Effect of Food Restriction on Plasma Cholecystokinin Levels and Exocrine Pancreatic Function in Rats

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Abstract. The objective of this study was to examine the effects of 10% food restriction on body weight, plasma cholecystokinin (CCK) levels, and exocrine pancreatic function in male Sprague-Dawley rats. A matched group of rats with unrestricted access to food served as controls. After ingesting the diets for 32 da, the rats were killed and blood obtained for plasma cholecystokinin, glucose, and insulin determinations. To evaluate pancreatic function, the pancreases were removed, weighed, and digested with collagenase to isolate pancreatic acini, which were incubated with maximal stimulating dose of CCK. The fraction of amylase that was released into the medium was measured. To explore the role of membrane receptors in exocrine pancreatic secretion, CCK receptor affinity and CCK receptor capacity were determined by radioligand binding assays in isolated, purified membranes from pancreatic acini. Compared to the control group, rats with 10% food restriction showed (a) reduced body weight gain, (b) increased pancreatic weight, (c) increased plasma CCK level, and (d) no significant changes in plasma glucose or insulin levels. The food-restricted group showed a reduction of pancreatic function, assessed by measuring amylase release in response to maximal CCK stimulation; the amylase release was diminished by 35% in the food-restricted group. In isolated acinar cell membranes from food-restricted rats, CCK receptor affinity and capacity were reduced by 23% and 16%, respectively, compared to controls. These results indicate that consumption of less food than normal affects pancreatic function by a mechanism that evidently involves CCK release and down-regulation of CCK receptors. The data suggest that CCK plays an important physiological role in the adaptation to eating less food, and thereby to the lowering of body weight in rats and, possibly, in other animals. (received 7 May 2001; accepted 30 June 2001)

Keywords: food restriction, cholecystokinin release, cholecystokinin receptors, radioligand binding

Introduction

Cholecystokinin (CCK) is an important regulator of food intake in rats and influences pancreatic functions in response to a meal [1]. The other important physiological roles of CCK are the control of gastrointestinal functions through integration of absorptive and metabolic processing of nutrients [2] via stimulation of pancreatic endocrine secretion [3] and pancreatic growth [4]. CCK-mediated stimulation of pancreatic growth is regulated by pancreatic expression of growth-related enzymes [5,6]. In a previous study from our laboratory, we observed that exposure of rats to 0.77 mM nicotine via drinking water for 28 da caused reduced body weight, associated with 10% decrease of food intake and impaired pancreatic function, compared to controls [7]. We did not determine whether or not the reduction in body weight was due to a direct action of nicotine on food intake or to an action of nicotine on endogenous regulatory agents that modulate food intake, body weight, and pancreatic function. The current study examined the effects of 10% food restriction, alone, on body weight and plasma CCK levels and tested whether or not changes of pancreatic function are correlated with those measurements.
Materials and Methods

Reagents. Cholecystokinin-octapeptide (CCK-8) was from Peninsula Laboratories (Belmont, CA). Purified collagenase (Type CLSPA, 675 U/mg) was from Worthington Biochemical Co (Freehold, NJ). Minimal Eagle’s medium amino acid supplement was from GIBCO (Grand Island, NY). The insulin assay kit was from Radioassay System Laboratories, Inc. (San Francisco, CA). 125I-BH-CCK-8 (2000 Ci/mmol) was from DuPont (Boston, MA). Ketamine-HCl was from Parke Davis (Morris Plains, NJ) and acepromazine maleate was from Aveco (Fort Dodge, IA). Reagents used to isolate pancreatic acinar cells, and for all assays, were analytical grade.

Animals. Eighteen male Sprague-Dawley rats (starting body weight, 150 g) were divided into two groups. Group 1 (control) received food (Purina rat chow) ad libitum. The amount of food eaten by rats in Group 1 was determined daily and the rats in Group 2 were given 10% less than that amount, (average food reduction, 2.2 g). All rats had free access to water. The animals were housed individually in screen-bottomed cages at 22°C for 32 da in a room with 12-hr light/dark cycle. Throughout the experiment, body weight, food consumption, and fluid intake were monitored daily.

After 32 da, rats were fasted for 24 hr, anesthetized with ketamine-HCl (50 mg/kg body weight), and killed by decapitation. Blood samples were collected in tubes containing Trasylol (100 KIE/ml) and heparin (10 U/ml) and kept on ice. After centrifugation at 4°C at 1,500 g for 15 min, plasma was stored at –80°C prior to assays of glucose, insulin, and CCK.

Isolation of pancreatic acini. Immediately postmortem, the pancreas was removed and stripped of fat and lymph tissue. Dispersed pancreatic acini were prepared by enzymatic digestion according to the method of Williams et al [8], as described elsewhere [9]). Two ml aliquots of acini in fresh HR buffer at a density of 0.2-0.4 mg acinar protein/ml were placed in polycarbonate Erlenmeyer flasks and incubated at 37°C with 10−10 M CCK for 30 min. Amylase released in the medium was measured using Procion yellow starch as substrate [10] and was expressed as a percentage of the initial amylase content of the acinar cells. Protein concentration was determined by the Bradford method [11].

Membrane preparation. Membranes were purified from isolated acinar cells by a modification of the procedure of Steigerwalt and Williams [12]. Briefly, the acinar cell membranes were purified with buffer A [0.3M sucrose, 10 mM HEPES, 1 mM benzamidine, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), pH adjusted to 7.4, and sonicated in buffer B (1.35 M sucrose, 10 mM HEPES, 1 mM benzamidine, 0.5 mM PMSF). Buffer A was layered over the pellet and centrifugation continued at 4°C for 2 hr at 100,000 g. Membranes were collected from the interface between these two solutions and stored at −80°C until further assay.

Receptor binding. Binding of CCK to acinar cell membranes was determined by a method described previously [12,13]. The membranes (protein = ~0.2 mg/ml) were suspended in buffer containing 10 mM HEPES, 120 mM NaCl, 4.7 mM KCl, 1.0 mM MgCl2, 1.0 mM EGTA, and 5 mg/ml soybean trypsin inhibitor (pH 7.4). Varying amounts of unlabeled CCK-8 and a fixed amount of 125I-BH-CCK-8 (10 pM) were added and the flasks were incubated (150 min, 37°C). Following incubation, aliquots were filtered through GF/B filter (Whatman Ltd, UK). The filters were placed in glass tubes and counted for 2 min in a gamma counter (Auto-gamma Spectrometer 5650, Packard Instruments). Competitive inhibition of 125I-BH-CCK-8 binding by unlabeled CCK-8 in isolated acinar membranes was determined. Receptor affinity and capacity were calculated by a standard computer program [14,15].

Assays of CCK, glucose, and insulin. Plasma CCK concentration was measured by a specific radioimmunoassay, as previously reported and validated [16,17]. Plasma glucose was measured by an o-toluidine method [18]. Plasma insulin was measured with specific insulin antibody supplied with the assay kit (Radioassay System Laboratories, San Francisco, CA).
Calculations. Data were expressed as means ± SEM and analyzed by ANOVA and t-test. A p value ≤0.05 was considered significant.

Results

Initially, the average body weight of all rats was 150 g; when the experiment ended on day 32, the average body weight of rats on food-restricted diet was 303 ± 3 g, versus 330 ± 8 g in control rats (p<0.05), (Table 1). Daily weight gain by food-restricted rats was slightly low beginning on day 2 of the study, but these values were reduced significantly from day 14 of the study, when compared to the control group (Fig. 1). Food intake in controls rats averaged 24.4 ± 0.05 g versus 22.2 ± 0.8 g in food-restricted group (p <0.05) (Table 1). Water intake (29 ± 0.02 ml/day and 28± 0.03 ml/day, respectively) did not differ

Fig. 1. Growth curves for the control and food-restricted groups: body weight (g) versus time (da) during the experimental period. Data are plotted as means ± SEM. The body weights of the control and food-restricted groups differed significantly (p <0.05) from da 14 until the end of the experiment.

Table 1. Effects of food restriction on metabolic and hormonal parameters. Data are means ± SEM (n = 9).

<table>
<thead>
<tr>
<th>Parameter and units</th>
<th>Control group</th>
<th>Food-restricted group</th>
</tr>
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<tbody>
<tr>
<td>Food intake (g/da)</td>
<td>24.4 ± 0.05</td>
<td>22.2 ± 0.8*</td>
</tr>
<tr>
<td>Fluid intake (ml/da)</td>
<td>29 ± 0.02</td>
<td>28 ± 0.03</td>
</tr>
<tr>
<td>Body weight at end (g)</td>
<td>330 ± 8</td>
<td>303 ± 3*</td>
</tr>
<tr>
<td>Pancreas weight (g)</td>
<td>1.04 ± 0.06</td>
<td>1.47 ± 0.05*</td>
</tr>
<tr>
<td>Pancreas wt (mg/g bw)</td>
<td>4.08 ± 0.20</td>
<td>4.86 ± 0.15*</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>135 ± 1.0</td>
<td>139 ± 2.0</td>
</tr>
<tr>
<td>Plasma insulin (µU/L)</td>
<td>16 ± 0.3</td>
<td>17 ± 0.4</td>
</tr>
<tr>
<td>Plasma CCK (pM)</td>
<td>49 ± 5</td>
<td>74 ± 6*</td>
</tr>
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*, p < 0.05, significantly different from control group.
between the groups. Average pancreas weight (1.5 ± 0.05 and 1.4 ± 0.06 g) and relative pancreas weight (mg/g body weight) (4.86 ± 0.15 and 4.08 ± 0.20, respectively) were higher in food-restricted rats, compared to controls. Plasma glucose and insulin levels were not significantly different in the 2 groups. Plasma CCK level in control rats averaged 49 ± 5 pM versus 74 ± 6 pM in food-restricted rats (p < 0.05).

Pancreatic function was assessed by the release of amylase (% initial content) in response to a maximal dose of CCK-8 (10–10 M), as determined in previous studies [9,13] (Fig. 2). Amylase release was reduced 35% in the food-restricted group, compared to controls (p < 0.05), (Fig. 2A); however, when expressed as units/mg protein, amylase release in food-restricted rats was only 9% less than in the controls (p >0.05) (Fig. 2B).

The effects of food restriction on CCK-binding to CCK-receptors were assessed in isolated acinar cell membranes by a competitive radioligand-binding assay (Fig. 3). Competitive inhibition curves were generated with 10pM of 125I-BH-CCK-8. With increasing amounts of CCK-8, there was a substantial decrease of 125I-BH-CCK-8 binding. Scatchard plot generated as bound/free versus bound radioactivity from specific 125I-BH-CCK-8 binding data showed the presence of a single class of binding sites. The binding affinity (Kd) for membranes from the control group was 44.7 ± 4.3, compared to 34.3 ± 6.7 for the food restricted group (p >0.05). The binding capacity (B max) for the control group was 639 ± 161 fmol/mg protein, versus 534 ± 114 for the food-restricted group (p >0.05). The calculated binding-affinity and binding-capacity of CCK receptors were reduced 23% and 16%, respectively, in the food-restricted group when compared to control group. However, these values in the food-restricted group did not differ significantly from those in the controls.
Discussion

Body weights of rats in the control and food-restricted groups did not differ significantly during the first fortnight, but thereafter rats on the food-restricted diet weighed significantly less than the controls. The relative reduction of body weight in food-restricted rats remained stable from day 14 to day 32 when the study was terminated. Plasma levels of glucose and insulin were not significantly different in the 2 groups, showing that decreased food consumption and diminished body weight gain did not affect these metabolic parameters. These observations support previous findings that such food restriction with reduced body weight did not affect the basal plasma glucose level [19,20].

Plasma levels of CCK were significantly greater in rats on the food-restricted diet, compared to controls (Table 1). The increased endogenous levels of CCK in response to food-restriction may reflect a neurohormonal adaptive response. The role of CCK as a satiety hormone in the control of food intake and body weight gain has been documented in the literature [21-23].

Pancreatic weight (mg/g of body weight) was significantly higher in the food-restricted group, compared to controls (Table 1). The pancreatic hypertrophy may be due to pancreatic hyperstimulation by increased plasma CCK levels. The present findings are consistent with a recent study in rats with moderate food restriction. After ip injection of biologically active CCK-octapeptide the rats developed pancreatic hypertrophy, increased brown adipose weight, increased metabolism, and slight decrease in growth [24].

Amylase release (expressed as % of initial acinar cell content) in response to maximal stimulating dose of CCK was significantly less in food-restricted rats, compared to controls. Amylase content of acinar cells (expressed as amylase activity/mg of protein) was not different in the 2 groups. These results are consistent with the decreased exocrine pancreatic secretion reported in rats in response to exogenous CCK [13] or endogenously CCK in jaundiced rats [25], suggesting that pancreatic function is sensitive to high levels of circulating CCK and may be suppressed by downregulation of its receptors. Indeed, the present results from membrane binding studies show that CCK receptor capacity and CCK receptor affinity were reduced by 16% and 23%, respectively, in food-restricted rats, compared to control rats; however, these values were not significantly different in the 2 experimental groups.

In rats, it has been shown that chronic treatment with CCK leads to significant decrease in CCK receptors, as demonstrated by in vivo whole cell receptor binding studies with 125I-CCK-33 [26]. These results suggest that a rise in plasma CCK, as found in the current study, may be responsible for downregulation of CCK receptors and subsequent decrease in exocrine pancreatic secretion [26]. It is likely that 16% reduction in CCK receptor capacity and 23% reduction in CCK receptor affinity, as found in this study, is sufficient to induce alterations in pancreatic function in food-restricted rats. Direct hormone receptor-dependent downregulation was demonstrated in cultured human lymphocytes when chronic exposure of lymphocytes to insulin decreased the insulin receptor concentration [27]. These studies suggest that the specificity of hormone-receptor interactions affects the affinity and receptor capacity, inducing functional alterations. In earlier studies from our laboratory, the interaction of CCK with acinar cells was shown to be receptor-mediated, time-dependent, and temperature-dependent [28].

Thus, it appears that providing rats with 10% less food than their regular intake affects body weight and CCK-mediated regulation of pancreatic growth and function. These findings, however, do not rule out the possibility that other regulating peptides and chemical substances may be involved in the physiological changes that occur in food-restricted rats, compared to rats that consume food ad libitum.

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References
