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Abstract

The effects of dietary consistency and water content on the amylase activity in parotid glands and pancreas were investigated in adult rats. The parotid amylase activity was markedly lower in rats fed a liquid diet than in rats fed a powdered or control diet for 2 weeks. The parotid amylase activity in rats fed the powdered diet was not different from in the rats fed the pelleted diet. The pancreatic amylase activity was slightly but statistically significantly lower with the liquid diet than with the pelleted diet. These results suggest that in adult rats the stimuli by the contact of the dry diet to the oral mucosa are more important for the maintenance of parotid amylase activity than those induced by mastication of dry diet. The intra-oral sensation induced by feeding the pelleted diet appears to be necessary for the maintenance of pancreatic amylase activity.

Key words: Pelleted diet, Powdered diet, Liquid diet, Parotid amylase, Pancreatic amylase.

Introduction

Amylase activity in the parotid glands of adult rats fed a liquid diet is reduced¹⁻³, and change to a solid pelleted diet leads to recovery of the amylase activity⁴. This suggests that the mastication of food is important for the maintenance of amylase activity in the parotid glands of adult rats.

In young rats, the amylase activity of parotid glands is reduced when they are fed a powdered...
form of standard pelleted diet⁴, confirming that parotid amylase activity is sensitive to diet consistency and mastication. However, in adult rats, the gland weight and amylase activity of parotid glands are not different in rats fed a powdered form of standard pelleted diet and in rats fed the pelleted diet⁵. Recently, it was also observed that the amylase activities of parotid and von Ebner's glands were not statistically different in rats fed a powdered diet and in rats fed standard pelleted diet⁶.

The study here investigates the effects of dietary consistency and water content on amylase activity in the parotid glands and pancreas of adult rats to clarify the role of intra-oral sensation in the maintenance of parotid and pancreatic amylase activity in adult 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.

**Animals**

Male Wistar rats, 6 weeks old and weighing 160–180g (Shizuoka Laboratory Animal Center), were housed individually in an air-conditioned room (22 ± 2 °C, lights on from 8 a.m. to 8 p.m.) and fed a commercial pelleted diet (Oriental MF, Oriental Yeast) and water *ad libitum* for 2 weeks prior to the feeding experiment.

**Feeding experiment**

At 8 weeks of age, the rats were divided into three groups for the two week experiment: one group was fed a standard pelleted diet (Oriental MF), a second group was fed a powdered form of the standard pelleted diet (Oriental MF powdered), and a third group was fed a liquid diet, prepared daily by mixing two parts of water with one part of the powdered diet. Food and water were given *ad libitum*, and body weight, food and water intakes were measured daily. At the end of the experimental period, the rats were deprived of food overnight to eliminate the diurnal variation in parotid amylase activity correlated with the nocturnal eating habits of rats⁷. On the following morning, between 10 and 12 a.m., the rats were killed by cervical dislocation and bled. Both parotid, submandibular, and sublingual glands, and also the whole pancreas were quickly removed, and rinsed in ice cold 0.9% saline, and weighed. Both parotid glands and the whole pancreas were 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 2,000g for 20 min at 4 °C, and the supernatant was used for the assay of amylase activity. The amylase activity of the parotid glands and pancreas was determined by the blue starch method as described by Ceska et al.⁸.

**Statistics**

Analysis of variance was performed on the data from the three groups. *Post hoc* individual comparisons were made with the Scheffé t test.
Results

The weight gain, food and water intakes were highest in rats fed the liquid diet. The weight gain, food, and water intakes were not different for the rats fed the powdered diet and the pelleted diet (Table 1).

The parotid glands of the rats fed the powdered diet were slightly smaller, the submandibular glands were slightly larger, and the sublingual glands were markedly larger than those of the rats fed the pelleted diet. The weight of the parotid and sublingual glands of the rats fed the liquid diet was smaller than that of the rats fed the pelleted diet. The submandibular gland weight of the rats fed the liquid diet was not different from that of the rats fed the pelleted diet (Table 2). The weight of the pancreas was similar for all three groups (Pelleted, 1.049±0.015 (SEM) g; Powdered, 1.009±0.018g; Liquid, 1.071±0.032g).

The parotid amylase activity was markedly lower in the rats fed the liquid diet than in the other two groups. The parotid amylase activity in the rats fed the powdered diet was not different from that in the rats fed the pelleted diet. The pancreatic amylase activity was

Table 1  Body weight, and food and water intakes of rats fed the standard pelleted diet, powdered diet, or liquid diet for 2 weeks

<table>
<thead>
<tr>
<th></th>
<th>Pelleted diet</th>
<th>Powdered diet</th>
<th>Liquid diet</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=25</td>
<td>n=15</td>
<td>n=9</td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>281±3</td>
<td>284±3</td>
<td>279±5</td>
<td>NS</td>
</tr>
<tr>
<td>Weight gain (g/2 weeks)</td>
<td>63±2</td>
<td>59±3</td>
<td>74±5\textsuperscript{ab}</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>21.7±0.3</td>
<td>21.3±0.4</td>
<td>24.6±0.5\textsuperscript{ab}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water intake (ml/day)</td>
<td>31.7±0.9</td>
<td>32.9±1.4</td>
<td>51.3±1.0\textsuperscript{ab}</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SEM.
* : weight of standard pelleted diet eaten.
** : includes excess water consumed with the liquid diet.
a : Significantly different from the group fed the standard pelleted diet (p<0.05).
b : Significantly different from the group fed the powdered diet (p<0.05).

Table 2  Weight of the major salivary glands of rats fed the standard pelleted diet, powdered diet, or liquid diet for 2 weeks

<table>
<thead>
<tr>
<th></th>
<th>Pelleted diet</th>
<th>Powdered diet</th>
<th>Liquid diet</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=25</td>
<td>n=15</td>
<td>n=9</td>
<td></td>
</tr>
<tr>
<td>Parotid gland weight (mg)</td>
<td>407±8</td>
<td>361±12\textsuperscript{a}</td>
<td>259±13\textsuperscript{ab}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>gland weight/body weight (mg/g)</td>
<td>1.28±0.02</td>
<td>1.14±0.04</td>
<td>0.78±0.04\textsuperscript{ab}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Submandibular gland weight (mg)</td>
<td>449±9</td>
<td>480±12</td>
<td>463±10</td>
<td>NS</td>
</tr>
<tr>
<td>gland weight/body weight (mg/g)</td>
<td>1.40±0.02</td>
<td>1.52±0.03\textsuperscript{a}</td>
<td>1.39±0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sublingual gland weight (mg)</td>
<td>75±2</td>
<td>111±2\textsuperscript{a}</td>
<td>64±3\textsuperscript{ab}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>gland weight/body weight (mg/g)</td>
<td>0.24±0.01</td>
<td>0.35±0.01\textsuperscript{a}</td>
<td>0.19±0.01\textsuperscript{ab}</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SEM.
a : Significantly different from the group fed the standard pelleted diet (p<0.05).
b : Significantly different from the group fed the powdered diet (p<0.05).
Table 3  Amylase activity in parotid glands and pancreas of rats fed the standard pelleted diet, powdered diet, or liquid diet for 2 weeks

<table>
<thead>
<tr>
<th></th>
<th>Pelleted diet (n=25)</th>
<th>Powdered diet (n=15)</th>
<th>Liquid diet (n=9)</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid amylase activity (U/gland)</td>
<td>30700±1200</td>
<td>26900±1500</td>
<td>8700±1200&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>74.9±2.2</td>
<td>74.4±3.2</td>
<td>33.1±2.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pancreatic amylase activity (U/gland)</td>
<td>12000±700</td>
<td>9900±1300</td>
<td>8900±400&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>11.5±0.6</td>
<td>10.4±1.3</td>
<td>8.3±0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SEM.

a: Significantly different from the group fed the standard pelleted diet (p<0.05).
b: Significantly different from the group fed the powdered diet (p<0.05).

slightly lower in the rats fed the liquid diet than in the rats fed the pelleted diet. The pancreatic amylase activity in the rats fed the powdered diet was not different from that in the rats fed the pelleted diet (Table 3).

Discussion

There were no differences in the weight gain, food and water intakes in the rats fed the powdered diet and the rats fed the standard pelleted diet. Diet consistency had no effect on the body growth of adult rats. Both Hall and Schneyer<sup>11</sup> and Sreebny and Johnson<sup>21</sup> prepared the liquid diet daily by mixing five parts of water with one part of powdered diet, and here the weight gain of the rats fed the liquid diet was not lower even when these rats ate significantly less than the rats eating the control pelleted diet<sup>20</sup>. In the experiment here, the liquid diet was made daily by mixing two parts of water with one part of powdered diet and the rats fed this liquid diet showed increases in food intake and weight gain. This suggests that the liquid diet here is easier to eat for the rats than that prepared by Hall and Schneyer<sup>11</sup> and Sreebny and Johnson<sup>21</sup>

The parotid glands of the rats fed the powdered diet weighed about 10% less than those of the rats fed the standard pelleted diet, while the weight of the sublingual glands was markedly higher. The reduction in parotid gland weight in the rats fed the powdered diet is not as large as that in the rats fed the liquid diet. The reduction in mastication appears to induce small decreases in parotid gland weight in the rats fed a powdered diet<sup>10</sup>

Murai et al.<sup>10</sup> showed that the weight of sublingual glands increased in mice fed a powdered diet. The mice fed the powdered diet had significantly higher amounts of glandular acetylcholine but lower amounts of glandular norepinephrine. It was suggested that the increased contact of diet with oral mucosa may stimulate the parasympathetic nervous system, and induce the increased weight of the sublingual glands in mice and rats fed a powdered diet.

The rats fed the liquid diet showed the largest decrease in parotid gland weight, while the sublingual glands exhibited smaller reductions. Parotid glands are most sensitive to a liquid diet among the three major salivary glands<sup>10,11</sup>. The added water in a liquid diet inhibited the
effect that a powdered diet would have on sublingual gland weight, and added to the effect that a powdered diet would have on parotid gland weight. Both the water and the decrease in mastication would decrease the weight of parotid glands in rats fed a liquid diet.

The parotid amylase activity in the rats fed the powdered diet was not different from that in the rats fed the pelleted diet. These results agree with previous studies, and suggest that masticatory stimulation of the mechanoreceptors is not necessary for the maintenance of parotid amylase activity in rats. Parotid amylase activity was markedly lower in the rats fed the liquid diet. The increased water content but not the decreased mastication would seem to decrease parotid amylase activity in rats fed the liquid diet. It may be hypothesized that the stimuli by the contact of dry diet with oral mucosa are more important for the maintenance of parotid amylase activity than the stimuli induced by mastication of dry diet.

Although sapid components of the powdered diet would be more readily available for gustatory stimulation in the liquefied form, taste stimuli alone in the liquid diet could not maintain the parotid amylase activity in rats. Taste stimuli of parotid glands are thought to be transmitted via sympathetic nerve fibers in rabbits, and intact sympathetic nerve pathways are thought to be required for normal reflex secretion of acinar granules in parotid glands of rats. It is considered that both the activation of sympathetic nerve pathways induced by taste stimuli and that of parasympathetic nerve pathways induced by the contact of dry diet with the oral mucosa are necessary for the maintenance of amylase activity in the parotid glands of rats.

Asztély et al. suggested that masticatory stimulation of the mechanoreceptors is responsible for the decrease in parotid amylase content of rats in response to eating pelleted diet in the presence of adrenoceptor and muscarinic receptor blockers. However, they did not observe the response of parotid glands to eating powdered diet. In the absence of adrenergic blockers, both the taste stimuli and the stimuli by the contact of dry diet with oral mucosa seem to be more important for the secretion of parotid amylase than the masticatory stimulation of mechanoreceptors. In order to further clarify the role of the masticatory stimulation of the mechanoreceptors for the secretion of parotid amylase during feeding, it is necessary to determine the responses to eating powdered diet in both the presence and absence of adrenoceptor and muscarinic receptor blockers.

Pancreatic amylase activity was unaffected by the feeding with the powdered diet, while it decreased in the rats fed the liquid diet. Intra-oral sensation induced by feeding the pelleted diet appears to be important in maintaining pancreatic amylase activity. The synthesis and secretion of pancreatic amylase is known to be regulated by insulin. Further study is necessary to clarify the relation between intra-oral sensation and insulin secretion during feeding of diets with different textures.
Acknowledgments

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References