Reversible Impairment of Glucose-Induced Insulin Secretion in SHR/N-*cp* Rats

Genetic Model of Type II Diabetes

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The SHR/N-*cp* rat is a new genetically obese model for non-insulin-dependent diabetes mellitus. Expression of the diabetes is enhanced by a high-sucrose (54%) diet. After 4 wk on the diet, the *cp/cp* rats weigh significantly more than their +/? controls, have postprandial hyperglycemia (>400 mg/dl), and are hyperinsulinemic, with immunoreactive insulin (IRI) levels 10- to 20-fold greater than controls. Total pancreatic IRI tends to be increased 1.6-fold in the *cp/cp* rats (although not significantly). There is no increase in pancreatic proinsulin content as a percent of total IRI. Studies of in vitro pancreatic function were carried out with the isolated nonrecirculating perfused pancreas method. The *cp/cp* rats (n = 10) showed impaired or absent IRI responses to 16.5 mM glucose, whereas +/? rats (n = 9) responded with classic biphasic curves. Comparison of insulin secreted in 20 min revealed a >53% decrease in IRI secretion in *cp/cp* rats ($P < .05$). A paradoxical hypersecretion of IRI at glucose concentrations of 0–2.7 mM was noted in *cp/cp* but not lean rats, i.e., 1.8 ± 0.2 mU/min IRI in *cp/cp* rats vs. 0.04 ± 0.007 mU/min in +/? rats. Perfusion of pancreases for 45 min with buffers containing no glucose resulted in restoration of a normal biphasic IRI response to 16.5 mM glucose in the *cp/cp* rats, whereas response in the lean rats was markedly reduced. Brisk IRI responses to 10 mM arginine in buffers with no glucose also occurred in *cp/cp* but not +/? rats. Glucagon secretion was relatively suppressed in the *cp/cp* rats. These findings are similar to those reported in glucose-infused normal rats and suggest that hyperglycemia per se may be responsible for the impaired β-cell responses to glucose in *cp/cp* rats. *Diabetes.* 37:398–404, 1988

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A new animal model for non-insulin-dependent diabetes mellitus has been developed (1,2). The congenic rat strain, spontaneously hypertensive/NIH-corpulent (SHR/N-*cp*), inherits obesity as an autosomal recessive trait. Because the corpulent rats do not reproduce, heterozygotes are mated and yield corpulent (*cp/cp*) and lean rats that are either +/*cp* or +/+. It is currently not possible to identify the heterozygotes except by breeding experiments. It is interesting that the hypertension characteristic of the original strain of SHR rats is significantly diminished or absent in the *cp/cp* rats, but retained in the lean rats. Diabetes and obesity are most pronounced when the weanling rats are provided with a high-sucrose diet, although diabetes also occurs with the feeding of chow diets (3). The syndrome is well developed within 4 wk of sucrose feeding. The characteristics of the diabetes in the *cp/cp* rats include striking elevations in plasma insulin, normal fasting blood glucose, and glucose levels after a glucose load (350 mg/dl with insulin levels of 900 μU/ml). Despite diabetes duration of 4–10 wk, fasting plasma glucose levels remain normal (2,3). Glycosuria and proteinuria also occur. Pathologic changes include pancreatic islet hyperplasia and renal lesions resembling human glomerulosclerosis (4). Of special interest is the observation that certain lean animals may also become diabetic after 9–12 mo of sucrose feeding and show histologic findings similar to those of *cp/cp* rats (4).

To obtain information about the mechanisms involved in the development of hyperglycemia and extreme hyperinsulinemia, we focused on the pancreas, defining the insulin and proinsulin content and the secretory responses. For the latter studies, we used the in vitro pancreas perfusion technique (5). Our findings support the existence of abnormal glucose regulation of insulin release from the pancreas in the *cp/cp* rat, with evidence of paradoxical insulin secretion

at low blood glucose levels, i.e., an inability to shut off insulin secretion. The extreme degree of hypersecretion of insulin, as seen in the genetically obese but nondiabetic Zucker rats (6), was not found.

MATERIALS AND METHODS

Male and female SHR/N-*cp* rats ~5 wk of age were obtained and easily separated into *cp/cp* and lean groups primarily by body length and shape, because body weights are not a reliable index of phenotype in weanlings. Animals were individually housed and exposed to a 12-h light-dark cycle with free access to food and water unless otherwise noted. The diet consisted of 54% carbohydrate as sucrose (unless otherwise indicated) and contained 10% casein, 10% lactalbumin, 5.9% cellulose, 4% lard, 4% corn oil, 4% beef tallow, 4% coconut oil, 3.1% AIN76 (American Institute of Nutrition) salt mix, and 1% vitamin mix (7).

Plasma glucose and insulin levels were recorded once per week for wk 1–4 of diet in a group of four *cp/cp* rats and their controls. A separate group of four animals was used after 8 wk of diet. In these studies, data were obtained 6–8 h into the feeding (dark) cycle. Tail blood from unanesthetized animals was used. Glucose was measured by a glucose oxidase method.

Glucose and insulin levels were also measured in a separate group that was perfused after 4 wk of diet. Measurements were made at the time of perfusion, which was 6–8 h into the light cycle. Samples were obtained from anesthetized rats.

In some experiments LA/N-*cp* rats were also studied. These animals are obese nondiabetic and hyperinsulinemic (7), having the *cp* gene but lacking the SHR gene.

Isolated rat pancreas perfusions were carried out by the procedure of Fink et al. (8) in *cp/cp* and lean rats. Open nonrecirculating perfusions were used with Krebs bicarbonate buffer (pH 7.4) containing 3% dextran 40 (Pharmacia, Uppsala, Sweden), 1% bovine albumin (Sigma, St. Louis, MO), and various concentrations of glucose or arginine. Buffers were continuously oxygenated with 95% O_2/5% CO_2. Flow rates were 5–6 ml/min, remaining constant for at least 60 min and declining minimally by 10 min. Portal vein effluent was collected continuously in 1-min fractions into chilled tubes containing 10.5 mg Na-EDTA and 1000 KIU Trasylol (FBA, New York). All tubes were centrifuged to remove blood cells, and the supernatants were frozen for radioimmunoassay of insulin by a double-antibody method (9). Rat insulin standards (Lilly, Indianapolis, IN) were used. Glucagon was measured with the G-15 antibody of Jaspan (10). Each perfusion included a 10- to 15-min period of low or no glucose before stimulation with high concentrations of glucose or arginine.

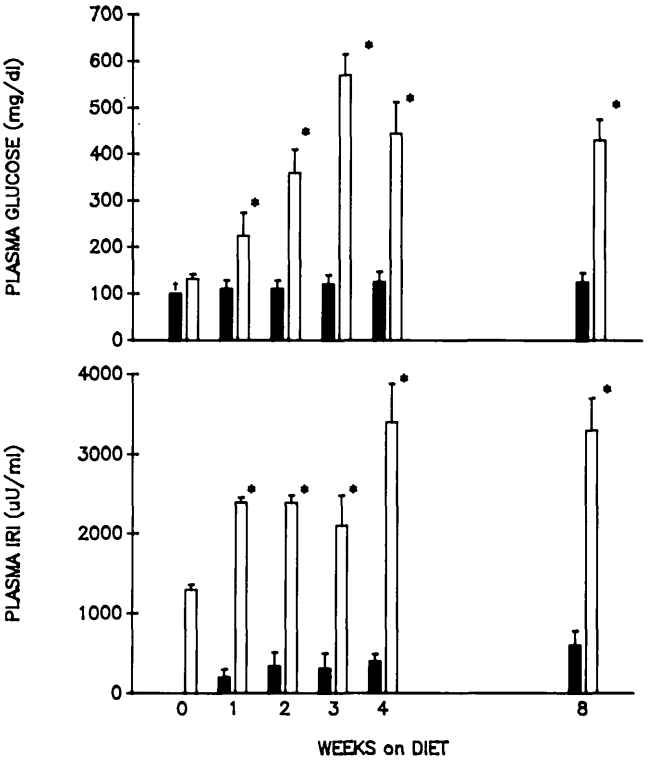

FIG. 1. Sequential changes in plasma glucose and immunoreactive insulin (IRI) in *cp/cp* (*open bars*) and lean control (+/?, *solid bars*) rats in fed state (8 h). Studies carried out weekly on 4 *cp/cp* and 4 +/? rats before and after sucrose diet. Note elevation of plasma insulin before hyperglycemia in *cp/cp* rats. Values are means ± SE. *$P < .05$.

In studies of the hormone content of the pancreas, animals were killed by guillotine after a stunning head blow, and the pancreas was removed, weighed, and extracted in cold acid alcohol (11). Insulin was assayed in the supernatant after centrifugation at low speed. Plasma and acid alcohol extracts of pancreas were examined for proinsulin content by Sephadex G-50 columns (12).

RESULTS

Figure 1 shows the time course of development of the diabetic state in *cp/cp* rats. Plasma glucose and insulin levels were obtained 8 h into the feeding cycle, when they were at their maximal levels as shown by around-the-clock measurements of sugars in rats on sucrose diets for 8 wk (data not shown). Note that in the *cp/cp* rats, glucose levels were

TABLE 1
Parameters in *cp/cp* and control rats after 4-wk sucrose diet

		Pancreas			Plasma		
	Body weight (g)	*n*	Insulin (U/g wt)	Insulin (U/pancreas)	*n*	Glucose (mg/dl)	Insulin (μU/ml)
cp/cp	483 ± 15*	6	3.01 ± 1.6	2.83 ± 0.59	8	310 ± 54*	950 ± 62*
+/?	340 ± 6	6	1.37 ± 0.3	1.75 ± 0.32	6	186 ± 16	210 ± 72

Values are means ± SE. Plasma samples obtained 6–8 h into light (nonfeeding) cycle.
*$P < .05$.

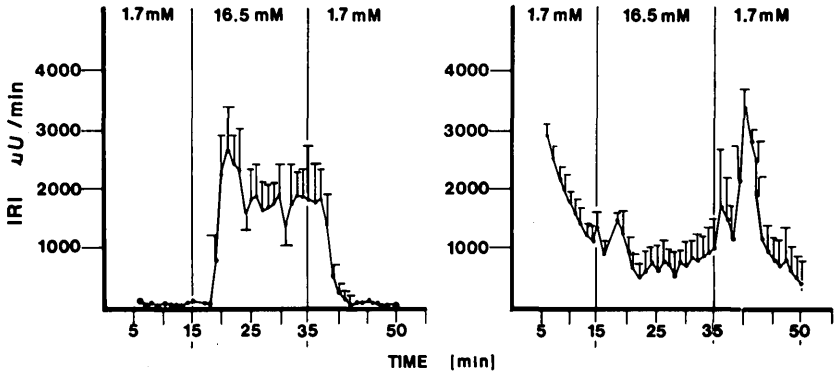

FIG. 2. Glucose-induced immunoreactive insulin (IRI) secretion in isolated perfused pancreases of 4 lean control (+/?, *left panel*), and 4 *cp/cp* (*right panel*) rats. Note hypersecretion of insulin at low glucose concentrations in *cp/cp* rats, whereas controls secrete insulin in high-glucose buffer. Values are means ± SE.

first elevated at the end of the 1st wk of diet and continued to rise progressively, reaching their highest levels by the 4th wk. Insulin levels in the *cp/cp* rats were elevated before the glucose levels became abnormal: the mean plasma insulin level was 1265 ± 81 μU/ml in the 5-wk-old rats before the start of the sucrose diet, rose progressively, and remained extremely elevated over the 8 wk of observation. Control rats after 8 wk on the sucrose diet were moderately hyperinsulinemic compared with most normal lean rats.

Table 1 compares the body weight and pancreatic insulin concentration and content in *cp/cp* rats and controls after 4 wk of diet. Plasma glucose and insulin levels in rats exposed to 6–8 h of daylight are also shown. The *cp/cp* rats were significantly heavier than their controls. Plasma glucose and insulin levels were significantly elevated in the *cp/cp* rats. The pancreatic concentration and content of insulin were both increased in the *cp/cp* rats; the differences were not statistically significant.

Sephadex gel filtration of plasma and pancreatic insulin from *cp/cp* and lean rats was carried out to determine what proportion of plasma and pancreatic insulin was made up by proinsulin. The major component of immunoassayable insulin was the normal 6000-M_r molecule in both lean and obese rats (data not shown). The considerable quantitative differences, however, did document hyperinsulinemia in the *cp/cp* rats.

Pancreas perfusions: glucose stimulus. Pancreases of *cp/cp* and lean rats were perfused with buffers with low concentrations of glucose (1.7 mM) followed by buffers with high concentrations (16.5 mM), and insulin secretion was measured. Figure 2 compares the responses of these animals. In lean rats there was a normal biphasic response to elevation of glucose and a rapid decrease in insulin release when the high-glucose stimulus was turned off. The response of the *cp/cp* rats was very different. Insulin secretion after high glucose was significantly reduced compared with that in lean controls. Lean rats released a total of 31,789 ± 3500 μU of insulin compared to 17,200 ± 1030 μU by the *cp/cp* rats. This calculation overestimates the response of the *cp/cp* rats, because the insulin level at the start of 16.5 mM glucose was 1000 μU/min and had not reached baseline. In the lean animals, the high-glucose perfusion started at insulin levels that were essentially zero.

In addition to the altered response to high glucose, the *cp/cp* rats showed a paradoxical response to the lowering of perfusate glucose levels. There was a striking increase in insulin release when the perfusate changed from 16.5 to 1.7 mM glucose. During this transition period, lean rats released 8145 ± 280 μU insulin, whereas the *cp/cp* rats released 21,030 ± 3270 μU. A similar paradoxical response of *cp/cp* rats to low glucose was also seen in the first 10 min of the perfusion. Table 2 quantifies the rates of insulin secretion during these perfusions and indicates the statistical significance of the differences between the *cp/cp* and lean rat responses.

Pancreas perfusions: arginine stimulus. To determine whether the altered insulin response to glucose in the *cp/cp* rats also occurs with other stimuli for insulin secretion, pancreases were perfused with 10 mM arginine in a buffer that contained no glucose. The results are shown in Fig. 3. Lean rats had no response to the arginine stimulus in the absence of glucose, as could be predicted from previous observations of normal rats (13). In contrast, the *cp/cp* rats responded with markedly increased insulin secretion. There was no paradoxical response to the discontinuation of the stimulus. However, as was seen with low glucose, there was increased insulin secretion in the first 10 min of perfusion with the zero-glucose buffer. Glucagon secretion in response

TABLE 2
Insulin secretion in pancreas perfusions in *cp/cp* and control rats

Perfusate glucose concentration (mM)	Total secretion (U)		Rate (μU/min)	
	cp/cp	+/?	*cp/cp*	+/?
2.7	16.59* ± 1.67	0.37 ± 0.06	1837* ± 180	41 ± 7
16.5	17.20* ± 1.03	31.79 ± 3.50	852* ± 53	1589 ± 175
2.7 after 16.5	21.03* ± 3.27	8.15 ± 0.28	1402 ± 218	543 ± 187

Values are means ± SE; n = 4 for each group of rats.
*P < .05 vs. +/? rats.

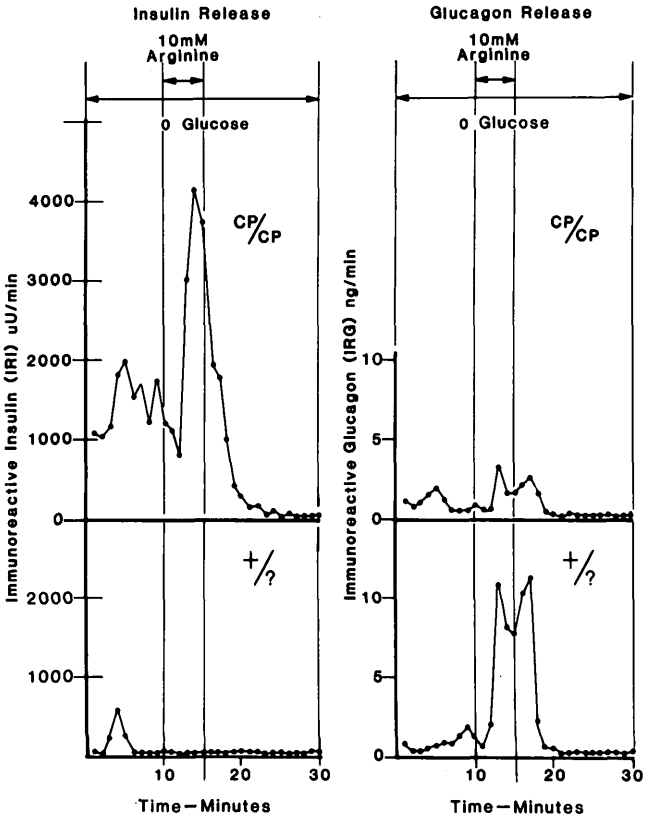

FIG. 3. Arginine-induced insulin and glucagon secretion in perfused pancreases of 2 lean (+/?) and 2 *cp/cp* rats. Note that perfusate buffer contains no glucose. In *cp/cp* rats there is large insulin response to arginine, whereas controls secrete no insulin in absence of glucose.

to arginine was the inverse of the insulin secretion. Lean rats showed significantly greater glucagon secretion compared with the *cp/cp* rats.

Pancreas perfusions: buffers without glucose. Figure 4 shows the insulin responses of the *cp/cp* and lean rats to an initial high-glucose stimulus, followed by a 45-min perfusion with buffer containing no glucose, and the subsequent insulin response to a second high-glucose stimulus. The *cp/cp* rats display an impaired response to the initial glucose stimulation. After the 45-min washout period, the *cp/cp* rats displayed a normal response to the high-glucose stimulation and a significantly increased quantitative release of insulin. Lean rats showed a normal response to the initial glucose stimulation; however, after the washout period, the response pattern did not change, although the total amount of insulin release decreased. The paradoxical insulin responses of the *cp/cp* rats to the switch from high to low glucose still persisted. Table 3 quantifies some of these data. There was no difference in the glucose content of the washout buffer between the *cp/cp* and lean rats when tested after perfusion. A similar study in a pair of *cp/cp* and control rats fed sucrose diets for 8 wk revealed responses identical to those described in rats fed sucrose diets for only 4 wk.

Effects of fasting on pancreas perfusions. The rats used in these studies were on sucrose diets for 8 wk. After an overnight fast, two pairs of *cp/cp* and control rats were perfused as in Fig. 2. There was no amelioration of the insulin secretory impairment or of the paradoxical response to low glucose in the initial 10 min.

Attempt at heterozygote identification. Because *cp/cp* rats have such elevated plasma insulin levels and such markedly abnormal insulin responses to glucose, it seems possible that the lean (*cp/+*) heterozygotes might be recognized by elevations of plasma insulin or by altered insulin secretory patterns to glucose. Twenty lean animals were examined for fasting insulin levels, and their pancreases were perfused with low and high concentrations of glucose by the method shown in Fig. 2. The mean fasting insulin level was 65 ± 22 μU/ml. Examination of the individual perfusions revealed no instances of impaired insulin responses, although several showed high levels of insulin in the initial low-glucose perfusion. There were no correlations between plasma insulin levels and perfusion responses in the low- or high-glucose periods. None of the *r* values were significant (Figs. 5 and 6).

Pancreas perfusion in genetically obese nondiabetic rats. Obese and control animals were fed sucrose diets, and after 4 wk, they were studied to determine their pancreatic responses to glucose stimuli. The data obtained in two pairs of animals are shown in Fig. 7. It is clear that the response to high glucose is normal. On the other hand, the release of insulin at low glucose is obviously greater than normal, and the time required to shut off all secretion at the end of the glucose stimulation is 10 min, compared to 4 min for the controls.

DISCUSSION

The SHR/N-*cp* rats provide a new genetic model for obesity and non-insulin-dependent diabetes (1,2). Although many aspects of this model remain unexplored, it is clear that after 1–2 wk on a high-sucrose diet, the diabetic syndrome is firmly established. Our major findings were obtained with perfused isolated pancreases of the *cp/cp* rats and their lean nondiabetic controls. Perfusions conducted after 4 wk of diet revealed that the insulin response to elevated levels of glucose (16.5 mM) was markedly impaired in the diabetic animals. Insulin release was reduced by 53% compared with control rats. However, at the cessation of the glucose stimulus, a paradoxical rise in insulin secretion occurred. Again, paradoxically, insulin secretion was greatest at the low glucose levels that initiated the perfusions. Furthermore, *cp/cp* rats secreted large amounts of insulin in response to arginine, despite the absence of any glucose in the buffer,

TABLE 3
Effects of glucose-free washout period on glucose-stimulated insulin secretion

Time (min)	Perfusate glucose concentration (mM)	Insulin secretion (μU/min)	
		cp/cp	+/?
0–10	2.7	2438 ± 324	206 ± 51
10–25	16.5	930 ± 129	1959 ± 313
25–70	0	655 ± 135	149 ± 35
70–90	16.5	1668 ± 128*	575 ± 76*
90–100	0	1878 ± 143	345 ± 78

Values are means ± SE; $n = 6$ for *cp/cp* and $n = 5$ for +/? rats. *$P < .05$ for response to 16.5 mM glucose before and after no glucose in same perfusions.

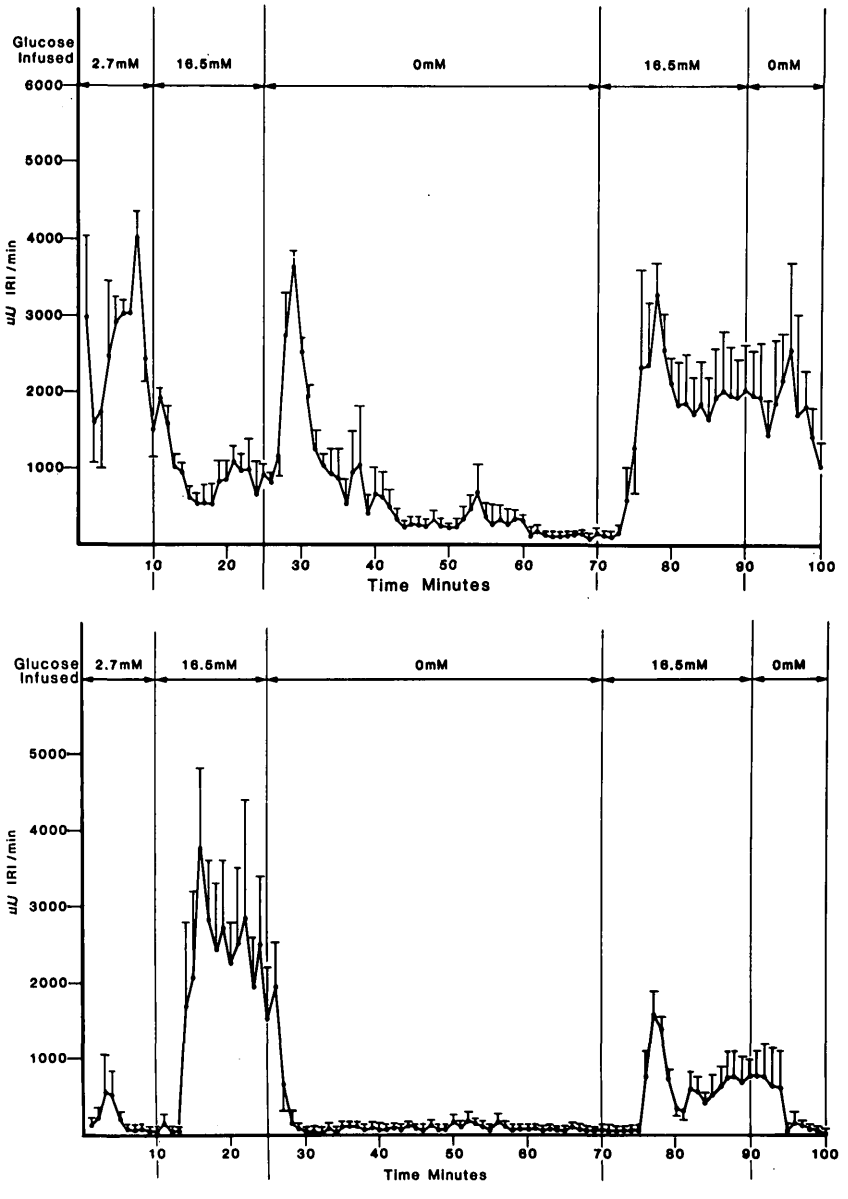

FIG. 4. Effect of perfusion of pancreases from *cp/cp* (*top*) and lean (+/?; *bottom*) rats with buffer containing no glucose. Initial response to high glucose compared with response to high glucose after washout period. Note return of normal response to high glucose in *cp/cp* rats after washout period. Values are means ± SE; *n* = 6 for each group.

whereas lean rats had no insulin response but a greater glucagon release under these conditions.

The glucagon and insulin responses of the *cp/cp* rat to arginine in the absence of glucose suggest that the islet cells are functioning in a manner consistent with a glucose-primed state. However, the altered insulin responses to glucose, both the decreased and the paradoxical, are more difficult to explain. Our findings are similar to those reported in two different nongenetic models of non-insulin-dependent diabetes, i.e., rats receiving streptozocin as neonates (14) and those with partial pancreatectomy (15), and are essentially identical to those in which normal rats were infused for 48–96 h with concentrated glucose solutions (16,17). In the latter studies, it was demonstrated that sustained hyperglycemia at a level of 200 mg/dl for at least 48 h, accompanied by continuous hyperstimulation of the islets by glucose infusions, was able to produce these alterations in insulin secretion. These changes in islet function persisted despite the fact that the plasma glucose had returned to <200 mg/dl after 48 h. Moreover, use of phloridzin during the last 2 days of the glucose infusion resulted in lowering of the plasma sugar and total normalization of the glucose-induced insulin response as measured in a perfused pancreas system. Leahy et al. (17) interpret these observations to support the concept that elevations of plasma glucose impair the function of the islets. They further suggest that the reversibility of the defect may be related to the relatively short period of hyperglycemia.

In our *cp/cp* rats, fed high-sucrose diets ad libitum, significant levels of hyperglycemia existed for >4 wk, yet almost total reversibility of the lesion occurred when the pancreas was perfused with buffer containing no glucose for 45-min periods. The insulin response to high glucose increased, and the normal biphasic pattern of response was restored. The paradoxical insulin release that takes place during the transition from a glucose-rich buffer to a glucose-free buffer still persisted but was somewhat attenuated. It is reasonable to assume that continued chronic stimulation of the β-cells by the elevated levels of plasma glucose had existed, as had extreme hyperinsulinemia.

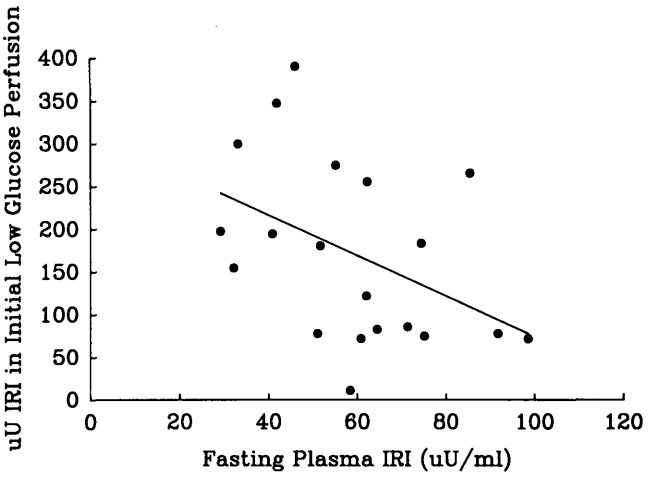

FIG. 5. Attempt to identify heterozygotes in lean animals. Fasting plasma immunoreactive insulin (IRI) in 18 rats plotted against total insulin secreted in 1st 10 min of pancreas perfusion (low-glucose stimulus). Perfusions performed as in Fig. 2. $r = -.432$, $P = .1$ (NS).

One possible explanation for the near normalization by glucose-free buffer is that an inhibitory substance released in high glucose is washed out. This substance does not appear to be free glucose. There is, however, considerable insulin released during the washout period in the *cp/cp* rats. The effect of insulin as an autocrine inhibitor of secretion is controversial but possible (18), although neonatal streptozocin-injected rats are significantly insulin deficient but can also be partially normalized by washout (19). Somatostatin could be this inhibitor. We have reported that pancreatic somatostatin content is threefold higher in *cp/cp* rats than in lean controls (3), and chemically diabetic animals also have increased levels of this δ-cell peptide (20). Unfortunately, we were unable to measure somatostatin in rat perfusion buffers.

Another, perhaps more likely, possibility we are considering is that chronic exposure of the islets to hyperglycemia may result in downregulation of the β-cell glucose transporters with upregulation or recycling of transporters by the washout procedure. A recent report by Kahn et al. (21) demonstrated that lowering plasma glucose levels with phloridzin in pancreatectomized rats resulted in a marked increase in glucose transporter activity in adipocytes. Thus, it appears that the glucose moiety (apparently independent of insulin) may be involved in the regulation of the number of glucose transporters in some tissues.

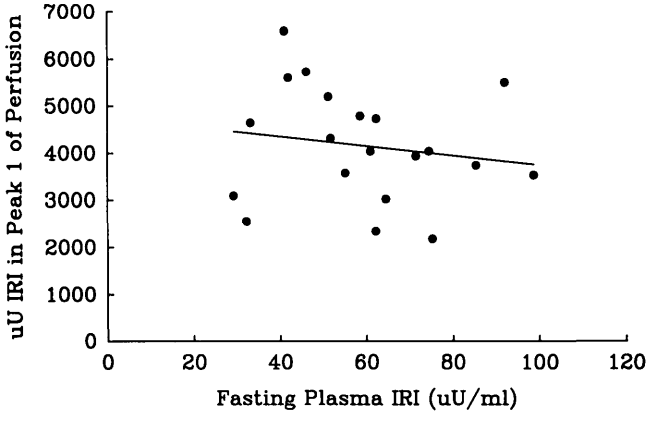

FIG. 6. Attempt to identify heterozygotes in lean animals. Fasting plasma immunoreactive insulin (IRI) in 20 rats plotted against total insulin secreted in 1st peak after high-glucose stimulus (16.5 mM glucose). Perfusions performed as in Fig. 2. $r = -.134$, P = NS.

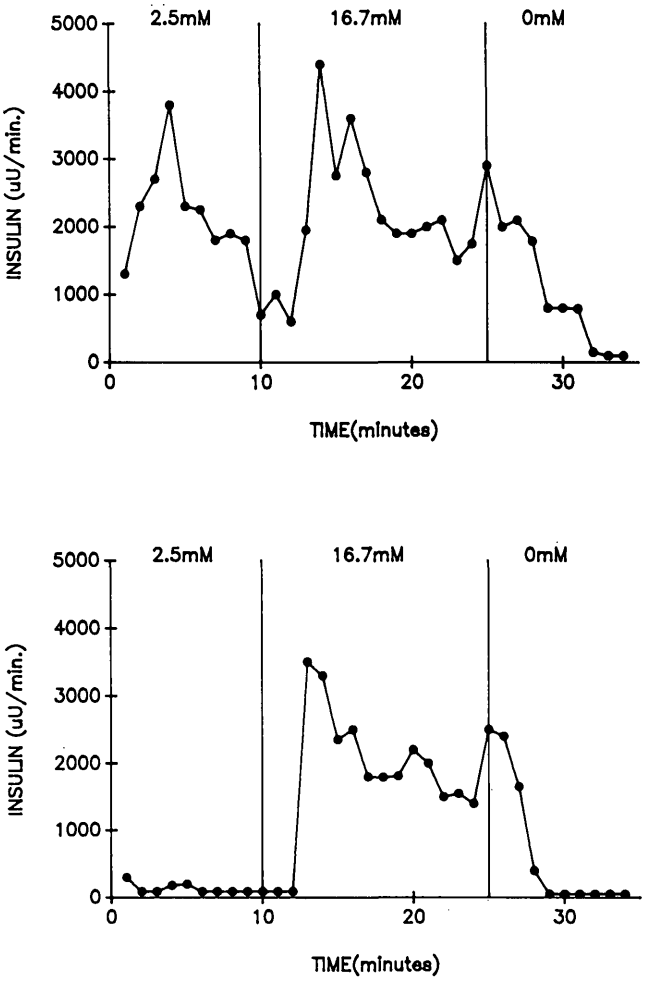

FIG. 7. Pancreas perfusions (as in Fig. 2) in 2 obese nondiabetic La/N-*cp* rats (*top*) and 2 lean controls (*bottom*). Note that responses to high glucose are normal in obese and lean rats. However, obese rats show increased insulin secretion at low glucose levels and slow decline of insulin secretion when high-glucose buffer is switched to low glucose.

The failure of fasting to reverse the insulin secretory abnormalities in our *cp/cp* rats was somewhat unexpected. It does not, however, rule out the role of plasma glucose in the development of these abnormalities. Perhaps a considerably longer period of normal plasma glucose levels (presumably occurring with fasting) may be required to provide the equivalent of washout of islets. It is pertinent to note that two different investigations have demonstrated a loss of ability of isolated islets to respond to high glucose concentrations after prolonged in vitro exposure to high glucose (22,23). Such desensitization is compatible with our findings and those of Leahy and Weir (14) but does not elucidate the mechanisms involved, except that because the phenomenon occurs with nonrecirculated perifusions of islets, a role for extraislet metabolites or hormones may be excluded.

Despite the markedly impaired in vitro insulin secretory

response to glucose of the perfused *cp/cp* rat pancreas, the in vivo response to ingestion of glucose was different. In the 30–60 min after oral glucose, *cp/cp* rats had significant increases in plasma insulin compared with lean controls (2). It seems likely that enteric factors, neural and/or humoral, were able to overcome the secretory impairment.

We were not able to identify *cp/+* heterozygotes by plasma insulin levels or pancreas perfusion responses to glucose. This contrasts with the clear differences found in Zucker rat heterozygotes, which are hyperinsulinemic, compared with lean homozygotes (24). The obese Zucker rats also showed more evidence of insulin hypersecretion in perfused pancreases than the *cp/cp* rats, despite the far greater plasma levels of insulin in the *cp/cp* rats (6). It is evident that studies of insulin degradation and plasma clearance must be carried out in the *cp/cp* rat, because lowered rates of clearance could explain the extremely high plasma insulin levels.

Note also that the excessive insulin secretion occurring at low glucose levels and the paradoxical shut-off response could also contribute to the hyperinsulinemia of the *cp/cp* rats, especially because fasting plasma glucose levels appear not to be elevated (2,3). Although Curry and Stern (6) found that the obese nondiabetic Zucker rat secreted insulin at lower glucose levels than normal, there was no failure of insulin response to elevated levels of glucose. Our preliminary studies with obese nondiabetic LA/N-*cp* rats, which were perfused exactly as our *cp/cp* rats, indicate that they also have no impairment in insulin response to high glucose levels. These data support the concept that significant levels of hyperglycemia may be necessary to produce the impaired insulin response to high glucose levels.

In conclusion, the SHR/N-*cp/cp* rat is a genetically diabetic obese rat that shows alterations in pancreatic function that resemble those induced by prolonged (48–96 h) glucose infusion in normal rats. It seems likely that the chronic elevation in plasma glucose may be responsible for the functional impairment. It is obvious that such a self-perpetuating phenomenon has important implications for the therapeutic approach to diabetes.

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