insulin-treated diabetic rats (Table 2). Three rats did not ex-

hibit improvement and showed persistent total insensitivity to glucose. Pretreatment plasma glucose levels were higher in the 3 diabetics than in the 7 others. Because the insulin dosage (5 U · kg^{-1} · day^{-1}) was the same in all 10 diabetic rats, it is reasonable that the mean plasma glucose levels during the 5-day insulin therapy remained significantly higher in these 3 than in the 7 others and that the plasma glucose decrease was not enough to reach approximately normal glucose values. Thus, the restoration of glucose-induced insulin release was not obtained. Consequently, the glucose range in which β-cell function is normal is probably very narrow.

A chronic decrease of plasma glucose levels in the diabetics is necessary to improve a glucose response. This is illustrated by the failure of the 1-day insulin therapy to activate a glucose-induced insulin release despite its effectiveness on plasma glucose levels, which agrees with a previous report (18). It appears, therefore, that in rats with NIDDM, the derangement of β-cell response to glucose is not intrinsic to the β-cells, because it can be reversed by changing the in vivo environmental conditions of the pancreas. This agrees with our previous data indicating that islets isolated from rats with NIDDM, once removed from chronic in vivo exposure to diabetic metabolic disorders, can behave like islets isolated from normal rats in terms of proinsulin biosynthesis and insulin release (14). Because the functional difference between the islets of diabetic rats and those of controls is not maintained in vitro when hyperglycemia is absent, we suggest that chronic hyperglycemia, even if mild, may be the factor that leads to loss of the glucose response in rats with NIDDM. In this context, note that rats treated with STZ at birth exhibit a better insulin response to glucose in vitro when tested during the weaning period than those tested as adults after long exposure to hyperglycemia (12). In patients with NIDDM, careful normalization of glycemia is accompanied by a partial return of the glucose-induced insulin response (20–24). Available morphological evidence in humans with NIDDM suggests that β-cell mass is reduced to ~35–60% of normal mass (25–27). The reduced β-cell mass of these patients may be stressed by long-term exposure to hyperglycemia in the same manner as in the rat with NIDDM, which may lead to selective loss of β-cell sensitivity to glucose.

When starting this study, we hypothesized that lowering plasma glucose might also improve the potentiating effect of glucose on the response to arginine. The results support our hypothesis, because after the 1-day insulin therapy the response to arginine in diabetic rats returned to levels similar to those in controls; it was again amplified at a high glucose level, but potentiation remained lower than in control rats. The same conclusions apply qualitatively to diabetic rats treated for 5 days. However, the absolute values of responses to arginine (in the absence or presence of glucose) are lower than those obtained after the 1-day treatment (Table 2). One possibility is that chronic insulin administration inhibits endogenous insulin response. Indeed, in pilot experiments we used a higher insulin dosage (10 U · kg^{-1} · day^{-1}) than in this protocol (5 U · kg^{-1} · day^{-1}), and insulin release in response to 16 mM glucose or 19 mM arginine was completely curbed. In mildly diabetic Chinese hamsters, suppression of insulin release has been documented after 4 wk of insulin treatment (28). Although the cause of the decrease in insulin release is not entirely clear, suppression via insulin administration (29,30) or by a catecholamine response to mild hypoglycemia (31) is possible. In either case, both the hypersensitivity to arginine and the "glucose blindness" of the response to arginine result from hyperglycemia in rats with NIDDM, and these defects are reversible by as little as 24 h of lowered plasma glucose. These conclusions agree with a previous report (18) and are further supported by the finding that 48 h of hyperglycemia in normal rats similarly impaired the ability of glucose to modulate the insulin response to arginine (32). Additionally, our data indicate that insulin therapy improves the priming effect of glucose on a second stimulation with glucose. The return of the glucose priming effect was hardly detectable after the 1-day insulin therapy but was clearly present after the 5-day insulin treatment.

In summary, this study provides the first evidence that insulin treatment improves glucose-induced insulin secretion in the neonatal STZ-D rat model of NIDDM. A return of the priming effect of glucose on glucose-stimulated insulin secretion was also seen. The 5-day insulin therapy alone corrected these defects. Moreover, the hyperresponse of arginine-stimulated insulin secretion in the model and the loss of the glucose-potentiating effect on arginine-stimulated insulin secretion were both reversed by the 1-day insulin treatment. The underlying mechanism at the level of the β-cell for the glucose-response recovery after prophylactic insulin treatment remains to be determined. In light of our preliminary observations suggesting that glucose metabolism is impaired in the β-cells of rats with NIDDM (33), it is possible that exogenous insulin may regulate diabetic β-cells directly and/or indirectly by correcting disturbances in circulating metabolites (thereby correcting hyperglycemia at first appearance) or hormones. How long improved insulin secretory responses persist after a period of insulin treatment remains to be investigated.

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