Effect of streptozotocin-induced diabetes on the activities of trypsin-like protease and amylase in the salivary glands and serum of rats

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Abstract: The body weight and the activities of secretory enzymes in the salivary glands and in serum were examined in male Wistar rats following the induction of streptozotocin diabetes. Amylase activity in the salivary glands and serum of diabetic rats was reduced to about half compared with controls. Trypsin-like protease activity in diabetic rats slightly decreased in the submandibular gland. Insulin treatment of diabetic rats restored the activities of amylase and protease to their respective control levels in the salivary glands. These data show that the enzyme in acinar cells of the salivary glands is more affected than that in duct cells by streptozotocin-induced diabetic rats.

Introduction

A decrease in the amount of saliva and in the salivary flow rate has been observed in diabetes1-3. Liu and Lin4 have reported that alloxan diabetes retarded body growth and growth of salivary glands, and insulin treatment prevented the retardation. Murakami5 has shown that the decreases in activities of several enzymes for carbohydrate metabolism were observed in the submandibular gland of diabetic rats. Some studies have reported that the activities of peroxidase6, a-amylase7-10 and N-acetyl-β-glucosaminidase11 in the submandibular or parotid gland of diabetic rats were also lower than in controls. Sato et al.12 have shown that streptozotocin-induced diabetes significantly reduced lysozyme activity in the submandibular, sublingual and parotid glands. Recent works have demonstrated that the levels of kallikrein in the submandibular gland of diabetic rats is lower than that of normal rats13,14. Iwata15 and Takai et al.16 found degenerative changes in duct cells in the submandibular glands of diabetic rats, however, the degree of damage was more marked in acinar cells than duct cells. We previously reported the distribution and secretion of trypsin-like protease in the submandibular gland of normal rats17. In this study, we investigated the effect of streptozotocin-induced diabetes on the activities of trypsin-like protease and amylase in various tissues, i.e., the submandibular, sublingual, and parotid glands, pancreas, liver and serum.

Materials and Methods

Male Wistar rats, 9-10 weeks of age, were used in this study. Diabetes was induced in rats (fasted for 17 hrs) by a single intravenous injection of streptozotocin (55 mg/kg body weight; Sigma, St. Louis, Missouri, U. S. A.) freshly dissolved in 5 mM citrate buffer (pH 4.5). Control rats received citrate buffer only. Diabetic rats 7 days after streptozotocin treatment received a daily subcutaneous injection of insulin (10 units/day, Sigma) for one...
Table 1 Effect of insulin treatment on serum glucose and body weight

<table>
<thead>
<tr>
<th>Days after injection of streptozotocin</th>
<th>Body weight (g)</th>
<th>Serum glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Control (16)</td>
<td>293.6</td>
<td>328.9</td>
</tr>
<tr>
<td></td>
<td>±9.2</td>
<td>±9.1</td>
</tr>
<tr>
<td>Diabetes (17)</td>
<td>285.6</td>
<td>261.6*</td>
</tr>
<tr>
<td></td>
<td>±9.9</td>
<td>±9.4</td>
</tr>
<tr>
<td>Diabetes+Insulin (8)</td>
<td>298.5</td>
<td>274.0</td>
</tr>
<tr>
<td></td>
<td>±9.9</td>
<td>±10.8</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SEM with the number of animals in parentheses.
7-Day diabetic rats were treated with insulin for 7 days.
Control vs Diabetes; * p<0.001
Diabetes vs Diabetes+Insulin; b p<0.001

The effects of streptozotocin-induced diabetes and insulin treatment on the body weights and concentration of serum glucose are shown in Table 1. The body weight of control rats gradually increased, however, that of diabetic rats decreased. The body weight of insulin-treated diabetic rats increased 20.2% over that of the untreated diabetic rats. The concentration of serum glucose in 7-day diabetic rats was 476.7 ± 17.6 mg/dl and thereafter continuously about 4 times higher than those of control animals. Serum glucose of insulin-treated diabetic rats decreased by approximately one-half compared with that of untreated diabetic rats, but still remained about 2-fold higher than the control value.

Table 2 shows trypsin-like protease activity in the submandibular and sublingual glands.
Table 3  Effect of insulin treatment on amylase activity in the serum, salivary glands, pancreas, and liver

<table>
<thead>
<tr>
<th></th>
<th>SE</th>
<th>SM</th>
<th>SL</th>
<th>PT</th>
<th>PC</th>
<th>LI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(16)</td>
<td>261.4±9.1</td>
<td>0.076±0.007</td>
<td>0.117±0.018</td>
<td>1654±76</td>
<td>821±59</td>
<td>0.153±0.005</td>
</tr>
<tr>
<td>Diabetes(17)</td>
<td>164.9±17.0^a</td>
<td>0.042±0.006^a</td>
<td>0.039±0.004^a</td>
<td>895±98^a</td>
<td>6±2^a</td>
<td>0.011±0.001^a</td>
</tr>
<tr>
<td>Diabetes(8) + Insulin</td>
<td>277.8±22.3^b</td>
<td>0.077±0.008^b</td>
<td>0.123±0.016^b</td>
<td>1721±58^b</td>
<td>292±42^b</td>
<td>0.027±0.008^c</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SEM with the number of animals in parentheses.

7-Day diabetic rats were treated with insulin for 7 days. All animals were killed on day 14 following the injection of streptozotocin.

SE, Serum; SM, Submandibular gland; SL, Sublingual gland; PT, Parotid gland; PC, Pancreas; LI, Liver

* One unit of amylase activity is defined as the amount of enzyme catalyzing a 1.0 change in the absorbance at 620 nm per 30 min per 1.0 mg of tissue or 1.0 ml of serum at 37°C.

Control vs Diabetes; ^a p<0.001
Diabetes vs Diabetes+Insulin; ^b p<0.001, ^c p<0.05

and pancreas of control, diabetic and insulin-treated diabetic rats. In a previous study, there was little activity detected in the parotid gland, serum and liver, so we did not assay the activity in these tissues. Protease activity in the submandibular gland of diabetic rats decreased to 75.8% compared with that of the control. Insulin treatment restored the activity in the submandibular gland to the control level. The protease activity in the diabetic pancreas decreased to 25.0% compared with controls, but the activity did not recover after insulin treatment. There was a large variation in protease activity in the pancreas of individual rats. The activity in the sublingual gland was almost the same among the three groups.

Table 3 shows the effect of insulin treatment on amylase activity. Amylase activity was reduced in the submandibular, sublingual and parotid glands, and serum of diabetic rats compared with controls (to 55.3%, 33.3%, 54.1% and 63.1%, respectively). Insulin treatment restored the amylase activity in the salivary glands and serum to their respective control levels. Amylase activity in the pancreas of diabetic rats was significantly reduced (to only 0.8% of the control), and the activity was recovered to 35.5% after insulin treatment. Amylase activity in the liver of diabetic rats was also significantly reduced (to 7.2%), and insulin allowed recovery to only 17.6% compared with the controls.

**Discussion**

In this study, we investigated the effect of streptozotocin-induced diabetes on the activities of amylase and trypsin-like protease in various glands and in serum, and on body weight. Streptozotocin-induced diabetes resulted in a significant decrease in body weight (Table 1), parotid and submandibular gland weights, and protein content in the glands, but no such decreases in the sublingual gland (data not shown). Trypsin-like protease activity in diabetic rats slightly decreased in the submandibular gland, but not in the sublingual gland (Table 2). Kurahashi et al. indicated that three major salivary glands had different responses to insulin deficiencies in the cholesterol metabolism. They showed that radioactive acetate incorporation into the cholesterol decreased in the submandibular and parotid glands, but not in the sublingual gland (data not shown). Trypsin-like protease activity in diabetic rats slightly decreased in the submandibular gland, but not in the sublingual gland (Table 2). Liu and Lin reported that alloxan-induced diabetes resulted in a significant decrease in body weight and in that of parotid and submandibular glands, furthermore, insulin treatment prevented the retardation of body and glandular growth and prevented defective changes in the glands. Acinar cells in parotid and submandibular glands of dia-
betic rats were atrophic and filled with auto-
phagic vacuoles\(^9,19,20\)\). These histological
findings were not seen in the sublingual
gland\(^3\). Amylase is present in the acinar
cells in the parotid gland; and trypsin-like
proteases are thought to be in the granular
convoluted tubule cells of the submandibular
gland\(^21,22\)\). Our data showing a significant
reduction in the amylase activity in the sali-
vary glands and a slight reduction in the
protease activity in the submandibular gland
agrees with the histological findings by
Iwata\(^15\) and Takai \textit{et al.}\(^3\). These data show
that acinar cells in the salivary glands are
more affected than duct cells by streptozoto-
cin-induced diabetes.

Amylase activities in the submandibular,
sublingual and parotid glands, and serum of
diabetes were similarly reduced. Also the
activities in the salivary glands and serum
diabetic rats could be restored to their
respective control levels by insulin treatment
(Tables 3). From these observations, the de-
crease in the activity of the enzyme in the sali-
vary glands and serum of streptozotocin-
induced diabetic animals, appears to be caused
by insulin insufficiency. But, the activity in
the pancreas decreased further more than that
in the salivary glands. The restorative effect
of insulin treatment in the pancreas was smaller
than that in the salivary glands. It seems
that an insufficient dose of insulin caused a
insufficient restorative effect in the pancreas,
and this remains to be elucidated. Korc \textit{et al.}\(^23\)
have reported that insulin selectively
stimulates the synthesis of amylase mRNA
in the pancreas and parotid mRNA is not
significantly affected by either diabetes or in-
sulin. We do not know whether insulin has
a direct effect on the synthesis of amylase,
but it may be one of the factors that regu-
lates the level of the enzyme in the salivary
glands.

抄録：ストレプトゾトシンにより誘発された糖尿病ラットの顔下腺、舌下腺、耳下腺および血清中のアミ-
ラーゼとトリプシン様プロテアーゼ活性を測定し、インスリンの効果を検討した。糖尿病ラットの唾液腺
および血清中のアミラーゼ活性は、対照に比べて約半分に減少し、脳臓および肝臓中のアミラーゼ活性は著し
い減少を示した。糖尿病ラットのトリプシン様プロテアーゼ活性は顔下腺でわずかに減少したものであった。
インスリンの投与によって、唾液腺および血清中のアミラーゼ活性と、顔下腺中のトリプシン様プロテアーゼ
活性はそれぞれ対照ラットのレベルまで回復した。

以上のことから、ストレプトゾトシン誘発性糖尿病ラットの唾液腺では、腎臓中の酵素の方がより影響を
受けると考えられる。

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