Chronic Hyperglycemia Is Associated with Impaired Glucose Influence on Insulin Secretion

A Study in Normal Rats Using Chronic In Vivo Glucose Infusions

J. L. Leahy, H. E. Cooper, D. A. Deal, and G. C. Weir
Research Division, Joslin Diabetes Center, New England Deaconess Hospital, and Brigham and Women's Hospital, Boston, Massachusetts 02215

Abstract

We have proposed that chronic hyperglycemia alters the ability of glucose to modulate insulin secretion, and have now examined the effects of different levels of hyperglycemia on B cell function in normal rats using chronic glucose infusions. Rats weighing 220–300 g were infused with 0.45% NaCl or 20, 30, 35, or 50% glucose at 2 ml/h for 48 h, which raised the plasma glucose by 18 mg/dl in the 30% rats, 37 mg/dl in the 35% rats, and 224 mg/dl in the 50% group. Insulin secretion was then examined using the in vitro isolated perfused pancreas. Glucose-induced insulin secretion remained intact in the normoglycemic 20% glucose rats and it was potentiated in the mildly hyperglycemic 30% glucose rats. However, with even greater hyperglycemia in the 35% glucose group the insulin response to a high glucose perfusate was severely blunted, and it was totally lost in the most hyperglycemic 50% glucose rats. In a second protocol that examined glucose potentiation of arginine-stimulated insulin release, a similar impairment in the ability of glucose to modulate the insulin response to arginine was found with increasing levels of chronic hyperglycemia. On the other hand, the ability of a high glucose concentration to inhibit arginine-stimulated glucagon release was preserved in all glucose-infused rats, but the glucagon levels attained in response to the arginine at 2.8 mM glucose were much less in the 50% glucose rats than in all the other groups. These data clearly show that after 48 h of marked hyperglycemia, glucose influence upon insulin secretion in the rat is severely impaired. This model provides a relatively easy and reproducible method to study the effects of long-term hyperglycemia on B cell function.

Introduction

Glucose is well known to regulate insulin secretion by directly stimulating insulin release (1) as well as by modulating the response to many nonglucose secretagogues (2). However, this relationship is disrupted in two rat models with reduced B cell mass—those that received streptozocin as neonates and those with a partial pancreatectomy—in ways that are reminiscent of human non-insulin-dependent diabetes mellitus. Specifically, glucose-stimulated insulin secretion is lost in both of these models while the response to nonglucose agents is preserved (3–5). Moreover, glucose modulation of arginine-stimulated insulin release is also impaired (6). These findings provide strong evidence for a link between a reduction in B cell mass and subsequent abnormalities in the function of the remaining cells, but the cause of these abnormalities is unknown. One possiblity is that chronic hyperglycemia occurs after the B cell reduction and that this has a direct deleterious effect on the function of the remaining cells.

The present study was designed to compare the effects of different levels of hyperglycemia over a period of 48 h on B cell function in normal rats using an adaptation of the glucose infusion method of Cole and Logothetopoulos (7). Our results show that chronic B cell stimulation, which is associated with hyperinsulinemia but not hyperglycemia, is not associated with changes in B cell responsiveness. On the other hand, the infusion of larger amounts of glucose that produce greater degrees of hyperglycemia are associated with progressive loss of the B cell sensitivity to glucose. These findings suggest that chronic hyperglycemia is the cause of the B cell functional defects described in these models.

Methods

Chronic intravenous infusion method. Male Sprague-Dawley rats weighing 220–300 g (Charles River Breeding Laboratories, Inc., Wilmington, MA) were used in all studies. An indwelling jugular venous catheter was placed by exposing and cannulating the external jugular vein with a 3-cm length of 0.012 × 0.025 in. sialastic tubing (Dow Corning Corp., Midland, MI) connected to a 10-cm length of 0.023 × 0.038 in. polyethylene tubing (Clay Adams, Parsippany, NJ); it was then tunneled underneath the skin to the back and exteriorized between the shoulders. It was flushed with a saline solution containing 30 U heparin sulphate/ml. The next day, a solution containing 20, 30, 35, or 50% glucose (wt/vol) or the diluent 0.45% NaCl was begun and infused into the catheter at 2 ml/h for a total of 48 h using a Sage syringe pump (Orion Research Inc., Cambridge, MA) and an infusion device. This device consisted of a swivel above the cage and a hollow metal cable that ran from it and was attached to the animal by way of a metal neck ring and a Velcro vest (Emdie Instruments, Goochland, VA) such that the rats were unrestrained and had free access to food and water. Blood for plasma glucose measurements was obtained by tail snipping at 0, 24, and 48 h and assayed using a Beckman Glucose Analyzer II (Beckman Co., Brea, CA). Blood for the plasma insulin concentration was obtained at similar time points by interrupting the flow to the jugular catheter for 2 min and then withdrawing 0.5 ml of blood and placing it in a heparin-coated 6 × 50-mm glass tube after carefully discarding 0.2 ml that was removed before the sample. A similar volume of saline was then replaced in the rat to minimize volume changes, and the infusion was restarted. The plasma was separated by centrifugation and stored at −20°C before assay. At the end of the infusion, the animals and the tubing were carefully checked to make sure that no leakage had occurred. A group of noninfused controls, which were normal rats taken

Presented in part at the 45th Annual Meeting of the American Diabetes Association, Baltimore, MD, 16–18 June 1985.

Please address all correspondence and reprint requests to Dr. Leahy, Research Division, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215.

Received for publication 12 July 1985 and in revised form 14 November 1985.

J. Clin. Invest.

0021-9738/86/03/0908/08 $1.00
Volume 77, March 1986, 908–915

from the general population immediately before study, was also studied along with the infused rats.

Pancreatic insulin and glucagon content. After the infusion, rats were decapitated and the pancreata removed, cleared of lymph nodes, blotted, weighed, and homogenized in cold acid ethanol using an Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). The volume was adjusted to 8 ml and was then stored at −20°C pending assay.

In vitro isolated perfused pancreas. The technique of the in vitro isolated perfused pancreas has been described previously (8). The perfusate was a modified Krebs-Ringer bicarbonate buffer containing 4% dextran T_{70} (Sigma Chemical Co., St. Louis, MO), 2 mM Ca, and 0.2% bovine serum albumin fraction V (Sigma Chemical Co.). After bubbling with 95% O_2 and 5% CO_2 for 20 min, glucose was added to the desired concentration and the pH was adjusted to 7.4; it was then placed in a reservoir maintained by water bath at 38°C. Alternate glucose concentrations and the 10 mM arginine (Eastman Co., Rochester, NY) were added to a second reservoir or driven by a syringe pump by means of a sidearm syringe, which added only 0.2 ml to the baseline flow rate of 3 ml/min. Immediately before delivery to the animal, the perfusate was passed through an artificial lung (9) to insure adequate oxygenation. After the surgery the animal was placed under a heat lamp and the temperature constantly monitored and maintained at 37°C. The body cavity was covered by a gauze pad kept moist with warmed saline to prevent the preparation from drying out. After a 20-min equilibration period, samples were collected at predetermined time points for 30 s into chilled tubes containing 4 mg EDTA and maintained on ice before storage at −20°C. The data from all samples collected are shown in the figures and were used in the tables. The protocols for each study are shown at the top of the corresponding figure.

Radioimmunoassay. The insulin concentration in the samples obtained with the perfused pancreas was measured using charcoal separation (10) and rat insulin standards (Eli Lilly, Indianapolis, IN). The plasma-insulin concentration was instead measured using double antibody separation (11). Glucagon was measured using 04A antibody (purchased from Dr. Roger Unger, Southwestern Medical School, Dallas, TX) and charcoal separation (8).

Data presentation and statistical methods. The insulin concentrations of the individual samples from the perfused pancreas studies are depicted on the figures as single points with brackets that represent the mean concentrations and standard error of the mean (mean±SEM) of the values from each group. The values in the tables represent the mean concentrations for that perfusate condition and were determined by calculating the mean result for each animal and then calculating the mean±SEM for that group. Statistical significance was determined using the unpaired and paired two-tailed Student's t test (12).

Results

Plasma glucose and insulin concentrations. The plasma glucose and insulin concentrations of the different groups measured after 24 and 48 h of infusion are listed in Table I. The plasma glucose of the 0.45% NaCl-infused rats did not vary at any point tested averaging 146±5 mg/dl at 48 h, which is slightly greater than the 134±3 mg/dl (n = 30) measured in the noninfused control rats immediately before study with the perfused pancreas. Also, values in the 20% glucose-infused group were not significantly different than those of the infused controls at either time point. On the other hand, at 48 h the plasma glucose was raised 18 mg/dl in the 30% glucose rats and 37 mg/dl in the 35% glucose rats. The 50% glucose caused marked hyperglycemia throughout the infusion. It should be noted that marked hyperinsulinemia was present in all the glucose-infused groups, and that the levels in the 20% glucose rats were not significantly different from those in the most hyperglycemic 50% glucose group at either time point. Therefore, the effects of chronic hyperinsulinemia with normoglycemia in the 20% group can be compared with those from chronic hyperinsulinemia with various levels of hyperglycemia in the other groups.

Pancreatic insulin and glucagon content. The average insulin and glucagon content per pancreas in each group after 48 h of infusion are listed in Table II. The insulin content of the 20% and 30% glucose rats was greater than that of the infused controls, although only the 20% group reached statistical significance. On the other hand, while the result of the 35% glucose rats was identical to that of the saline-infused rats, the value in the 50% glucose rats was significantly decreased. Similar results were obtained when the data was expressed as insulin concentration (insulin content/milligram pancreas, data not shown). The glucagon contents of all the groups were similar, except that of the 20% glucose rats that was slightly increased.

Effects of acute changes in glucose concentration on in vitro insulin release. The protocol and results are shown in Figs. 1 and 2. The perfusate used for the equilibration period and for the baseline sampling contained 16.7 mM glucose, which was then decreased to 2.8 mM for 10 min and then returned to 16.7 mM for 15 min.

The pattern of response in a normal animal to this protocol can be determined by examining the results of the noninfused control group in Fig. 1. As expected, they showed a rapid suppression of insulin release to a very low level after the glucose reduction and a marked biphasic response when the high glucose perfusate was returned. The pattern of response in the infused controls was qualitatively very similar, but it is evident that glucose-stimulated insulin secretion is partially suppressed in this group, even though the mean concentrations were not statistically different from those of the noninfused group (Table III). The results of the glucose-infused rats are shown in Fig. 2. Insulin release in the 20% glucose rats was similar to that of the infused controls at all time points. On the other hand, the baseline secretory rate and the fall in insulin concentration after the glucose reduction in the 30% glucose group was similar to that of the saline-infused rats, but the second phase insulin release caused by the 16.7 mM glucose was markedly increased. The baseline insulin concentration of the 35% glucose group was less than that of the infused controls. After the glucose reduction, a small transient increase in insulin release was found, but then the concentration fell to a level similar to that of the saline-infused rats. The insulin response to the high glucose perfusate was again significantly less than that of the infused controls at all points tested. The baseline secretion rate of the 50% glucose rats was similar to that of the saline-infused rats, but after the glucose reduction a marked paradoxical stimulation of insulin release was found. It should be noted that no increase in insulin release occurred when the high glucose perfusate was returned.

Effect of glucose on in vitro arginine-stimulated insulin release. The protocol and results are shown in Figs. 3 and 4. The baseline perfusate contained 2.8 mM glucose and then 10 mM arginine was added. After reequilibration at 2.8 mM glucose, the glucose was increased to 16.7 mM and arginine was added again.

As expected, the noninfused control rats (Fig. 3) had a much larger response to the arginine at the high glucose background with the mean insulin concentration being more than 17 times greater than that attained at the low glucose level (Table IV), which clearly demonstrates the normal effect that glucose had in modulating arginine-stimulated insulin release. The pattern of response in the infused controls was similar, as was that in the 20% glucose rats (Fig. 4) at all time points tested. In the 30%

Table I. Plasma Glucose and Insulin Concentrations after 24 and 48 h of Infusion

	Plasma glucose				Plasma insulin		
Time (hours)	0	24	48		0	24	48
	mg/dl	*mg/dl*	*mg/dl*		*μU/ml*	*μU/ml*	*μU/ml*
Animals (*n*)							
0.45% NaCl (16)	145±4	140±5	146±5	(6)	31±10	17±3	8±3
20% Glucose (15)		148±5	152±6	(5)		192±44*	127±27*
30% Glucose (13)		226±16*	164±4‡	(5)		166±14*	129±10*
35% Glucose (13)		325±19*	183±13‡	(5)		200±52‡	193±28*
50% Glucose (17)		466±27*	370±25*	(6)		263±26*	195±29*

Plasma glucose and insulin concentrations are expressed as mean±SEM. Statistical significance was determined using the unpaired two-tailed Student's *t* test and was calculated by comparing the results from the 0.45% NaCl-infused rats with the corresponding results from the other groups.
* $2P < 0.001$; ‡ $2P < 0.05$.

glucose rats the baseline secretion rate and the response to the first arginine challenge were equivalent to those of the infused controls. However, the 16.7 mM glucose caused much greater insulin release, averaging 14.1±2.64 ng/ml vs. only 2.51±0.50 ng/ml in the saline-infused rats ($2P < 0.001$). A marked response was then seen to the second arginine challenge. In the 35% glucose rats, the arginine, when given at 2.8 mM glucose, caused a large insulin release such that the mean insulin concentration increased only twofold when the arginine was given at 16.7 mM glucose, even though the insulin levels attained were not different than those of the infused controls. The baseline secretory rate of the 50% glucose rats was greater than that of the saline-infused controls. A very marked insulin response was found to the arginine at 2.8 mM glucose, which was greater than that to the second arginine challenge. It should be noted that the mean insulin level attained in this group in response to the arginine at 2.8 mM glucose was significantly greater than that obtained in the infused controls when it was given at 16.7 mM glucose (50.4±6.52 vs. 20.8±6.93 ng/ml, $2P < 0.05$).

Effect of glucose on in vitro arginine-stimulated glucagon release. The mean glucagon concentrations caused by the arginine at 2.8 and 16.7 mM glucose taken from the perfusions shown in Figs. 3 and 4 are listed in Table IV. It is evident that the 16.7 mM glucose has a marked inhibitory influence on arginine-stimulated glucagon release in all groups. It should be noted that the mean glucagon concentrations of all the groups are similar at both glucose levels, except that of the 50% glucose rats, which was significantly decreased at the low glucose concentration.

Effect of previous hyperglycemia on in vitro arginine-stimulated insulin release. The protocol and results are shown in Fig. 5. The baseline perfusate contained 7.8 mM glucose and then 10 mM arginine was added. After reequilibration at 7.8 mM glucose, the glucose was increased to 32.8 mM for 30 min, and then returned to 7.8 mM for 20 min. A second arginine challenge was then given.

The noninfused control rats showed a marked biphasic insulin response to the initial arginine challenge, and then, after reequilibration at 7.8 mM glucose, the glucose increase caused a marked biphasic release. However, it should be noted that after the return to 7.8 mM glucose the insulin concentration only fell to 9.24±2.90 ng/ml, which is much greater than the mean baseline level attained before the initial arginine challenge (2.38±0.33 ng/ml). The second arginine challenge caused a sim-

Table II. Pancreatic Insulin and Glucagon Concentrations after 48 h of Infusion

	Insulin content	Glucagon content
	μg/pancreas	*μg/pancreas*
Animals (*n*)		
0.45% NaCl (7)	81.5±9.70	4.06±0.21
20% Glucose (7)	113±10.0*	5.06±0.26*
30% Glucose (8)	112±13.6	5.25±0.50
35% Glucose (7)	77.2±9.66	4.29±0.47
50% Glucose (7)	29.7±3.20‡	4.56±0.42

Hormone content is expressed as mean±SEM. Statistical significance was determined using the unpaired two-tailed Student's *t* test and was calculated by comparing the results from the 0.45% NaCl-infused rats with the corresponding results from the other groups.
* $2P < 0.05$;
‡ $2P < 0.001$.

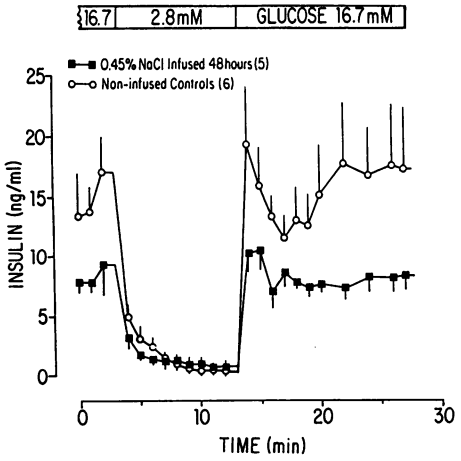

Figure 1. Effects of an acute reduction and then increase in the perfusate glucose concentration on insulin secretion in rats infused with 0.45% NaCl for 48 h and assessed using the in vitro isolated perfused pancreas. Noninfused controls were normal rats taken from the general population immediately before study.

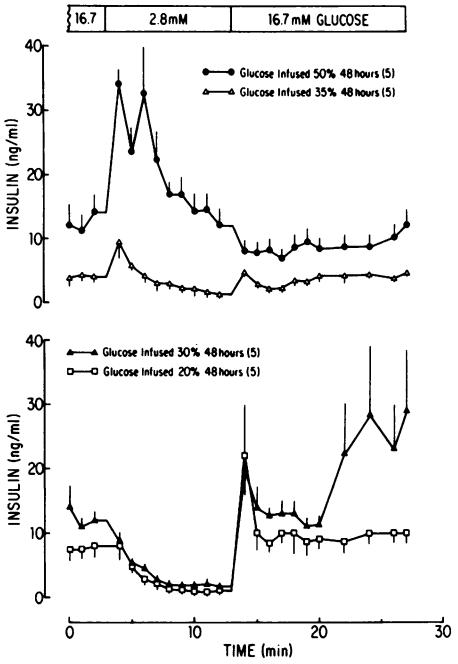

Figure 2. Effects of an acute reduction and then increase in the perfusate glucose concentration on insulin secretion in rats infused with 20, 30, 35, or 50% glucose for 48 h and assessed using the in vitro isolated perfused pancreas.

ilar insulin response to that of the first. The saline-infused controls showed a blunted second phase release to the initial arginine challenge as well as partially suppressed glucose-induced insulin secretion compared with the noninfused controls. After the return to 7.8 mM glucose, the insulin release of this group also failed to suppress fully as it fell to only 4.23±0.53 ng/ml compared with the baseline value of 1.08±0.20 ng/ml. The response to the second arginine challenge was also very similar to that of

Table III. Effects of Acute Changes in Glucose Concentration on In Vitro Insulin Secretion after 48 h of Infusion

	Mean insulin concentration		
	16.7 mM glucose	2.8 mM glucose	16.7 mM glucose
Animals (*n*)	*ng/ml*	*ng/ml*	*ng/ml*
Noninfused (6)	14.7±2.74	1.62±0.41	15.4±3.40
0.45% NaCl (5)	8.33±1.19	1.28±0.59	8.28±0.47
20% Glucose (5)	7.80±1.71	2.62±0.84	10.6±1.77
30% Glucose (5)	12.2±1.66	1.33±0.52	17.8±2.70*
35% Glucose (5)	4.08±0.92*	1.33±0.66	3.51±0.60‡
50% Glucose (5)	12.2±2.92	11.8±2.87*	8.63±1.80

Insulin concentrations are expressed as mean±SEM and were calculated using each sample obtained during that perfusate condition. Statistical significance was determined using the unpaired two-tailed Student's *t* test and was calculated by comparing the results from the 0.45% NaCl-infused rats with the corresponding results from the other groups.
* $2P < 0.05$;
‡ $2P < 0.001$.

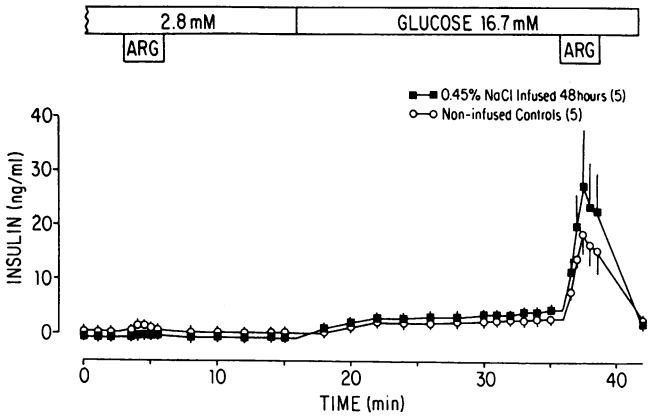

Figure 3. Effects of glucose and 10 mM arginine on insulin secretion in rats infused with 0.45% NaCl for 48 h and assessed using the in vitro isolated perfused pancreas. Noninfused controls were normal rats taken from the general population immediately before study.

the first. This protocol was only tested in the 20% and 50% glucose-infused rats. The response in the 20% glucose group to the first arginine challenge and to the 32.8 mM glucose was very similar to that of the infused controls. However, the mean insulin output to the second challenge was surprisingly less than that to the first (15.2±3.50 vs. 28.0±4.94 ng/ml, $2P < 0.02$). The baseline secretory rate of the 50% glucose group was much greater than that of the other groups. The initial arginine challenge caused a marked biphasic insulin response that averaged 53.6±7.62 ng/ml vs. only 24.4±4.85 in the saline-infused rats ($2P < 0.05$). After the arginine, the insulin concentration fell to a level much less than the baseline value, but then slowly rose

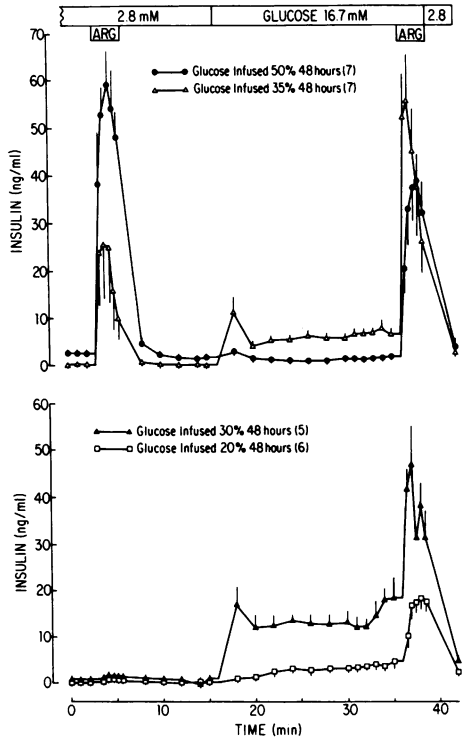

Figure 4. Effects of glucose and 10 mM arginine on insulin secretion in rats infused with 20, 30, 35, or 50% glucose for 48 h and assessed using the in vitro isolated perfused pancreas.

Table IV. Effects of Glucose on In Vitro Arginine-stimulated Insulin and Glucagon Secretion after 48 h of Infusion

	Mean insulin concentration		Mean glucagon concentration	
	2.8 mM glucose + 10 mM arginine	16.7 mM glucose + 10 mM arginine	2.8 mM glucose + 10 mM arginine	16.7 mM glucose + 10 mM arginine
Animals (*n*)	*ng/ml*	*ng/ml*	*ng/ml*	*ng/ml*
Noninfused (5)	0.83±0.29	14.3±2.04	2.09±0.55	0.33±0.11
0.45% NaCl (5)	0.26±0.15	20.8±6.93	1.78±0.46	0.19±0.08
20% Glucose (6)	0.43±0.15	15.8±1.83	1.84±0.31	0.13±0.03
30% Glucose (5)	1.32±0.51	38.1±4.24	2.27±0.70	0.53±0.22
35% Glucose (7)	17.9±7.54	43.2±7.69	1.32±0.27	0.27±0.06
50% Glucose (7)	50.4±6.52*	32.2±6.62	0.60±0.09‡	0.23±0.08

Insulin and glucagon concentrations are expressed as mean±SEM and were calculated using each sample obtained during that perfusate condition. Statistical significance was determined using the unpaired two-tailed Student's *t* test and was calculated by comparing the results from the 0.45% NaCl-infused rats with the corresponding results from the other groups. * 2 $P < 0.001$; ‡ 2 $P < 0.05$.

during the 32.8 mM glucose to reach a value that was indistinguishable from the baseline. After the return to 7.8 mM glucose, the insulin concentration showed a nonsignificant rise from 12.6±2.27 ng/ml to peak at 16.5±4.73 ng/ml. The response to the second arginine challenge was again smaller than that of the first.

Reversibility of the lost modulating effect of glucose on in vitro insulin secretion in 50% glucose-infused rats. To determine if the modulating influence of glucose on insulin secretion was restorable in the 50% glucose rats, a group was infused for 48 h with 50% glucose and then removed from the infusion devices. They were studied 72 h later with the perfused pancreas using the protocol from Fig. 2. The results are shown in Fig. 6 and it is evident that the pattern of response was identical to that of noninfused controls such that the paradoxical stimulation after the glucose reduction and the failure to respond to the high glucose perfusate were no longer present.

Discussion

The normal B cell rapidly responds to changes in the glucose concentration so that a decrease causes prompt suppression of insulin release while an increase causes a marked stimulation of secretion. Glucose also plays a critical role in modulating the B cell responses to many nonglucose secretagogues. Taken together, these findings suggest that insulin secretion is predominately controlled by the prevailing glucose concentration. However, we have recently described two rat models with a reduced B cell mass in which this relationship is disrupted. In the first, the neonatal streptozocin model (NSZ),[1] rats receive streptozocin at 2 d of age that causes a chronic hyperglycemia averaging 200 mg/dl once the rats reach ~6 wk (13). In the second, the partial pancreatectomy model (Px), rats undergo a 90% surgical pancreatectomy at 4 wk of age and they then develop a milder hyperglycemia averaging 160–180 mg/dl within one week (5). In both of these models, in vivo and in vitro studies have demonstrated that glucose-induced insulin release is lost, while release to nonglucose agents is preserved (3–5). Moreover, the glucose-modulating effect on arginine-stimulated insulin release is also impaired in both, although to a greater degree in the more hyperglycemic NSZ rats (6). These findings provide strong evidence for an association between a reduction in B cell mass and subsequent defects in the function of the remaining cells, but the cause of these abnormalities has not been elucidated. We have previously proposed that chronic exposure of the remaining B cells to elevated glucose concentrations might be the explanation (3, 14).

The findings of the present study provide further support for this hypothesis, for the ability of glucose to stimulate insulin secretion was increased in the very mildly hyperglycemic 30% glucose rats, but then progressively decreased as the level of chronic hyperglycemia rose. Moreover, the unusual paradoxical release of insulin to the acute glucose reduction and the marked insulin response to arginine at 2.8 mM glucose that are present in the 50% glucose rats are similar to patterns of insulin release that have previously been described in the NSZ model (6, 15), which suggests that the pathophysiologic events that are occurring

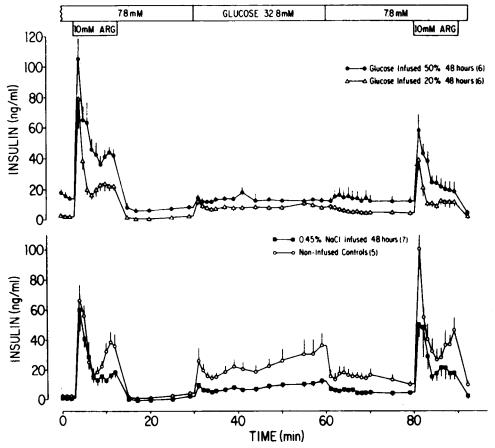

Figure 5. Effects of a 30-min exposure to 32.8 mM glucose on a subsequent response to 10 mM arginine in rats infused with 20 or 50% glucose or 0.45% NaCl for 48 h and assessed using the in vitro isolated perfused pancreas. Noninfused controls were normal rats taken from the general population immediately before study.

1. *Abbreviations used in this paper:* NSZ, neonatal streptozocin model; Px, 90% pancreatectomy model.

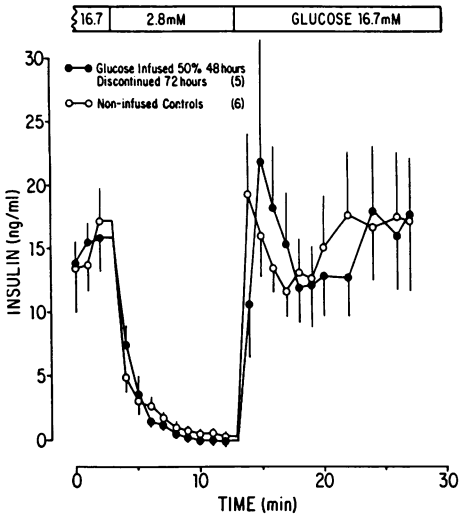

Figure 6. Effects of an acute reduction and then increase in the perfusate glucose concentration on insulin secretion in rats 72 h after an infusion with 50% glucose for 48 h and assessed using the in vitro isolated perfused pancreas. Noninfused controls were normal rats taken from the general population immediately before study.

in both groups are identical. The marked hyperinsulinemia of these rats may seem at odds with the findings in the NSZ model in which hyperglycemia is accompanied by hypoinsulinemia (13), but Brodsky and Heuck (16) have shown that with longer periods of glucose-infusion the plasma-insulin response falls to levels that are indistinguishable from those of controls.

The results obtained with arginine are also consistent with the view that chronic hyperglycemia can alter insulin secretion. We have previously suggested that chronic hyperglycemia may cause a "potentiated" state in the B cell such that exposure to arginine even at trivial glucose levels causes a marked response (14). Indeed, a very large amount of insulin was released by the most hyperglycemic rats when arginine was given on a background glucose concentration of 2.8 mM. Moreover, this finding is likely not unique for arginine since a similar hyperresponse has also been demonstrated in the NSZ model for leucine (17), isoproterenol (3), and 3-isobutyl-1-methylxanthine (6) as well as for arginine (6, 17). However, the potentiation of this response by the high glucose perfusate in each group can only be estimated from the results in this study, for the magnitude of the insulin levels attained to the second arginine challenge as well as to the 16.7 glucose alone was likely suppressed by the initial arginine exposure (18).

Therefore, we suggest that chronic hyperglycemia is the factor that leads to the altered B cell responses in the glucose-infused rats as well as the models with reduced B cell mass. Indeed, the results in Fig. 5 clearly demonstrate that normal rats exposed to 32.8 mM glucose for only 30 min do not fully suppress insulin release when the glucose is then returned to 7.8 mM. Yet alternatives other than chronic hyperglycemia must be considered. It is well known that insulin inhibits endogenous insulin release (19, 20) and one might wonder if the marked hyperinsulinemia of the most hyperglycemic rats exerts a suppressive influence. However, marked hyperinsulinemia is present in all the glucose-infused rats, including the 20% glucose rats in which in vitro insulin responsiveness remains intact. Moreover, similar patterns of in vitro insulin release have been described in the NSZ model that is hypoinsulinemic (13). It also seems unlikely that excess catecholamine release from hyperglycemia or volume depletion is playing any part in the B cell changes, for a global suppression of insulin release would be expected. Instead, the plasma-insulin concentrations in all the glucose-infused rats are comparable, and the in vitro insulin responses of the 50% glucose rats to 7.8 mM glucose and to arginine are significantly greater than those of the saline-infused group. Moreover, alpha receptor stimulation increases glucagon release (15, 21), and the response to arginine in the 50% glucose rats is instead less than that of the other groups.

We therefore propose that chronic hyperglycemia is associated with changes in B cell responsiveness, and, once established, a vicious cycle begins, for insulin responses eventually become impaired, perhaps leading to worsening of the hyperglycemia. Several studies in human non–insulin-dependent diabetes mellitus have shown an improvement in insulin secretion after various periods of close diabetic control (22–24), which suggests that a similar sequence of events may also occur in man.

An interesting aspect to the results in this study is the paradoxical insulin release that occurs in the 50% rats after a decrease from 16.7 to 2.8 mM glucose, but not when the glucose was reduced from 32.8 to 7.8 mM. Previous studies in the NSZ rats have shown that this response is not due to the osmolarity change (15). One possible mechanism could be a factor that is released during the sudden reduction of perfusate glucose and that causes the insulin response. Indeed, a recent study (25) has confirmed an earlier observation (26) that catecholamines are locally released by acute hypoglycemia in the perfused rat pancreas preparation, so perhaps another factor may also be released. Another possiblity stems from recent observations on the effects of glucose on B cell intracellular calcium. Hellman et al. (27) have suggested that glucose stimulates calcium influx into the cell thereby raising the intracellular calcium level, but it also promotes calcium uptake by the organelles and stimulates calcium efflux from the cell, which tends to decrease cytoplasmic calcium. Glucose-induced insulin release is thought to be caused by an increase in the free cytoplasmic calcium concentration so that the calcium influx into the cell would normally predominate. However, they have observed glucose-induced inhibition of insulin release in alloxan diabetic rats (27), a phenomena that has also been observed in some human diabetics (28), and they have postulated that in these pathological situations calcium uptake into organelles predominates over intracellular influx such that cytoplasmic calcium falls (27). It follows that an acute lowering of glucose might then mobilize these calcium stores and cause an insulin response. Further studies are needed to explore these possibilities.

It is also noteworthy that the direct modulating effect of glucose on insulin secretion is totally restored in the 50% glucose rats 72 h after the infusion. Similar attempts to study the reversibility of B cell defects in diabetic animal models using established therapeutic modalities have been hard to interpret, perhaps because of the difficulty in chronically establishing perfect glucose control (14, 29, 30). While it remains to be proven that these results have relevance to situations with longer periods of hyperglycemia, they at least suggest that B cell changes associated with short-term hyperglycemia are totally reversible.

The findings in this study also suggest that chronic hyperglycemia has an inhibitory effect on arginine-stimulated glucagon release, for the response to arginine at 2.8 mM glucose is much less in the 50% infused rats than in the other groups. We have previously shown in the NSZ model in which the A cell mass is

comparable to that of the controls (13) that the glucagon response to arginine at 2.8 mM glucose is only about one-half of that found in the controls (14), which lends further credence to this concept.

The pancreatic insulin content of the 50% rats was markedly reduced, which is comparable to a similar result obtained in the Logothetopoulos model after 24 h of infusion (31). Insulin content is dependent on insulin biosynthesis on the one hand and on insulin release and intracellular degradation on the other. It has been well established with both in vitro and in vivo techniques that insulin synthesis is increased in markedly hyperglycemic glucose-infused rats after 24 and 72 h of infusion (32, 33) and the results from this study show that insulin release is also markedly increased throughout the infusion period. We don't know how much intracellular degradation is occurring. It is then of interest that the pancreatic insulin contents of the various groups differ so much in spite of plasma insulin levels that are not very dissimilar. It may be that the insulin concentrations do not reflect the secretion rate because insulin clearance is somehow altered under these different conditions. These results suggest that with the infusion of 20% or 30% glucose the rate of biosynthesis exceeds that of secretion, whereas with more hyperglycemia the reverse occurs. Yet it must be remembered that these infusions are only given for 48 h and that the effects of more prolonged hyperglycemia may differ (34). One finding that is clear, however, is that the quantitative insulin response to the arginine at 2.8 mM glucose in the 50% group is larger than that of the control groups at 16.7 mM glucose, even with the marked decrease in insulin content, and that the insulin levels attained in the 20% rats are similar to those of the controls, even though the insulin content is increased. These results suggest that the quantitative insulin response under most circumstances is not determined by insulin content.

In summary, these results suggest that 48 h of very mild hyperglycemia tended to potentiate glucose-induced insulin secretion and did not alter the modulating effect of glucose on arginine-stimulated insulin release. On the other hand, with greater degrees of hyperglycemia the direct influence of glucose to acutely alter insulin secretion decreased such that it was most impaired in the more hyperglycemic 50% group. Moreover, a progressive alteration of the glucose influence on arginine-stimulated insulin release was also found with increasing levels of hyperglycemia. These findings are very similar to patterns of insulin secretion that have been described in the Px and NSZ rat models, and they strongly suggest to us that chronic hyperglycemia is the factor that causes many of the defects noted in the B cell function of these groups. It remains to be seen whether a similar sequence of events occurs in man, but it is very interesting that a recent study has demonstrated that insulin secretion in normal subjects fails to appropriately suppress during hypoglycemia after just 12 h of hyperglycemia (35).

The glucose infusion method therefore provides a way to study the effects of chronic hyperglycemia on normal B cells, obviating the need for streptozocin or animal strains with genetic abnormalities of glucose homeostasis. Also, it allows the effects of different levels of glycemia for different time periods to be assessed, making this model an important tool for further study.

Acknowledgments

The authors thank D. Poczatek for preparation of this manuscript.

The work has been supported by grant AM53449, formerly AM-20349 from the National Institutes of Health. Dr. Leahy is the recipient of a Research Career and Development award from the American Diabetes Association.

References

1. Grodsky, G. M., A. A. Batts, L. L. Bennett, C. Vcella, N. B. McWilliams, and D. F. Smith. 1963. Effects of carbohydrates on secretion of insulin from isolated rat pancreas. *Am. J. Physiol.* 205:638–644.

2. Levin, S. R., G. M. Grodsky, R. Hagura, D. F. Smith, and P. H. Forsham. 1972. Relationships between arginine and glucose in the induction of insulin secretion from the isolated perfused rat pancreas. *Endocrinology.* 90:624–631.

3. Weir, G. C., E. T. Clore, C. J. Zmachinski, and S. Bonner-Weir. 1981. Islet secretion in a new experimental model for non-insulin-dependent diabetes. *Diabetes.* 30:590–595.

4. Trent, D. F., D. J. Fletcher, J. M. May, S. Bonner-Weir, and G. C. Weir. 1984. Abnormal islet and adipocyte function in young B-cell-deficient rats with near-normoglycemia. *Diabetes.* 33:170–175.

5. Bonner-Weir, S., D. F. Trent, and G. C. Weir. 1983. Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J. Clin. Invest.* 71:1544–1553.

6. Leahy, J. L., S. Bonner-Weir, and G. C. Weir. 1984. Abnormal glucose regulation of insulin secretion in models of reduced B-cell mass. *Diabetes.* 33:667–673.

7. Cole, E., and J. Logothetopoulos. 1974. Glucose oxidation ($^{14}CO_2$ production) and insulin secretion by pancreatic islets isolated from hyperglycemic and normoglycemic rats. *Diabetes.* 23:469–473.

8. Weir, G. C., S. D. Knowlton, and D. B. Martin. 1974. Glucagon secretion from the perfused rat pancreas. *J. Clin. Invest.* 54:1403–1412.

9. Hamilton, R. L., M. N. Berry, M. C. Williams, and E. M. Severinghaus. 1974. A simple and inexpensive membrane "lung" for small organ perfusion. *J. Lipid Res.* 15:182–186.

10. Albano, J. D. M., R. P. Ekins, G. Maritz, and R. C. Turner. 1972. A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moities. *Acta Endocrinol.* 70:487–509.

11. Morgan, C. R., and A. Lazarow. 1963. Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, subdiabetic, and diabetic rats. *Diabetes.* 12:115–126.

12. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, IA. 1–593.

13. Bonner-Weir, S., D. F. Trent, R. N. Honey, and G. C. Weir. 1981. Responses of neonatal rat islets to streptozotocin. Limited B-cell regeneration and hyperglycemia. *Diabetes.* 30:64–69.

14. Leahy, J. L., S. Bonner-Weir, and G. C. Weir. 1985. Abnormal insulin secretion in a streptozotocin model of diabetes: effects of insulin treatment. *Diabetes.* 34:660–666.

15. Leahy, J. L., and G. C. Weir. 1985. Unresponsiveness to glucose in a streptozotocin model of diabetes: inappropriate insulin and glucagon responses to a reduction of glucose concentration. *Diabetes.* 34:653–659.

16. Brodsky, G. M., and C. C. Heuck. 1975. Der einflub von glukoseinfusionen auf die proinsulinund insulinsynthese in der Langerhansschen Inseln bei der ratte. *Endokrinologie.* 66:46–55.

17. Giroix, M.-H., B. Portha, M. Kergoat, D. Bailbe, and L. Picon. 1983. Glucose insensitivity and amino-acid hypersensitivity of insulin release in rats with non-insulin-dependent diabetes. A study with the perfused pancreas. *Diabetes.* 32:445–451.

18. Nesher, R., L. Waldman, and E. Cerasi. 1984. Time-dependent inhibition of insulin release: glucose-arginine interactions in the perfused rat pancreas. *Diabetologia.* 26:146–149.

19. Iverson, J., and D. W. Miles. 1971. Evidence for a feedback inhibition of insulin on insulin secretion in the isolated, perfused canine pancreas. *Diabetes.* 10:1–9.

20. Service, F. J., R. L. Nelson, A. H. Rubenstein, and V. L. W. Go. 1978. Direct effect of insulin on secretion of insulin, glucagon, gastric

inhibitory polypeptide, and gastrin during maintenance of normoglycemia. *J. Clin. Endocrinol. Metab.* 47:488–493.

21. Samols, E., and G. C. Weir. 1979. Adrenergic modulation of pancreatic A, B, and D cells. α Adrenergic suppression and β adrenergic stimulation of somatostatin secretion, α adrenergic stimulation of glucagon secretion in the perfused dog pancreas. *J. Clin. Invest.* 63:230–238.

22. Vague, P., and J. P. Moulin. 1982. The defective glucose sensitivity of the B cell in noninsulin dependent diabetes. Improvement after twenty hours of normoglycemia. *Metabolism.* 31:139–142.

23. Andrews, W. J., B. Vasquez, M. Nagulesparan, I. Klimes, J. Foley, R. Unger, and G. M. Reaven. Insulin therapy in obese, non-insulin-dependent diabetes induces improvements in insulin action and secretion that are maintained for two weeks after insulin withdrawal. *Diabetes.* 33:634–642.

24. Garvey, W. T., J. M. Olefsky, J. Griffin, R. F. Hamman, and O. G. Kolterman. 1985. The effect of insulin treatment on insulin secretion and insulin action in type II diabetes mellitus. *Diabetes.* 34:222–234.

25. Hisatomi, A., H. Maruyama, L. Orci, M. Vasko, and R. H. Unger. 1985. Adrenergically mediated intrapancreatic control of the glucagon response to glucopenia in the isolated rat pancreas. *J. Clin. Invest.* 75: 420–426.

26. Christensen, N. J., and J. Iversen. 1973. Release of large amounts of noradrenaline from the isolated perfused canine pancreas during glucose deprivation. *Diabetologia.* 9:396–399.

27. Hellman, B. 1985. B-Cell cytoplasmic Ca^{2+} balance as a determinant for glucose-stimulated insulin release. *Diabetologia.* 28:494–501.

28. Hellman, B., R. Hällgren, H. Abrahamsson, P. Bergsten, C. Berne, E. Gylfe, P. Rorsman, and L. Wide. 1985. The dual action of glucose on the cytosolic Ca^{2+} activity in pancreatic B cells. Demonstration of an inhibitory effect of glucose on insulin release in the mouse and man. *Biomed. Biochim. Acta.* 44:63–70.

29. Clark, A., E. Brown, T. King, R. I. Vanhegan, and R. C. Turner. 1982. Islet changes induced by hyperglycemia in rats. Effect of insulin or chlorpropamide therapy. *Diabetologia.* 31:319–325.

30. Frankel, B. J., F. G. Schmid, and G. M. Grodsky. 1979. Effect of continuous insulin infusion with an implantable seven-day minipump in the diabetic chinese hamster. *Endocrinology.* 104:1532–1539.

31. Logothetopoulos, J., M. Kaneko, G. A. Wrenshall, and C. H. Best. 1964. Zinc, granulation and extractable insulin of islet cells following hyperglycemia or prolonged treatment with insulin. *In* Structure and Metabolism of the Pancreatic Islets. S. E. Brolin and B. Hellman, editors. Pergamon Press, Oxford. 333–344.

32. Zucker, P., and J. Logothetopoulos. 1975. Persisting enhanced proinsulin-insulin and protein biosynthesis (^{3}H-leucine incorporation) by pancreatic islets of the rat after glucose exposure. *Diabetes.* 24:194–200.

33. Logothetopoulos, J., and N. Valiquette. 1984. Hormonal and non-hormonal protein biosynthesis in the pancreatic beta cell of the intact rat after prolonged hyperglycaemia. *Acta Endocrinol.* 107:382–389.

34. Orland, M. J., R. Chyn, and M. A. Permutt. 1985. Modulation of proinsulin messenger RNA after partial pancreatectomy in rats. Relationship to glucose homeostasis. *J. Clin. Invest.* 75:2047–2055.

35. Dimitriadis, G., P. Cryer, and J. Gerich. 1985. Prolonged hyperglycaemia during infusion of glucose and somatostatin impairs pancreatic A- and B-cell responses to decrements in plasma glucose in normal man: evidence for induction of altered sensitivity to glucose. *Diabetologia.* 28:63–69.