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Starch digestion by parotid amylase in the gastrointestinal tract of rats with pancreatitis

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Abstract: Amylase secretion from parotid glands and the pancreas into the gastrointestinal tract and starch digestion by amylase in the gastrointestinal tract during feeding were investigated in rats with pancreatitis. Pancreatitis increased the amylase activity of parotid glands in the fasting state. The total amylase activity in the gastric contents after feeding tended to be higher in the rats with pancreatitis than in the control rats, and about one third of the ingested starch was digested in the stomachs of both groups. In the rats with pancreatitis, amylase secretion from the pancreas decreased markedly; thus, amylase activity in the small intestine and in the intestinal contents after feeding was about 3% in the control rats and mainly consisted of the parotid-type. However, the percentage of residual starch decreased from 86.8% in the pelleted diet to 21.4% in the intestinal contents. These results suggest that amylase secreted from the parotid glands moved from the stomach to the small intestine and played a physiological role in starch digestion in the gastrointestinal tract of rats with pancreatitis.

抄録：膵炎を誘発させたラットを用い、摂食時の耳下腺および膵から胃腸管内へのアミラーゼ分泌およびこれらのアミラーゼによるデンパン消化について検討した。膵炎は絶食時の耳下腺アミラーゼ活性を増加させた。摂食後の胃内容中アミラーゼ活性は、対照ラットと比較し膵炎ラットにおいて高い傾向にあり、両群ともに胃において摂食したデンパンの約3分の1が消化された。膵炎ラットでは、膵からのアミラーゼ分泌が著明に減少し、摂食刺激時の小腸および腸内容アミラーゼ活性は対照群の約3%であり、その活性は主として耳下腺型であった。しかしながら、残存するデンパンの割合は固形飼料中の86.8%から、小腸内では21.4%に減少していた。これらの結果から、膵炎ラットでは、耳下腺から分泌し、胃から小腸へ移動した耳下腺型アミラーゼが、胃腸管におけるデンパン消化に生理的な役割を果たしていることが示唆された。

Introduction

Electrophoretic patterns of amylase isozymes have shown that most of the amylase isozyme in the small intestine and in the intestinal contents of normal rats after feeding is of the pancreatic-type, and indicated that amylase secreted from the pancreas mainly acts in the small intestine of normal rats during feeding¹. On the other hand, in diabetic rats, the reduction in amylase activity in the parotid glands is not as significant as in the pancreas in the fasting state; the ratio of parotid-type amylase activity to total amylase activity in the intestinal content after feeding has been shown to be about 60%¹, and parotid duct ligation markedly reduced both amylase activity and starch
digestion in the gastrointestinal content of diabetic rats after feeding\textsuperscript{3}). These results suggest that amylase, secreted from the parotid glands and transported from the stomach to the small intestine, plays a physiological role in starch digestion in the gastrointestinal tract of diabetic rats during feeding.

Ligation of the common bile duct close to the orifice into duodenum is known to induce acute pancreatitis\textsuperscript{3}) and reduce the secretion of pancreatic digestive enzymes\textsuperscript{4}). Bile entering the pancreas through the pancreatic ducts by bile duct ligation may induce a more severe exocrine pancreatic insufficiency in rats. The amylase secretion from the pancreas is reduced in pancreatitis as well as diabetes. However, the response of the parotid glands to pancreatitis is considered to be different from that to diabetes.

In order to clarify the adaptive changes in parotid glands and the physiological role of parotid-type amylase in starch digestion in the gastrointestinal tract of rats with pancreatitis, we investigated the amylase activity in the parotid glands and pancreases before and after feeding, the amylase secretion from the parotid glands and pancreases into the gastrointestinal tract, and the starch digested by amylase in the gastrointestinal tract during feeding in both control and bile duct-ligated rats.

**Materials and Methods**

All animal protocols followed the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

1. **Animals**

Fifty male Wister rats, 7 weeks old and weighing 190~210 g (Shizuoka Laboratory Animal Center), were housed individually in an air-conditioned room (23±2°C, lights on from 8 a.m. to 8 p.m.) and fed a commercial pellet diet (Oriental Yeast) and water *ad libitum* for 2 weeks prior to use.

2. **Experimental procedure**

At 9 weeks of age, the rats were laparotomized under sodium pentobarbital anesthesia (50 mg/kg, intraperitoneally, Abbott Laboratories), and pancreatitis was induced by double ligations of the common bile duct close to the orifice into the duodenum, with excision of the duct according to the method of Kakizaki *et al.*\textsuperscript{3}), and then the abdomen was closed. A sham operation was performed similarly, but without bile duct ligation and excision. The rats were kept individually in metabolic cages. Body weight and food intake were measured every day.

Fourteen days after the operation, the rats of both groups were deprived of food overnight. On the following morning, half of each group was used as the fasting controls, while the other half was allowed to eat for 1 h, and the amount of food and water consumed was measured. The rats were killed by cervical dislocation after the 1 h feeding, and bled. Plasma was obtained from trunk blood by centrifugation.

The whole pancreas, both parotid glands, stomach, and small intestine were quickly removed. The whole pancreas and both parotid glands were rinsed with ice-cold 0.9% saline, weighed, and homogenized with ice-cold 0.02 M phosphate buffer (pH 7.0) containing 0.05 M NaCl in a Potter-Elvehjem glass homogenizer with a Teflon pestle. The homogenate was centrifuged 2,000 g for 20 min at 4°C, and the supernatant was used for the assay of amylase activity.

The stomachs of the fed rats were dissected, and the contents removed, weighed and diluted with four volumes of ice-cold 0.9% saline and homogenized. The pH was measured at 4°C, and the contents were neutralized with 100 mM Na<sub>2</sub>HPO<sub>4</sub> solution. The intestinal contents of the fed rats were collected into a vessel by manual compression of the intestine, and weighed. The lumen of the intestine was rinsed with 5 ml of ice-cold 0.9% saline, and the effluent was added to the collected intestinal content. The intestinal content was further diluted with two volumes of ice-cold 0.9% saline, homogenized and the pH measured at 4°C. The small intestine from which the contents were removed was weighed, and homogenized with ice-cold 0.02 M phosphate buffer (pH 7.0) containing 0.05 M NaCl in a Potter-Elvehjem glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 2,000 g for 20 min at 4°C, and the supernatant was used for the assay of amylase activity.

The suspensions of gastric and intestinal contents of
the fed rats were centrifuged at 2,000 g for 20 min at 4°C. The supernatant was stored, and the precipitate suspended in 10 ml of ice-cold 0.9% saline, homogenized and centrifuged. This procedure was repeated three times. The combined supernatant was used for the assay of amylase activity and soluble sugar concentration. Ten ml of water and 12 ml of 60% perchloric acid were added to the final precipitate. The mixture was kept cool by immersion in ice. Stirring was continued for 20 min, then 8 ml of water added, before the mixture was centrifuged. The aqueous starch solution was stored, and the extraction of starch by perchloric acid was repeated two more times. The combined extract was used for the assay of starch concentration. Both the soluble sugar and starch in the pellet diet were extracted using the same procedure as in obtaining the gastric and intestinal contents.

3. Assays

The plasma bilirubin concentration was determined by the Evelyn-Malloy method (Bilirubin-Test, Wako Pure Chemical Industries), the plasma insulin concentration by radioimmunoassay, with rat insulin used as a standard (Incstar Co.), and the plasma glucose concentration was determined by the glucose oxidase method (Glucose C-Test, Wako Pure Chemical Industries). The amylase activity of the plasma, tissue, gastric, and intestinal contents was determined by blue starch method described Ceska et al.\(^\text{39}\). The soluble sugar and starch concentrations in the diet, gastric, and intestinal contents were determined by glucose-anthrone sulfuric acid reaction\(^\text{40}\), and expressed as mg glucose.

4. Estimation of residual starch and absorption of carbohydrate

The following particulars were determined: 1) Starch in diet (%) ; 2) residual starch in the gastric contents (%) ; 3) gastric emptying of carbohydrate (mg) ; 4) residual starch in the small intestinal contents (%) ; 5) intestinal absorption of carbohydrate (mg), as follows :

$$1) \text{starch in diet (mg/g)} = \frac{\text{starch in diet (mg/g)}}{\text{total carbohydrate in diet (mg/g)}} \times 100$$

where total carbohydrate in diet (mg/g) = starch in diet (mg/g) + soluble sugar in diet (mg/g)

$$2) \text{residual starch in the gastric content (mg)} = \frac{\text{residual starch in the gastric content (mg)}}{\text{total carbohydrate in the gastric content (mg)}} \times 100$$

$$3) \text{gastric emptying of carbohydrate (mg)} = \text{total carbohydrate intake (mg)} - \text{total carbohydrate in the gastric contents (mg)} = \text{total carbohydrate in diet (mg/g)} \times \text{food intake (g)}$$

$$4) \text{residual starch in the small intestinal content (mg)} = \frac{\text{residual starch in the intestinal content (mg)}}{\text{gastric emptying of carbohydrate (mg)}} \times 100$$

and

$$5) \text{intestinal absorption of carbohydrate (mg)} = \text{gastric emptying of carbohydrate (mg)} - \text{total carbohydrate in the intestinal content (mg)}$$

5. Separation of amylase isozymes

Amylase isozymes in plasma, tissues, and in gastric and intestinal contents were separated on a thin layer of 7.5% polyacrylamide gel by electrophoresis using a method modified from Otsuki et al.\(^\text{17}\). Here, 2-amino-2-methyl-1, 3-propanediol\(^\text{50}\) (Wako Pure Chemical Industries) was used as the buffer instead of tris (hydroxymethyl) amino methane as in the standard buffer system, to clearly separate the more basic components of the amylase isozymes. Eight microliters of each sample (1 U/ml) was applied to the gel, and electrophoresis was carried out at 4°C for 16 h at 1 mA/cm. After the electrophoresis, the gel plate was incubated for 30 min at 37°C in a starch solution (1 g soluble starch dissolved in 100 ml of boiling 0.02 M phosphate buffer, pH 7.0, containing 0.05 M NaCl), followed by incubation of the plate alone at 37°C for 5 h. The gel plate was soaked in dilute acetic acid (50 ml/l) for 5 min before staining with an iodine/potas-
sodium iodide solution (30 g of KI and 13 g of I₂/l). The area where amylase activity was present was identified as a light yellow band against a dark blue background. The stained gels were photographed, and the photographs were scanned with a densitometer (Densitron, PAN-802, Joko) using a 0.2 × 10-mm slit beam (500 nm). The proportions of isozymes were calculated from the ratio of the area using a built-in integrator.

6. Statistics
Analysis of variance was performed on the date from the four groups. Post hoc individual comparisons were made with a Bonferroni t test. Either Student’s t-test or Chochran’s t-test was used to analyze the differences between the two groups.

Results

1. Body weight and food intake (Fig. 1)
The food intake of the bile duct-ligated rats decreased markedly immediately after the operation, resulting in a decrease in body weight. Recovery of food intake of the bile duct-ligated rats was gradual from the second to the seventh day after the operation. From the seventh to the 14th day after the operation, both body weight gain and food intake were similar for the bile duct-ligated, and the sham-operated control rats.
2. Plasma bilirubin concentration and amylase activity (Fig. 2)

Continuous bilirubinuria was observed after the bile duct ligation. In the bile duct-ligated rats, both the plasma bilirubin concentration and amylase activity were higher than in the sham-operated control rats in the fasting state. The 1h feeding significantly increased their levels in the bile duct ligated rats, but not in the sham-operated control rats. The electrophoresis showed that the amylase isozyme in the plasma of the sham-operated control rats was mostly of the parotid-type in both the fasting and fed states, while the elevated plasma amylase activity observed in the bile duct-ligated rats was of the pancreatic-type in both the fasting and fed states (data not shown).

3. Amylase activity in the pancreas and parotid glands (Fig. 3)

In the fasting state, the amylase activity in the pancreas of bile duct-ligated rats was markedly lower than in the sham-operated control rats, while the amylase activity in the parotid glands of bile duct-ligated rats was significantly higher than in the sham-operated control rats. The amylase activity in the parotid glands of the bile duct-ligated rats did not differ from that of the sham-operated control rats after feeding. The reduction in the amylase activity in the parotid glands during the 1h feeding appears higher in the bile duct-ligated rats than in the sham-operated control rats.

Table 1 Carbohydrate intake: weight, pH, amylase activity, soluble sugar, and residual starch in gastric contents: and gastric emptying of carbohydrate in sham-operated controls and bile duct-ligated rats

<table>
<thead>
<tr>
<th>Carbohydrate intake (mg)</th>
<th>Weight (g)</th>
<th>pH</th>
<th>Amylase activity (U/g)</th>
<th>Soluble sugar (mg/g)</th>
<th>Residual starch (mg/g)</th>
<th>Residual starch (%)</th>
<th>Gastric emptying of carbohydrate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (10)</td>
<td>1,811±150</td>
<td>5.86±0.32</td>
<td>5.52±0.03</td>
<td>311±38</td>
<td>69±2</td>
<td>89±3</td>
<td>56.2±1.3</td>
</tr>
<tr>
<td>Bile duct-ligated (14)</td>
<td>1,961±106</td>
<td>7.78±0.43</td>
<td>5.41±0.04</td>
<td>340±31</td>
<td>61±3</td>
<td>81±5</td>
<td>57.0±0.7</td>
</tr>
<tr>
<td>P vs. Sham-operated</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means±SE. Numbers of animals in parentheses. NS: not significant.
Table 2  Weight, pH, amylase activity, soluble sugar, and residual starch in intestinal contents; and intestinal absorption of carbohydrate in sham-operated controls and bile duct-ligated rats

<table>
<thead>
<tr>
<th>Intestinal content</th>
<th>Weight (g)</th>
<th>pH</th>
<th>Amylase activity (U/g)</th>
<th>Soluble sugar (mg/g)</th>
<th>Residual starch (mg/g)</th>
<th>Residual starch (%)</th>
<th>Intestinal absorption of carbohydrate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (10)</td>
<td>3.18±0.24</td>
<td>7.16±0.03</td>
<td>2,280±159</td>
<td>23±1</td>
<td>12±1</td>
<td>4.6±0.5</td>
<td>780±138</td>
</tr>
<tr>
<td>Bile duct-ligated (14)</td>
<td>3.58±0.24</td>
<td>7.43±0.04</td>
<td>68±18</td>
<td>17±1</td>
<td>49±2</td>
<td>21.4±1.7</td>
<td>609±55</td>
</tr>
<tr>
<td>P vs. Sham-operated</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are means±SE. Numbers of animals in parentheses. NS: not significant

4. Starch in diet: carbohydrate intake; weight, pH, amylase activity, soluble sugar, and residual starch in gastric contents; and gastric emptying of carbohydrate (Table 1)

Starch and soluble sugar in the diet were 402 mg/g and 61 mg/g, respectively. Therefore, total carbohydrate in the diet was 463 mg/g, and starch (%) was 86.8. The amylase activity (U/g) in the gastric content was similar for both the sham-operated controls and the bile duct-ligated rats. However, the weight of the gastric contents after the 1h feeding was significantly higher in the bile duct-ligated rats than in the sham-operated control rats. Therefore, the total amylase activity in the gastric contents tended to be higher in the bile duct-ligated rats (2,726±346 (SE) U, n=14) than in the sham-operated control rats (1,805±227 (SE) U, n=10) (0.05<P<0.1). Carbohydrate intake during the 1h feeding and residual starch in the gastric contents, and gastric emptying of carbohydrate by the 1h feeding were similar for both the sham-operated controls and the bile duct-ligated rats, while the pH of the gastric content was significantly lower in the bile duct-ligated rats than in the sham-operated control rats.

5. Amylase activity in the small intestine; weight, pH, amylase activity, soluble sugar, and residual starch in intestinal contents; and intestinal absorption of carbohydrate (Table 2)

After the 1h feeding, the amylase activity in the small intestine was markedly lower in the bile duct-ligated rats (8±1 (SE) U/g, n=10) than in the sham-operated control rats (272±30 (SE) U/g, n=14) (P<0.001). The amylase activity of the intestinal content was markedly lower in the bile duct-ligated rats than in the sham-operated control rats. In the bile duct-ligated rats, the amylase activity in the small intestine and intestinal contents was about 3% in the control rats, while the percentage of residual starch in the intestinal content was significantly higher in the bile duct-ligated rats than in the sham-operated control rats. The weight of the intestinal contents after the 1h feeding did not differ between the two groups. The pH of the intestinal content was significantly higher in the bile duct-ligated rats than in the sham-operated control rats. The intestinal absorption of carbohydrate did not differ between the two groups.

6. Amylase isozymes (Fig. 4 and Table 3)

In the sham-operated control rats, the amylase isozyme in the gastric contents was of the parotid-type, while most of the amylase isozyme in the small intestine and in the intestinal contents was of the pancreatic-type. On the other hand, most of the amylase isozyme in the small intestine and in the intestinal contents of the bile duct-ligated rats was of the parotid-type.

7. Plasma insulin and glucose concentrations (Fig. 5)

In the fasting state, the plasma insulin concentration of bile duct-ligated rats did not differ from that
Fig. 4 Amylase isozyme determined in parotid glands (a, b), gastric content (c, d), pancreas (e, f), small intestinal content (g, h), and small intestine (i, j) of sham-operated controls (A), and bile duct-ligated rats (B) after 1 h of feeding. O: origin, +: anode.

Table 3 Parotid amylase as a percentage of total amylase for small intestine and intestinal content of sham-operated controls and bile duct-ligated rats.

<table>
<thead>
<tr>
<th></th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small intestine</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>3.8±0.6 (10)</td>
</tr>
<tr>
<td>Bile duct-ligated</td>
<td>74.2±6.4 (14)</td>
</tr>
<tr>
<td>P vs. Sham-operated</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means±SE. Numbers of animals in parentheses.

of the sham-operated control rats, while the plasma glucose concentration of bile duct-ligated rats was lower than in the sham-operated control rats. After

Fig. 5 Plasma insulin (A) and glucose (B) concentrations of sham-operated controls (n=13 or 10) and bile duct-ligated rats (n=13 or 14) before and after 1 h of feeding. *P<0.01 vs. sham-operated controls.

the 1 h feeding, the plasma insulin concentration did not differ between the two groups, while the plasma glucose concentration of bile duct-ligated rats was lower than in the sham-operated control rats.

Discussion

Both the continuous bilirubinuria after the bile duct ligation and the increased plasma bilirubin concentration of the bile duct-ligated rats in the fasting state indicate that the bile duct was obstructed throughout the experimental period. Pancreatic-type amylase appeared in the plasma of the bile duct-ligated rats, and increased with feeding. Further, the pancreas of these rats showed both reduced amylase activity in the fasting state and reduced amylase secretory response to the feeding stimulation. Ligation of pan-
creatic ducts have been reported to result in only partial exocrine pancreatic insufficiency with the amylase activity being diminished only 50~60%. Bile entering the pancreas through the pancreatic ducts by bile duct ligation close to the orifice into the duodenum in this study may induce pancreatitis with reduced secretion of pancreatic amylase in rats.

The parotid amylase activity in the fasting state was higher in the rats with pancreatitis than in the control rats. White et al. showed that parotid amylase activity is increased in exocrine pancreatic insufficient rats induced by a copper deficient diet. The increased amylase activity in the parotid glands of rats with pancreatitis in this study may be due to either exocrine pancreatic insufficiency alone or to the combined effect of exocrine pancreatic insufficiency and cholestasis. The plasma level of cholecystokinin (CCK) is known to increase in both exocrine pancreatic insufficiency and in bile and pancreatic juice diversions in rats. Hormonal factors including CCK are possible mediators for the hyperfunction of the parotid glands in rats with pancreatitis.

The amylase activity in the parotid glands after the 1h feeding did not differ between the rats with pancreatitis and the control rats. Since the parotid amylase activity in the fasting state was higher in the rats with pancreatitis than in the control rats, the amylase secreted from the parotid glands during the 1h feeding appears higher in the rats with pancreatitis than in the control rats. Indeed, the total amylase activity in the gastric contents tended to be higher in the rats with pancreatitis than in the control rats.

About one third of the ingested starch was digested in the stomachs of both the rats with pancreatitis and the controls. Parotid-type amylase seemed to work in the oral cavity and stomach of the rats with pancreatitis, similar to that in the control rats. Rosenblum et al. showed that in the acidic environment of the human stomach, salivary-type amylases were protected from inactivation by the mixtures of substrates and their hydrolyzed products. At moderately acidic pH in the rat stomach, parotid-type amylase seems to maintain its activity, similar to that in humans.

Most of the amylase isozyme in the small intestine and the intestinal contents of control rats was of the pancreatic-type, indicating that pancreatic amylase is the main starch digestive enzyme in the small intestine of normal rats. Although the amylase activity in the small intestine and the intestinal contents after the 1h of feeding was markedly lower in the rats with pancreatitis than in the control rats, most of the amylase isozyme in the small intestine and the intestinal contents of the rats with pancreatitis was of the parotid-type. This result is similar to that found in humans which shows that the contribution of salivary isoamylase in duodenal aspirates is higher in patients with severe pancreatic exocrine insufficiency than in patients with normal exocrine pancreatic function.

The percentage of residual starch decreased from 86.8 in the pellet diet to 21.4 in the intestinal contents. Further, the intestinal absorption of carbohydrate and plasma insulin concentration after the 1h feeding did not differ between rats with pancreatitis and the controls. A small quantity of parotid-type amylase seems to digest a large part of the starch in the small intestine of rats with pancreatitis.

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References

4) Curtis, K. J., Gaines, H. D. and Kim, Y. S.: Protein digestion and absorption in rats with pancre-