The Effect of Fasting, Diet, and Actinomycin D on Insulin Secretion in the Rat

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ABSTRACT The present studies were performed to elucidate the mechanisms responsible for the impairment of glucose-stimulated insulin secretion observed in fasting. Rats fasted for 48 hr displayed marked impairment in their insulin secretory response to both oral and intravenous glucose. Glucose-stimulated insulin secretion was restored within 24 hr by refeeding; actinomycin D given before refeeding blocked the expected return of normal glucose-stimulated insulin secretion despite adequate food intake. Fasted rats refed a diet devoid of carbohydrate failed to display a return of normal insulin secretory responsiveness to oral glucose in contrast to rats fed isocalorically a high carbohydrate diet. Differences in insulin secretion in fed, fasted, and fasted-refed rats could not be attributed to changes in pancreatic insulin content. There was no significant difference in the insulin secretory response to aminophylline of fed, fasted, or fasted-refed rats. The intermittent pulsing of fasted rats with hyperglycemic episodes by the injection of small amounts of glucose (500 mg) intraperitoneally every 8 hr ameliorated the impairment of glucose-stimulated insulin secretion characteristic of the fasting state. These results suggest that the impairment of glucose-stimulated insulin secretion during fasting and its restoration by refeeding are regulated by changes in a glucose-inducible enzyme system in the pancreatic beta cell.

INTRODUCTION

Starvation results in a decrease in the basal plasma insulin levels of all species thus far studied (1-4) and, where measured, in an impairment of glucose-stimulated

insulin secretion. The mechanism(s) whereby total caloric deprivation produces these changes in insulin secretion has not been defined. The purpose of this study was to elucidate the mechanism(s) responsible for the decrease in insulin secretion observed during fasting and to assess the importance of dietary composition on the restoration of normal insulin secretion upon refeeding. The results are compatible with the concept that the impairment of glucose-stimulated insulin secretion during fasting and its restoration on refeeding reflect changes in a glucose-inducible enzyme system in the pancreatic beta cell.

METHODS

Male Sprague-Dawley rats weighing between 250 and 400 g were used in all studies. After induction of anesthesia with sodium pentobarbital (4 mg/100 g body weight, intraperitoneally), a PE 50 catheter was threaded through a jugular vein into the right atrium for blood sampling and intravenous injection of substances to be tested. Plasma insulin was determined by radioimmunoassay (5) and glucose was measured by the Technicon AutoAnalyzer ferri-cyanide method (6).

Oral glucose tolerance tests were performed in anesthetized rats by placing glucose (125 mg/100 g body weight) directly into the stomach through a gastric tube and obtaining blood samples at frequent intervals for the following 90-120 min. Intravenous glucose tolerance tests were performed by injecting 500 mg glucose (1 ml of 50% glucose solution) over 1 min and obtaining blood samples at frequent intervals over 60-90 min. The plasma insulin secretory response to theophylline was determined by injecting intravenously 15 mg aminophylline in 0.5 normal saline and taking blood samples at 2- to 10-min intervals over a 30 min period.

The significance of dietary composition on the restoration of normal glucose-stimulated insulin secretion was assessed in rats fasted for 48 hr and then pair fed isocalorically high and low carbohydrate formula diets for 72 hr. The composition of these diets is listed in Table I. Since the low carbohydrate-high fat diet is less palatable to rats than the high carbohydrate diet, pair feeding was accomplished by determining the caloric intake of the low carbohydrate-fed rats and then offering a comparable caloric amount of the high carbohydrate diet the following day to another group of animals.

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TABLE I
Composition of High and Low Carbohydrate Diets Used
in Pair-Feeding Experiment*

	High carbohydrate, 4.16 cal/g	Low carbohydrate, 4.77 cal/g
	%	%
Sucrose	68	0
Casein	18	18
Vegetable oil	8	45
Yeast	2	4
Salt	4	4
Amphocel	0	29

* Composition expressed as per cent by weight.

Pancreatic insulin content was determined by preparing acid alcohol extracts of the pancreas (7) and measuring the insulin content by radioimmunoassay using three different dilutions of the pancreatic extract. In each instance, the slope obtained with varying dilutions of extract was identical with the standard curve.

Since methods have not been developed which permit the direct quantitation of pancreatic insulin secretion, the results are presented as either insulin secretion (i.e. Σ insulin), represented by the area circumscribed by the plasma insulin response curve above the base line level and expressed as μ unit-minutes ml^{-1} , or as the ratio of this value to the area circumscribed by the corresponding plasma glucose curve (i.e. insulinogenic index).

RESULTS

Effects of fasting and refeeding on plasma insulin secretory response to oral and intravenous glucose. The plasma insulin secretory responses of fed and 48-hr

fasted rats to oral glucose are shown in Fig. 1. As noted previously, basal insulin levels decreased from 26 ± 6 $\mu\text{U}/\text{ml}$ in fed rats to 9 ± 2 $\mu\text{U}/\text{ml}$ in fasted animals. The insulin response to oral glucose in the fed group is characterized by a prompt 3- to 4-fold increase in plasma insulin to peak levels of 88 ± 6 $\mu\text{U}/\text{ml}$. In contrast, fasted animals exhibited a barely detectable rise in plasma insulin. Fed rats also displayed a prompt rise in plasma glucose with a rapid return to baseline levels, whereas fasted rats manifested carbohydrate intolerance with persistent hyperglycemia (~ 250 $\text{mg}/100$ ml) throughout the period studied. When expressed in terms of total insulin release, the fasted rat secreted less than 16% (62 ± 11 $\mu\text{U}\cdot\text{min ml}^{-1}$) of the amount released by the fed animal (400 ± 62 $\mu\text{U}\cdot\text{min ml}^{-1}$). Since the plasma glucose levels were significantly higher in the fasted animal than the fed rat, the impairment of insulin secretion during fasting is even greater when this factor is taken into consideration by expressing insulin secretion in terms of insulinogenic index: fed rats 0.55 ± 0.08 ; fasted rats 0.05 ± 0.01 .

Insulin secretion after oral glucose represents a composite response mediated by two factors: (a) an entero-insular stimulus (8, 9) and (b) a glycemic stimulus. To determine whether the decrease in glucose-stimulated insulin secretion produced by fasting reflects a primary change in pancreatic beta cell function or is secondary to an alteration in the enteroinsular stimulus, the insulin secretory response to intravenous glucose (500 mg) was also examined in fed and fasted animals (Fig. 2). Fed rats exhibited a prompt increase in plasma insulin to peak levels of 166 ± 12 $\mu\text{U}/\text{ml}$ compared to a peak re-

FIGURE 1 Plasma insulin and glucose responses to oral glucose (125 $\text{mg}/100$ g body weight) in fed and 48-hr fasted rats. Data are plotted as mean $\pm$ SEM and the number of rats studied is indicated in parentheses.

FIGURE 2 Plasma insulin and glucose responses to intravenous glucose (500 mg) in fed and 48-hr fasted rats. Data are plotted as mean $\pm$ SEM and the number of rats studied is indicated in parentheses.

sponse of $46 \pm 9 \mu\text{U/ml}$ in the fasted group. Total insulin secretion in the fed group ($326 \pm 41 \mu\text{U-min ml}^{-1}$) was more than twice that seen in fasted animals ($124 \pm 30 \mu\text{U-min ml}^{-1}$). When expressed as the insulinogenic index in order to include the contribution of the greater glycemic stimulus seen in the fasted group, insulin secretion in the fasted rat (0.23 ± 0.04) was less than 25% of that seen in the fed animals (0.93 ± 0.21).

The effect of refeeding on insulin secretion is shown in Fig. 3. In 48-hr fasted rats refed Purina Laboratory Chow ad lib., glucose-stimulated insulin secretion ($160 \pm 22 \mu\text{U-min ml}^{-1}$) returned in 7 hr to 40% of control values ($400 \pm 62 \mu\text{U-min ml}^{-1}$) and complete restoration was observed after 24 hr.

Effect of actinomycin D on insulin secretion in fasted-refed rats. To determine whether the effects of fasting

FIGURE 3 The effect of fasting and refeeding on the plasma insulin secretory response to oral glucose expressed as insulin secretion (Σ insulin) and the insulinogenic index (see Methods section). Values represent mean $\pm$ SEM and the number of animals studied is indicated in parentheses.

FIGURE 4 The effect of actinomycin D (100 $\mu\text{g}/\text{kg}$ body weight, intraperitoneally) on plasma insulin responses to oral glucose in fasted-refed rats. Data are plotted as mean $\pm$ SEM and the number of animals studied is indicated in parentheses.

and refeeding on insulin secretion reflected alterations in an inducible enzyme system in the pancreatic beta cell, 48-hr fasted rats were given actinomycin D (100 $\mu\text{g}/\text{kg}$ body weight intraperitoneally) and then allowed free access to Purina Laboratory Chow for 24 hr. Food intake and weight change over this period were identical in the actinomycin D-treated and fasted-refed control animals. The actinomycin D-treated animals exhibited an insignificant increment in plasma insulin (5 $\pm$ 14 $\mu\text{U}/\text{ml}$) after ingestion of oral glucose whereas the control group achieved peak plasma insulin increments of

FIGURE 5 The effect of actinomycin D on the plasma insulin secretory responses to oral glucose of fasted-refed rats expressed as insulin secretion (Σ insulin) and the insulinogenic index. Values represent mean $\pm$ SEM; number of animals studied is indicated in parentheses.

69 $\pm$ 11 $\mu\text{U}/\text{ml}$ (Fig. 4). Total insulin secretion in the actinomycin D-treated rats (47 $\pm$ 32 $\mu\text{U} \cdot \text{min}/\text{ml}^{-1}$) was less than 15% of control levels (324 $\pm$ 17 $\mu\text{U} \cdot \text{min}/\text{ml}^{-1}$). Glucose tolerance was severely impaired in the actinomycin D-treated rats with the blood sugar reaching levels of 400–500 mg/100 ml. As a consequence, when insulin secretion is expressed as the insulinogenic index, hormone secretory activity in the actinomycin D-treated rat (0.02 $\pm$ 0.01) was less than 3% of that seen in control rats (0.65 $\pm$ 0.19) as shown in Fig. 5. Actinomycin D given to fed rats 60–90 min before an oral glucose load does not significantly affect the insulin secretory response.

Effect of refeeding high and low carbohydrate diets on glucose-stimulated insulin secretion. Rats were fasted for 48 hr and then pair fed isocalorically high and low carbohydrate diets to determine the effect of dietary composition on the restoration of normal glucose-stimulated insulin secretion. Pair-fed animals manifested similar weight changes during fasting and refeeding over a 72 hr period. Rats fed the high carbohydrate diet exhibited normal insulin secretory responsiveness after 24 hr refeeding. On the other hand, refeeding a diet devoid of carbohydrate failed to restore insulin secretion to normal levels even after 72 hr (Fig. 6). Rats refed the low carbohydrate diet did display a transient improvement (i.e. first 24 hr) in glucose-stimulated insulin secretion, but this degree of secretory responsiveness was not sustained over the next 48 hr. The persistent and progressive impairment of insulin secretion in rats on the low carbohydrate-high fat diet is noted

FIGURE 6 Effect of refeeding high and low carbohydrate diets on the restoration of the plasma insulin secretory response to oral glucose. Values represent mean $\pm$ SEM and number of animals studied is indicated in parentheses.

FIGURE 7 Pancreatic insulin content of fed, fasted, fasted-refed, and actinomycin D fasted-refed rats. Values represent mean $\pm$ SEM and number of animals studied is indicated in parentheses.

regardless of whether insulin secretion is expressed as total insulin secretion (i.e. Σ insulin) or in terms of the insulinogenic index. Rats refed the high carbohydrate diet for 48–72 hr did not exhibit higher basal insulin levels than those refed this diet only 24 hr.

Effect of fasting, refeeding, and dietary composition on pancreatic insulin content. The insulin content of the pancreas was examined to determine whether the decreased plasma insulin responses observed during fasting and after various dietary manipulations were a consequence of depletion of pancreatic insulin or reflected impaired insulin secretion. No significant difference in

pancreatic insulin content was noted among the various groups of rats; pancreatic insulin content ranged from 1.5 to 2.0 U/g pancreas in all groups (Fig. 7).

Effect of aminophylline on insulin secretion in fed, fasted, and fasted-refed rats. Aminophylline provokes insulin secretion in the rat by increasing the intracellular level of cyclic adenosine 3',5'-monophosphate (10) and was used to determine whether the beta cell of the fasted rat is responsive to insulinogenic stimuli other than glucose. In fasted rats, aminophylline (15 mg/rat) elicited a prompt and marked increase in plasma insulin from basal levels of 22 ± 2 μ U/ml to peak levels of 116

FIGURE 8 The effect of aminophylline (15 mg i.v.) on the plasma insulin secretory responses of fed, fasted, and fasted-refed rats. Data plotted as mean $\pm$ SEM and number of animals is indicated in parentheses.

FIGURE 9 Plasma insulin secretory responses of fed, fasted, and fasted-refed rats to aminophylline expressed as insulin secretion (Σ insulin) and insulinogetic index. Values represent mean $\pm$ SEM and number of animals studied indicated in parentheses.

$\pm 30 \mu\text{U}/\text{ml}$ (Fig. 8). Although the absolute values for plasma insulin are lowest in fasted rats, there was no significant difference in the total increment of insulin secretion above base line noted in fasted animals as compared to rats fed regular Purina Rat Chow or high and low carbohydrate diets (Fig. 9). Actually, the insulin response of the fasted rat was greatest when expressed as the insulinogetic index, since the blood

FIGURE 10 Plasma insulin secretory responses of fasted-refed actinomycin D-treated rats to aminophylline. Values represent mean $\pm$ SEM and number of animals studied indicated in parentheses.

sugar level increased only slightly in the fasted group whereas marked rises were noted in all other animals. Comparable insulin secretory responses were noted in all fed groups whether expressed as insulin secretion or as the insulinogetic index. Actinomycin D did not affect the secretory response of the aminophylline-treated fasted-refed rat (Fig. 10).

FIGURE 11 The effect of intermittent hyperglycemia produced by intraperitoneal injection of 500 mg glucose every 8 hr on the plasma insulin secretory responses of 48-hr fasted rats to oral glucose. Insulin expressed as insulin secretion (Σ insulin) and insulinogetic index and represent mean $\pm$ SEM. Number of animals studied shown in circles.

Effect of intermittent parenteral glucose administration during fasting on glucose-stimulated insulin secretion. During the course of a 48 hr fast, rats were given glucose (500 mg intraperitoneally) every 8 hr to determine whether intermittent pulsing of a starved animal by hyperglycemic episodes would modify the impairment of glucose-stimulated insulin secretion seen with starvation (Fig. 11). Animals were studied 8 hr after the last injection of glucose. These animals received a total of 12 cal during the fast and their weight loss (40 ± 2 g) was comparable to that of totally starved animals (42 ± 4 g). The plasma insulin secretory response of this group ($243 \pm 67 \mu\text{U} \cdot \text{min}^{-1} \text{ml}^{-1}$) was significantly greater than that of 48-hr totally starved rats and 60% of the level seen in fed rats.

DISCUSSION

These studies demonstrate that starvation markedly impairs the ability of glucose to stimulate insulin secretion and that the defect responsible for this change resides in the pancreatic beta cell. The defect is not reflected in quantitative changes in total islet insulin content, since pancreatic insulin levels are the same in fed and fasted rats. Furthermore, the defect does not appear to involve the secretory mechanism itself, since rapid and normal mobilization of hormone is evoked by theophylline. Curry, Bennett, and Grodsky (11) have observed that glucose provokes a biphasic insulin secretory response in the perfused rat pancreas preparation and have attributed this pattern to the existence of two intracellular insulin pools; one is small and rapidly mobilizable, the other pool is much larger and equilibrates slowly with the first. A similar biphasic secretory response is also noted in portal vein insulin levels in the intact rat after intravenous theophylline (10). Simultaneous measurements of peripheral venous insulin levels after either intravenous glucose or theophylline do not show this biphasic pattern (reflecting, in all probability, a variety of factors including hepatic clearance of hormone and dilution of the hormone in the peripheral circulation), but they do demonstrate the attainment of peak levels shortly after the injection of the secretagogue. Since theophylline provoked comparable insulin responses in fed, fasted, and refed animals, it seems reasonable to conclude that the secretory defect associated with starvation is not represented by alterations in the size or function of the insulin pools.

There is now considerable evidence indicating that the chemoreceptor mechanism mediating the effects of glucose on insulin secretion involves the utilization of the sugar. For example, sugars which are metabolized by the islets (e.g., glucose and mannose) are effective insulin secretagogues, whereas nonmetabolizable sugars (e.g., galactose and D-xylose) are not. Furthermore, in-

hibition of glucose utilization by beta cells suppresses glucose stimulated release (12, 13). Since glucose phosphorylation rather than transport across the cell membrane appears to be the rate-limiting event in glucose utilization in the beta cell, attention might be focused on the initial phosphorylation process as a possible site of the glucoreceptor mechanism.

The pancreatic beta cell possesses many of the biochemical characteristics found in the hepatic parenchymal cell (14–17). Of particular relevance to the present study is the demonstration of hexokinase (14, 16), glucokinase (14, 16), and glucose-6-phosphatase (16, 17) activity in the beta cell. The temporal pattern for the disappearance and reappearance of insulin secretory responsiveness to glucose during starvation and refeeding is remarkably similar to that of hepatic glucokinase (18, 19). The ability of actinomycin D to block the restoration of normal insulin secretory responsiveness by refeeding is highly suggestive of the involvement of an inducible enzyme system. Finally, the finding that a high carbohydrate diet restores insulin secretory sensitivity to glucose whereas a noncarbohydrate diet does not and that intermittent hyperglycemia produced with calorically insignificant amounts of glucose prevents the effects of fasting on insulin secretory responsiveness lends further support to the concept of a glucose inducible enzyme system as the glucoreceptor mechanism. On the basis of these considerations, one would predict that glucose utilization by the beta cell is considerably reduced in the fasting state. However, such studies have not been reported. Although the discussion has focused on glucokinase, recent studies suggest that variations in hexokinase activity may also play a prominent role. At least four distinct forms of D-glucose-ATP-6 phosphotransferase (hexokinase) activity have been identified in rat liver; all other rat tissues, as well as human cells in culture, contain varying proportions of three "low K_m hexokinases" (20). Each type of hexokinase exhibits different kinetic properties. The activity of the type II form in human cell cultures is enhanced by glucose (21), whereas fasting results in a preferential decrease in this hexokinase in both rat liver and adipose tissue (22). Studies are currently in progress in this laboratory to identify the hexokinase-glucokinase profile of the rat beta cell and the effects of various nutritional and hormonal factors on this phosphorylation system.

These studies would also seem to bear potential relevance to the insulin secretory patterns seen in two clinical conditions, diabetes mellitus and obesity.

There is increasing evidence that a hypoinsulinemic response to glucose is a characteristic feature of many genetic diabetics even before the appearance of impaired carbohydrate tolerance (for review see Kipnis [23] and Luft [24]). These same individuals, however, may ex-

hibit normal insulin secretory responses to other secretagogues which act closer to the terminal events of the secretory system. For example, tolbutamide evokes a normal response in prediabetic (25) and mildly overt diabetic individuals (26) who exhibit a hypoinsulinemic response to glucose. This pattern is similar to that seen in the fasting rat with respect to glucose and theophylline. Furthermore, inhibition of glucose utilization does not inhibit the ability of tolbutamide to stimulate insulin release from the pancreas in vitro (13). It would seem of interest, therefore, to define the glucose phosphorylating system in the beta cells of nondiabetic and diabetic human subjects and the effects thereon of various nutritional and hormonal factors.

Hyperinsulinism is a characteristic feature of the markedly obese individual (26-28). This finding has generally been assumed to be secondary to the insulin antagonism characteristic of the obese state (29), but an alternative explanation may be offered on the basis of present studies: namely, that the hyperinsulinism of obesity is a result of increased carbohydrate intake by the markedly obese individual. In this context, the insulin resistance of the obese subject might be considered as an adaptation developed to protect against the occurrence of hypoglycemia secondary to excess insulin secretion. The observation that the elevated plasma insulin levels of obese individuals decrease progressively toward normal in prolonged starvation (30), even though they are still markedly overweight, is consistent with this thesis. On the basis of the present studies, one might predict that removing carbohydrate from the diet of the obese individual and substituting a diet consisting almost exclusively of fat, even in amounts sufficient to maintain obesity, might result in a progressive fall in the basal plasma insulin level. Preliminary studies in our laboratory have demonstrated such to be the case; namely, obese subjects exhibited a progressive fall in their basal plasma insulin levels when fed a 3500 cal diet consisting of 90% fat and 10% protein despite the maintenance of their obese state. Furthermore, Malaise, Lemonnier, Malaise-Lagae, and Mandelbaum (31) have recently demonstrated that glucose-stimulated insulin release was significantly less from in vitro rat pancreatic fragments obtained from rats fed a diet containing 40% fat in sufficient quantity to induce obesity than that seen with pancreas fragments obtained from control animals.

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