Comparison of Insulin Secretory Patterns in Obese Nondiabetic LA/N-*cp* and Obese Diabetic SHR/N-*cp* Rats

Role of Hyperglycemia

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Obese diabetic SHR/N-(*cp*/*cp*) rats are a genetic model for non-insulin-dependent diabetes mellitus. When SHR/N-*cp* rats are overtly diabetic, they are hyperinsulinemic and hyperglycemic in the fed state when consuming commercial chow or semipurified high-carbohydrate diets. Obese SHR/N-*cp* rats were hyperinsulinemic by 4 wk of age, although hyperglycemia did not appear until 3–4 wk later and was exacerbated by a high-sucrose diet (mean ± SE 1488 ± 238 μU/ml insulin and 425 ± 51 mg/dl glucose). The control SHR/N-*cp* rats (+/?) on the sucrose diet remained lean and normoglycemic. The obese diabetic SHR/N-*cp* rats showed three alterations in pancreas perfusion data (not present in control rats): *1*) paradoxically high insulin secretion at low glucose levels (2.5 mM), 2) secretion of insulin in response to arginine (10 mM) in the absence of glucose, and *3*) impaired response of insulin secretion to high glucose (16.7 mM). To determine whether hyperglycemia was responsible for the abnormalities of insulin secretion, perfusion studies were conducted in obese nondiabetic LA/N-*cp* rats and compared with the SHR/N-*cp* rats. The obese LA/N-*cp* rats resembled the corpulent SHR/N-*cp* rats in every way, except that they were normoglycemic on the sucrose diet. The obese LA/N-*cp* rats had two of the three alterations in insulin secretion shown by obese SHR/N-*cp* rats, lacking only the impaired response to high glucose, suggesting that hyperglycemia was required for that defect to occur. This finding is supported by the partial reversal of this defect in the obese SHR/N-*cp* rats after fasting for 48–72 h, when plasma glucose levels had been close to normal for >24–48 h. Additional evidence was obtained in younger, prediabetic obese SHR/N-*cp* rats that also showed the first two of the three defects and showed only a slightly abnormal response to high glucose. Paradoxically, high insulin secretion at low glucose levels and hypersecretion of insulin in response to arginine without glucose are early abnormalities associated with the development of obesity in these models, whereas decreased maximal response to high glucose develops later and is associated with hyperglycemia. *Diabetes* 38:691–97, 1989

Arginine	1 μM = 0.174 mg/dl	Glucose	1 mM = 18 mg/dl
Insulin	1 pM = 0.139 μU/ml		

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The obese SHR/N-*cp* rat (*cp*/*cp*) is an inbred genetic model of non-insulin-dependent diabetes (1). The diabetic syndrome is characterized by progressively increasing obesity and extreme hyperinsulinemia, both manifested 3–4 wk before the appearance of sustained hyperglycemia. When the animals were fed commercial rat chow or a semipurified diet containing 54% sucrose, which exacerbates hyperglycemia in several rodent models, blood glucose levels in the fed state reached >400 mg/dl (2,3), although they decreased to ≤200 mg/dl after an overnight fast. Using an in vitro isolated-pancreas perfusion technique, we found that pancreatic insulin secretion failed to increase in response to elevated concentrations of glucose (16.7 mM), whereas a paradoxical release of insulin occurred in response to low concentrations of glucose (0–5 mM) (2). In addition, the diabetic rats secreted insulin in response to arginine (10 mM) administered in a glucose-free buffer, although lean control rats (+/?) failed to secrete insulin under those conditions. We also demonstrated that perfusion of the diabetic pancreas for 45 min with buffer containing no glucose resulted in normalization of the insulin response to high glucose without affecting the paradoxical insulin response to low glucose (2).

Because recent reports have implicated hyperglycemia in the pathogenesis of similar insulin secretory abnormalities (4–7), we wanted to determine whether normalization of

plasma glucose in obese diabetic SHR/N-*cp* rats would reverse these alterations in insulin response to glucose and arginine. To achieve this, rats were fasted for 48–72 h, and insulin responses were measured subsequently in pancreas perfusions.

The availability of the obese LA/N-*cp* rat (8,9), a genetically inbred strain of an obese hyperinsulinemic but nondiabetic model with the same *cp* allele as the SHR/N-*cp* rat, provided an additional basis for evaluation of the effect of hyperglycemia on pancreatic islet function. This nonhyperglycemic animal could be compared with the diabetic rat. The obese LA/N-*cp* animals (*cp*/*cp*), when fed the same high-sucrose diet as our obese SHR/N-*cp* rats, did not become diabetic.

Finally, younger obese SHR/N-*cp* rats were studied before the development of hyperglycemia but after the appearance of hyperinsulinemia and obesity. These rats were labeled *prediabetic*.

MATERIALS AND METHODS

Corpulent diabetic SHR/N-*cp* and corpulent LA/N-*cp* nondiabetic rats and their lean littermates (+/?) were obtained from C. Hansen (National Institutes of Health, Bethesda, MD). The development of obesity in SHR/N (spontaneously hypertensive rat, NIH inbred strain) and LA/N rats (Albany crossed with a hooded rat, NIH inbred strain), requires homozygosity of the *cp* allele. The SHR/N-*cp* strain was obtained by crossing an SHR/N rat with an obese Koletsky rat, followed by extensive backcrossing to SHR/N to achieve congenicity (9,10). The LA/N-*cp* strain was obtained similarly. Genetic studies indicate that the Koletsky *f* (designated *cp* by Hansen) and *fa* are allelic, although these two independent mutations may not be identical (11).

At the age of 4–5 wk, all animals were placed on diets containing 54% carbohydrate as sucrose and 10% casein, 10% lactalbumin, 5.99% cellulose, 4% lard, 4% corn oil, 4% beef tallow, 4% coconut oil, 3.1% AIN salt mix (American Institute of Nutrition, Rockville, MD), and 1% vitamin mix (8). Animals were studied after 8 days to 10 wk on the diet.

Body weights and fed and fasted plasma glucose and insulin levels in the rats were recorded weekly and at the time the rats were killed. In certain experiments, obese and lean control SHR/N-*cp* and LA/N-*cp* rats were fasted for 48–72 h. Water was unrestricted. Fed and fasted animals were then subjected to pancreas perfusions as previously described (2,4). Open nonrecirculating perfusions of the pancreas were used with Krebs bicarbonate buffer (pH 7.4) containing 3% dextran T 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 essentially constant over the course of the perfusions and declining minimally by 100 min. Each perfusion included an initial 10- to 15-min period of low glucose levels.

Portal vein effluent was collected in 1-min fractions into chilled tubes containing 10.5 mg Na EDTA and 1000 KIU Trasylol (FBA, New York). All tubes were rapidly centrifuged to remove any erythrocytes that might be present, and the supernatants were frozen for radioimmunoassays of insulin and glucagon. Insulin was measured with a double-antibody procedure (12). Rat insulin standards were obtained from Lilly (Indianapolis, IN). Glucagon was measured with the G15 antibody (J. Jaspan, Univ. of Chicago, Chicago, IL) by the method of Unger et al. (13). Plasma levels of glucose were measured by a glucose oxidase procedure (Sigma).

Statistical significance was assessed with Student's two-tailed *t* test.

RESULTS

For a comparison of body weights and plasma glucose and insulin levels in the obese and lean LA/N-*cp* and SHR/N-*cp* rats, see Table 1. All obese rats were significantly heavier than control rats. In the fed state, obese rats showed significantly increased levels of plasma insulin when compared with lean control rats. Obese diabetic SHR/N-*cp* rats had significantly higher levels of plasma insulin and glucose than the obese LA/N-*cp* and lean control rats.

After a 72-h fast, plasma glucose levels in the obese SHR/N-*cp* rats had decreased by 48%, and insulin levels had decreased by 85%. Although plasma glucose levels were close to 200 mg/dl after the 72-h fast, at no time were the levels as low as those in the obese LA/N-*cp* or the lean control rats. Furthermore, insulin levels after fasting remained higher in all obese rats compared with their respective control rats.

PANCREAS PERFUSIONS IN FED RATS: RESPONSE TO GLUCOSE

LA/N-*cp* rats. For the mean insulin secretory responses of seven obese and seven lean rats as measured in isolated perfusions in which the pancreas was exposed first to 2.5 mM glucose, then to 16.7 mM glucose, and subsequently to glucose-free buffer, see Fig. 1. The patterns of secretion

TABLE 1
Body weight, plasma glucose, and insulin values in control and obese rats

	SHR/N-*cp*		LA/N-*cp*	
	Means ± SE	*n*	Means ± SE	*n*
Body weight (g)				
Control	387 ± 8	11	274 ± 12	11
Obese	483 ± 13	11	464 ± 17	17
Fed plasma				
Glucose (mg/dl)				
Control	174 ± 11	8	163 ± 9	11
Obese	425 ± 51	9	159 ± 9	17
Insulin (μU/ml)				
Control	258 ± 71		198 ± 21	
Obese	1488 ± 238		856 ± 102	
Fasted 48–72 h				
Glucose (mg/dl)				
Control	146 ± 12	4	89 ± 17	3
Obese*	235 ± 19	6	134 ± 3	3
Insulin (μU/ml)				
Control	100 ± 49		37 ± 9	
Obese	192 ± 26		193 ± 18	

Values are means ± SE for *n* rats. Sample sizes for insulin are same as for glucose. Bracketed comparisons are significantly different at $P < .05$.
*Of 6 obese rats, 4 were fasted for 48 h, and 2 were fasted for 72 h. Control rats for obese diabetic rats were fasted for 48 h.

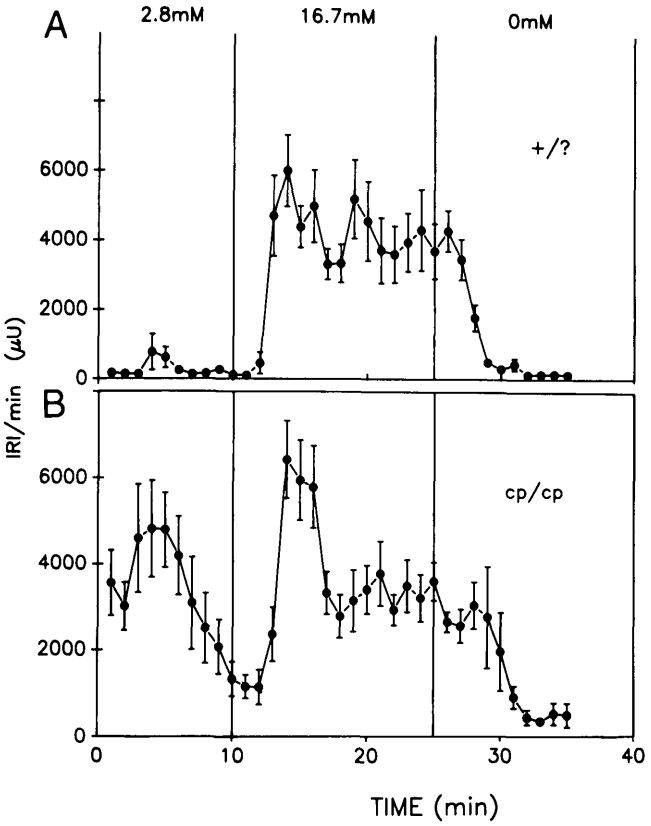

FIG. 1. Isolated-pancreas perfusions in 7 fed lean (+/?) control (*A*) and 7 obese (*cp/cp*) nondiabetic LA/N-*cp* (*B*) rats. Results are means ± SE for immunoreactive insulin (IRI) secretion in response to sequence of 3 glucose concentrations in perfusate (2.8, 16.7, and 0 mM glucose). At 2.8 mM glucose, obese rats secreted significantly more IRI than lean rats ($P < .001$).

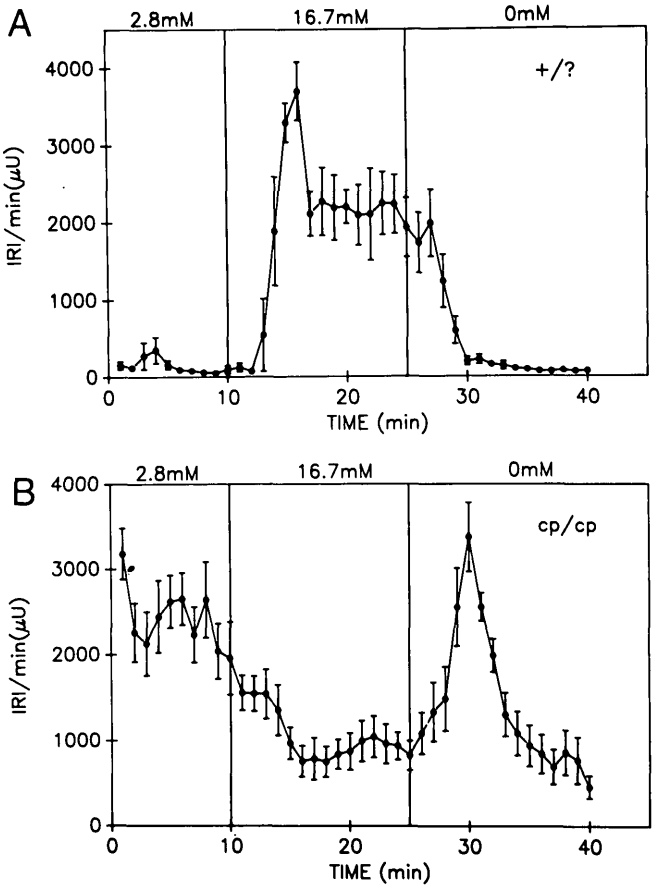

FIG. 2. Isolated-pancreas perfusions in 9 fed lean (+/?) control (*A*) and 9 obese (*cp/cp*) diabetic SHR/N-*cp* (*B*) rats. Results are means ± SE for immunoreactive insulin (IRI) secretion in response to sequence of 3 glucose concentrations in perfusate (2.8, 16.7, and 0 mM glucose). Rats received sucrose diet for periods of 4–9.5 wk. Lean responses differ significantly from responses of obese diabetic SHR/N-*cp* rats. $P < .001$ at 0 and 2.8 mM glucose; $P < .05$ at 16.7 mM glucose.

differed between the lean and obese rats. The obese rats showed a paradoxical response to low concentrations of glucose, with secretion of 33.9 mU of insulin in 10 min at 2.5 mM glucose, whereas controls secreted 2.8 mU of insulin. The response of the obese rats to high concentrations of glucose was biphasic and similar in pattern and quantitative secretion (34.9 mU in 15 min) to that in the lean controls (37.5 mU insulin). Mean insulin secretion rates in microunits per minute are shown in Table 2.

SHR/N-*cp* rats. For the results of pancreas perfusions with high and low concentrations of glucose in nine diabetic rats and 9 lean control rats, see Fig. 2. These obese diabetic

TABLE 2
Effect of fasting on insulin secretion from perfused pancreases of nondiabetic obese LA/N-*cp* and control rats

	Glucose concentration (mM)		
	2.5	16.7	0
Fed			
Control	280 ± 68*	3753 ± 397*	1136 ± 461
	$P < .001$		
Obese	3393 ± 362	3499 ± 385	1571 ± 336
	$P < .01$	$P < .01$	$P < .01$
Fasted 48 h	$P < .01$	$P < .001$	
Control	37 ± 8†	360 ± 117†	150 ± 55
	$P < .05$	$P < .01$	
Obese	1240 ± 310	1258 ± 227	246 ± 48

Values are means ± SE for fed (n = 7) and 48-h fasted (n = 3) rats. Mean rates of secretion (μU/min) for each of 3 glucose periods during pancreas perfusion are shown.
*$P < .001$ for values on same row.
†$P < .05$ for values on same row.

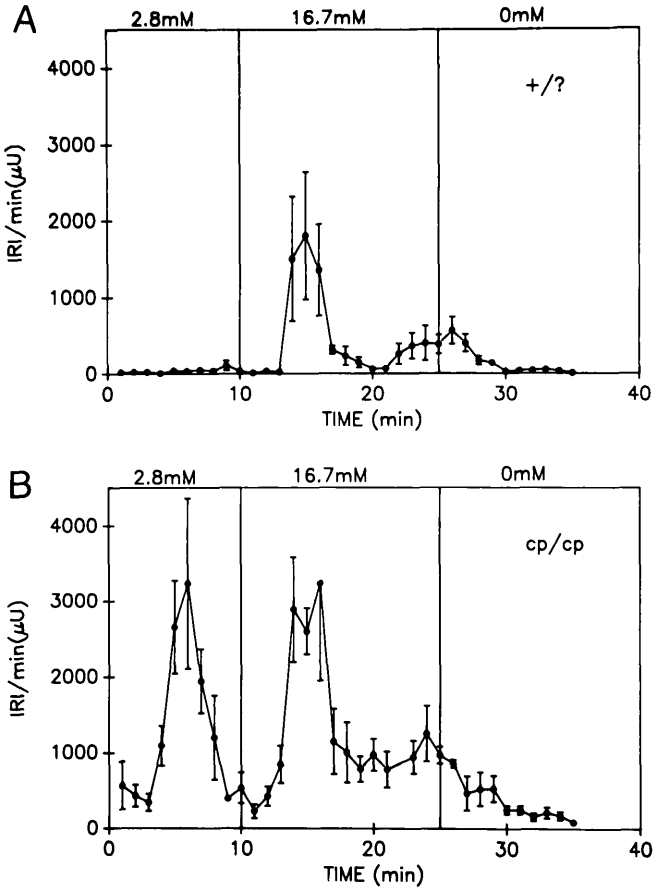

FIG. 3. Pancreas perfusions in obese (*cp/cp*) nondiabetic LA/N-*cp* rats fasted for 48 h. *n* = 3 for lean (+/?) rats (*A*); *n* = 3 for obese (*cp/cp*) rats (*B*). Obese rats secreted significantly more immunoreactive insulin (IRI) than lean control rats at 2.8 and 16.7 mM glucose ($P < .05$).

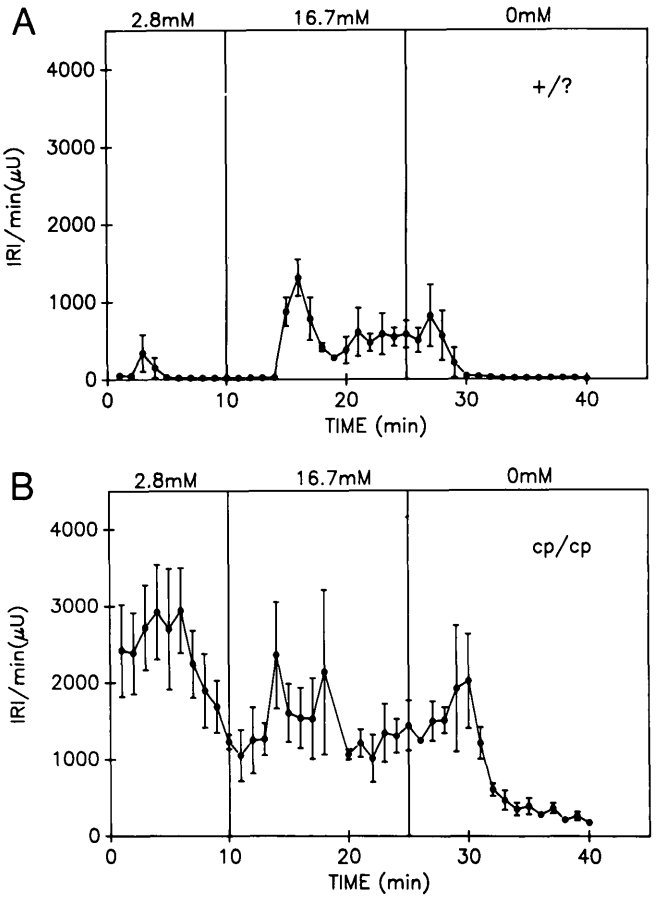

FIG. 4. Pancreas perfusions in lean (+/?) control (*A*) and obese (*cp/cp*) SHR/N-*cp* (*B*) rats fasted for 48–72 h. Lean controls represent mean of 3 animals fasted 48 h, whereas obese diabetic rats represent 2 rats fasted 48 h and 2 rats fasted 72 h. Significant differences persisted between lean and obese diabetic rats at all glucose concentrations ($P < .001$ at 2.8; $P < .001$ at 16.7; $P < .05$ at 0 mM glucose). Note difference in pattern of fasted cp/cp rats at 16.7 mM glucose compared with fed rats in Fig. 2. IRI, immunoreactive insulin.

rats had been on sucrose diets for 4–9.5 wk. Marked secretion of insulin at 2.5 and 0 mM glucose is seen in pancreases from the *cp/cp* rats. This pattern is similar to that observed in the obese LA/N-*cp* rats, although the obese diabetic SHR/N-*cp* rats show more clearly defined peaks in insulin secretion when the glucose level in the perfusate is reduced to 0 mM.

The major difference between the two strains of obese rats is in the response to 16.7 mM glucose (Figs. 1 and 2). The obese diabetic SHR/N-*cp* rats, as we have previously reported (2), showed no response or a decreased response to 16.7 mM glucose and no defined biphasic pattern, although the obese nondiabetic LA/N-*cp* rats had a normal biphasic pattern of response (Fig. 1).

TABLE 3
Effect of fasting on insulin secretion from perfused pancreases of diabetic obese SHR/N-*cp* and control rats

		Glucose concentration (mM)		
	n	2.5	16.7	0
Fed				
Control	9	143 ± 45*	1995 ± 296*	455 ± 85
		$P < .001$	$P < .05$	$P < .001$
Obese	9	2333 ± 243*	1086 ± 201*	1340 ± 97
			$P < .001$	
Fasted 48–72 h				
Control	3	94 ± 20*	491 ± 91*	419 ± 197
		$P < .001$	$P < .01$	$P < .05$
Obese†	4	2104 ± 67*	1369 ± 141*	1121 ± 138

Values are means ± SE. Mean rates of secretion (μU/min) for each of 3 glucose periods during pancreas perfusion are shown.
*$P < .001$ for values on same row.
†Includes 2 rats fasted for 48 h and 2 rats fasted for 72 h.

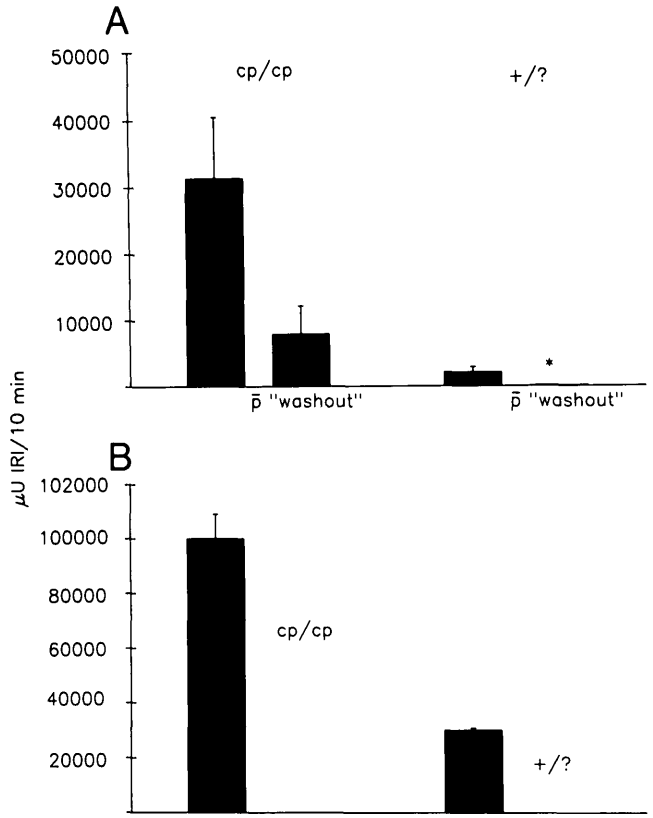

FIG. 5. Immunoreactive insulin (IRI) response of fed obese (*cp/cp*) and lean (+/?) nondiabetic LA/N-*cp* rats to 10 mM arginine without and with glucose during pancreas perfusion. *n* = 4 for glucose-free studies (*A*); *n* = 3 for 5.5 mM-glucose studies (*B*). Responses of obese animals were significantly greater under all conditions than control (+/?) responses ($P < .05$). Washout of perfusion with glucose-free buffer for 45 min before arginine stimulation decreased IRI responses in obese animals ($P < .05$). *Total absence of IRI secretion.

PANCREAS PERFUSIONS IN FASTED RATS

LA/N-*cp* rats. The effect of 48 h of fasting is shown in Fig. 3. Compared with the pattern in fed rats, the total amount of insulin secreted in response to glucose stimulation was significantly decreased (Table 2). In some of the lean animals after 48 h of fasting, insulin secretion was unmeasurable. In the obese animals, despite the reduction in secretion, paradoxical secretion at low glucose concentrations persisted (i.e., insulin secretion rates at 2.5 and 16.7 mM glucose were equivalent).

SHR/N-*cp* rats. Studies of fasted obese rats (Fig. 4) revealed that the pattern of insulin response to high glucose concentrations was changed in the direction of normal; a distinct first-phase insulin peak and possibly a second phase were found. However, there was no ablation of the paradoxical insulin secretion in low glucose even after 72 h of fasting. Although there was a significant reduction in insulin response to high glucose in the lean rats after the 48-h fast, the obese rats were resistant to the effects of fasting, with no significant reduction in the rate of insulin released in low or high glucose (Table 3).

RESPONSE TO ARGININE

LA/N-*cp* rats. The insulin responses to 10 mM arginine were compared in lean and obese LA/N-*cp* rats (Fig. 5). Without glucose, the obese rats responded to arginine by secreting large quantities of insulin, whereas the lean animals (as is true of normal lean rats) did not respond. Increasing the buffer glucose concentration to 5 mM intensified the insulin response of the obese rats to arginine, resulting in a biphasic response, whereas the lean rats also showed a small insulin response. The glucagon responses to arginine were similar in the obese and lean animals (7.42 ± 1.2 vs. 5.29 ± 1.0 ng/min, respectively).

The insulin response to arginine of the obese LA/N-*cp* rats was markedly attenuated by a prolonged (45-min) washout period with glucose-free buffer (Fig. 5). This washout period was intended to allow depletion of intracellular metabolites or reversal of other intracellular processes that were presumably altered by prolonged in vivo exposure to high glucose in the diabetic rats or to an overabundance of fuels, as in the obese LA/N-*cp* rats.

SHR/N-*cp* rats. During a 15-min exposure to arginine without glucose, obese diabetic SHR/N-*cp* rats secreted 2559 ± 356 μU/min of insulin, whereas lean control rats showed no response ($P < .01$), supporting our previous report (2). Glucagon secretion during this period was 8.28 ± 1.06 ng/min for obese and 10.77 ± 1.41 ng/min for lean animals (NS). Fasting for 72 h did not decrease the insulin response to arginine (2042 μU/ml in 2 rats).

PREDIABETIC ANIMALS: RESPONSES TO GLUCOSE

Because we observed that obese SHR/N-*cp* rats became hyperglycemic only after 3–4 wk on the sucrose diet, although they were already hyperinsulinemic at weaning, we decided to perfuse the rats during this relatively nonhyperglycemic (prediabetic) phase. After 8–17 days on the diet, plasma glucose levels were 228 ± 52 mg/dl in obese rats and 142 ± 21 mg/dl in the lean controls. Plasma immunoreactive insulin levels were 886 ± 73 and 315 ± 20 μU/ml, respectively. For the insulin responses to glucose in four young obese and four young lean controls, see Fig. 6. Obese animals showed biphasic insulin responses to 16.7 mM glucose. However, they already showed paradoxically high rates of insulin secretion in 0 or 2.5 mM glucose and could easily be identified as obese animals by that pattern. In two of the young obese rats studied, a significant insulin response to arginine was seen without glucose; this response was not seen in the two lean controls perfused at the same time (data not shown).

DISCUSSION

There may be three separate but probably related insulin secretory alterations in the SHR/N-*cp/cp* diabetic rat: *1*) the paradoxically elevated secretion in 0 or 2.5 mM glucose, *2*) the failure to respond to 16.7 mM glucose, and *3*) the responsiveness to arginine without glucose (in contrast to the normal requirement for glucose). To determine the role of hyperglycemia in the production of these insulin secretory abnormalities, we used pancreas perfusions to compare obese diabetic SHR/N-*cp* and obese nondiabetic LA/N-*cp* rats. Additional approaches included the study of younger, prediabetic obese rats before the development of hyperglycemia; the study of fasted rats, which have more normal blood glucose levels; and the use of a prolonged glucose-free washout of the perfused pancreas. The latter treatment was expected to deplete intracellular metabolites of glucose and to permit reversal of other possible intracellular effects of sustained hyperglycemia.

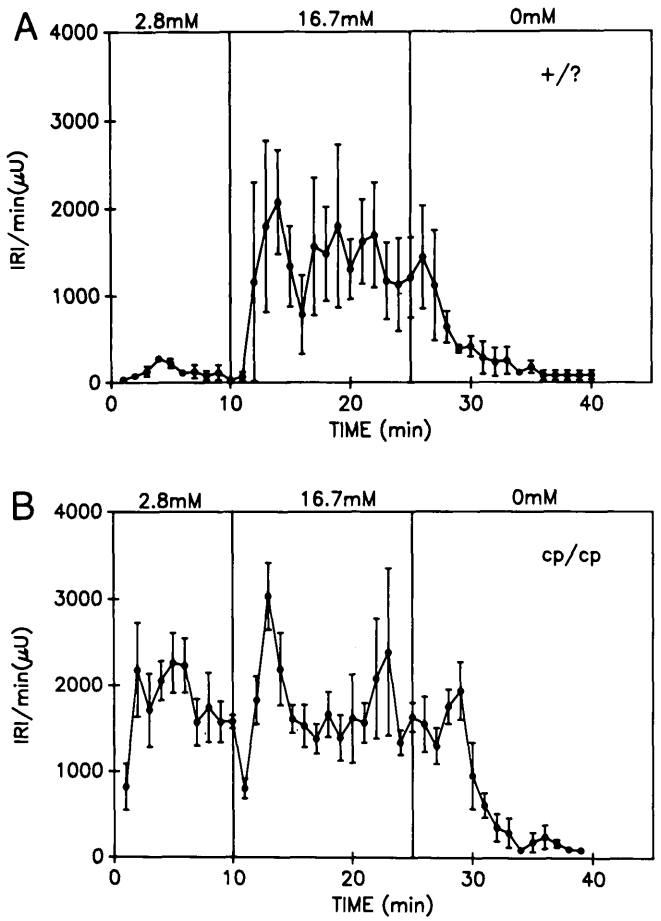

FIG. 6. Pancreas perfusions in young obese prediabetic SHR/N-*cp* rats fed sucrose diets for 8–17 days. *n* = 4 for lean (+/?) rats (*A*); *n* = 4 for obese *cp*/*cp* rats (*B*). Prediabetic rats secreted significantly more immunoreactive insulin (IRI) at 0 ($P < .05$) and 2.8 mM ($P < .01$) glucose than did lean control rats. Pattern and IRI levels secreted at 16.7 mM glucose were not significantly different from lean animals.

Thus far, we have observed two of the three defects in obese LA/N-*cp* animals as well as in younger, obese SHR/N-*cp* rats. In both cases, the third defect, namely the failure to respond to high glucose, was absent. The defective response to high glucose seen in older obese diabetic animals was completely reversed by in vitro washout (2) and was partially reversed by 48–72 h of fasting (Figs. 2 and 4), when plasma levels of glucose were close to normal. Thus, the evidence shows a close association of hyperglycemia with the failure of perfused pancreas to respond to high glucose.

None of the conditions tried (fasting, washout [2], or use of younger, prediabetic animals) was able to reverse the paradoxical insulin secretion in low glucose. The hyperresponse to arginine could be diminished but not totally normalized by glucose-free–buffer washout in vitro, and it persisted in 72-h-fasted obese diabetic SHR/N-*cp* rats. Thus, persistent hyperglycemia was not necessary to maintain these two defects in insulin secretion, and these defects were present in nonhyperglycemic, younger animals.

To understand the mechanism by which these phenomena develop, note that Leahy et al. (4–7) first demonstrated changes in insulin secretion in normal rats infused with 50% glucose for 48–96 h that were identical to the changes that we subsequently described in our diabetic rats (2). With phloridzin added to the glucose infusions, Leahy et al. were able to reduce the plasma glucose levels to normal and to completely reverse the paradoxical secretion of insulin at low glucose and the impaired insulin response at high glucose. However, phloridzin may act directly on the islets to reduce the rate of insulin secretion (14). Although they considered that the secretory defects were probably caused by hyperglycemia, they could not rule out the effect of excessive stimulation of insulin secretion.

In this study, the fasted obese diabetic SHR/N-*cp* rats showed an improvement in the response to high glucose, despite continued hypersecretion of insulin and paradoxical secretion in low glucose. Plasma glucose values were nearly normal. Thus, failure to respond to high glucose was associated with elevated plasma glucose rather than with hypersecretion of insulin. However, fasting, in addition to lowering plasma glucose, is associated with changes in numerous other metabolic and endocrine functions. It is possible that some of these other effects may be involved in the altered response of insulin to high glucose.

With regard to the paradoxical secretion of insulin at low glucose levels, note that insulin secretory responses to glucose in the obese nondiabetic Zucker (*fa*/*fa*) rat suggest a sensitization to glucose; the glucose-insulin dose-response curves are shifted to the left (15; N.R.V. and L.R., unpublished observations). These findings are similar to the paradoxical responses in obese LA/N-*cp* rats and suggest that sensitization to low glucose is somehow related to the abnormal function of the (still unidentified) *fa* and *cp* mutant gene products.

Our observations with those of Leahy et al. (4–7) suggest that overabundance of fuel for the islets may result in hypersecretion of insulin characterized by the three defects noted above, with paradoxical secretion and arginine sensitivity appearing earlier and the hyperglycemia and the high-glucose lesion appearing later. We hypothesize that hyperphagia results in increased fuel and in the secretion of gut neuropeptides and hormones that augment insulin secretion. This combination primes the islets (responsiveness to arginine) and sensitizes the islets to low levels of plasma glucose (paradoxical secretion). Finally, when hyperglycemia supervenes (increasing insulin resistance, additional genetic factors, dietary factors, and other unknowns), there ensues a series of intraislet metabolic events that decrease the V_{max} for glucose-insulin response. Hyperglycemia may act directly on the islet to cause the decrease in V_{max}; Bolaffi et al. (16) have demonstrated a reduction in insulin release during very prolonged (6–24 h) exposure of normal rat islets to high glucose.

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