Ligation of the parotid duct changes zymograms of amylase isozymes in the parotid gland and the serum of rats

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Introduction

Pancreatic amylase is apparently different from the amylase of the parotid gland of rats in its amino acid composition and its immunological properties. Adult rat serum contains only parotid type isozymes of amylase, whereas prenatal rat serum contains both pancreatic and parotid types. The electrophoretic mobility of rat liver amylase is quite different from that of either pancreatic or parotid amylase. Our previous studies indicated that not only serum but also submandibular and sublingual glands contained parotid type amylase in both pilocarpine-stimulated and unstimulated rats. Parotidectomized rats did not show an elevation of amylase activity in the serum after pilocarpine administration. This indicates that amylase in the parotid gland is a source of serum amylase. It is well known that a rise in the activity of salivary amylase isozymes in the serum occurs following inflammation due to surgery on the parotid gland, or in the presence of a parotid tumor. In non-inflammatory experimental sialadenitis produced by repetitive isoproterenol injection, increases of isozyme activities can be also observed in the serum.

Little is known about the release of the isozymes from the parotid gland into the serum in drug-stimulated and unstimulated rats. The purpose of this investigation is to investigate the relationship between the amylase isozymes of the parotid gland and the serum after ligation of the excretory ducts of the rat parotid gland.

Materials and Methods

Male Wistar rats weighing 200-250 g had free access to a commercial pelleted diet (Oriental MF, Oriental Yeast Co. Ltd.) and water. Bilateral excretory ducts of the parotid gland were ligated at the distance of one cm from the oral cavity under anaesthesia with sodium pentobarbital (16 mg/kg, Abbott Laboratories). Animals were killed 24 hr, and 2 and 4 weeks after the operation. The parotid gland was removed, weighed, and homogenized in saline using a Polytron (Kinematica, GmbH) homogenizer. Serum was prepared by centrifugation. Amylase isozymes in the serum and in the parotid gland were separated by electrophoresis in a Cello-gel (Chemotron). A discontinuous buffer system was used; the anolyte was 0.1 M Tris-HCl (pH 8.8) buffer and the catholyte was 0.025 M borate-HCl (pH 8.2) buffer. Two μl of the sample were applied to the Cello-gel and horizontal electrophoresis was carried out at 15°C for 70 min at one mA per cm. After electrophoresis, the gel was incubated for 30-40 min at 37°C on muddy insoluble blue starch (Daichi Pure Chemical Co. Ltd). The gel plate was soaked in methanol for 5 min to stop the enzyme reaction then the gel was transferred into a mixture of methanol : phenol : acetic acid (90 : 8 : 2) to render the gel transparent. Three minutes later the gel was laid on a glass plate and was left in room temperature. The area in which
Bilateral excretory ducts of the parotid glands were ligated. Animals were killed one day (1 D), 2 weeks (2 W) and 4 weeks (4 W) after the operation. Sera from IN and 1 D were diluted 1 : 4 with saline. Two μl of each serum were applied to the Cellogel to separate the amylase isozymes by electrophoresis.

Fig. 1 Isoamylase patterns from sera of intact (IN) and operated rats. Bilateral excretory ducts of the parotid glands were ligated. Animals were killed one day (1 D), 2 weeks (2 W) and 4 weeks (4 W) after the operation. Sera from IN and 1 D were diluted 1 : 4 with saline. Two μl of each serum were applied to the Cellogel to separate the amylase isozymes by electrophoresis.

Results and Discussion

Electrophoretic zymograms of serum and parotid gland amylase in control and duct-ligated rats are shown in Figure 1 and 2. Serum amylase (Fig. 1) in control rats was separated into one major active band (SE-1) and four minor active bands (SE-2, SE-3, SE-4 and SE-5). The electrophoretic patterns clearly indicate increased amylase activity in bands SE-1 and SE-2 in the serum 24 hr after the operation. The serum amylase activity of the operated rats was several-fold higher than that of control rats (Table 1). Zymograms of the isozymes in the serum 2 and 4 weeks after the operation were similar to those of control rats.

Only one highly active band (PT-1) of amylase was detected in the parotid gland (Fig. 2) in control rats. In the duct-ligated parotid, three active bands (PT-0, PT-1 and PT-
Fig. 2 Isoamylase patterns from the parotid gland of intact (IN) and operated rats. Bilateral excretory ducts of the parotid gland were ligated. Animals were killed one day (1D), 2 weeks (2W) and 4 weeks (4W) after the operation. Thirty mg of the parotid gland were homogenized with 2 ml of saline. Samples from IN and 1D were diluted 100 and 10 times with saline, respectively. Two µl of each sample were applied on the Cellogel.

2) were clearly observed after 24 hr, and a different three (PT-1, PT-2 and PT-3) after 2 and 4 weeks. The amylase activity in the parotid gland 2 and 4 weeks after the operation was about one hundredth of that of control rats (Tab. 1). Other bands were not detected around bands SE-2, and over PT-0 to PT-2 in the experiments designed to detect the possible existence of some other bands.

Electrophoretic patterns of the amylase activity of the operated rats are significantly different from those in the intact parotid gland. Band PT-0 was detected in the duct-ligated parotid gland 24 hr, and band PT-3 in the gland 2 and 4 weeks after the operation. Also some change in the zymograms of amylase isozymes was observed 2 and 4 weeks after the operation. Band PT-1 was the major one in the gland after 2 weeks, but band PT-2 appeared larger after 4 weeks. Only band PT-1 was detected in the control parotid gland, and the activity of this band decreased after duct ligation. In another experiment we observed that the mobility of
Table 1 Amylase activity in the serum and parotid gland after ligation of the parotid duct

<table>
<thead>
<tr>
<th></th>
<th>before ligation</th>
<th>1 day</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>serum</td>
<td>269±7(12)</td>
<td>1413±131(6)</td>
<td>297±7(7)</td>
<td>243±13(5)</td>
</tr>
<tr>
<td>parotid gland</td>
<td>1725±118(12)</td>
<td>1800±271(6)</td>
<td>9±2(7)</td>
<td>13±4(5)</td>
</tr>
</tbody>
</table>

Results were as the mean±SEM, with number of animals in parentheses. *One unit of amylase activity is defined as the amount of enzyme catalyzing the change in 1.0 unit of absorbance at 620 nm per 30 min per 1.0 ml of the serum and 1.0 mg of the parotid gland.

band SE-2 is similar to that of band PT-2. Our previous experiment indicates that amylase activity in the rat serum is increased a little by electrical stimulation of the parotid gland, and that the elevation of the serum amylase activity is enhanced in parotid duct-ligated rats*. One day after the duct ligation, the serum amylase had a very high activity indicating an increase in the release of parotid amylase into the blood stream within 24 hr. We do not have a reasonable explanation for the appearance of band PT-0 in the gland one day, and bands PT-2 and PT-3 in the gland 2 and 4 weeks after the ligation. If amylase isozymes PT-0, PT-2 and PT-3 are synthesized from isozyme PT-1, these isozymes should be observed in the parotid gland of control rats. Although there is no evidence, one could speculate that the atrophied parotid cells after ligation release certain proteases from lysosomes and that the proteases might partially digest amylase PT-1 resulting in the production of PT-0, PT-2 and PT-3. The activity of amylase SE-2 in the serum 2 and 4 weeks after the ligation was only a little higher than that of control rats indicating that amylase PT-2 was not easily released into the blood stream from the duct-ligated gland.

Five amylase isozymes were detected in rat serum. One (SE-2) of them shows a very high activity one day after ligation. These zymograms of amylase isozymes are not a sufficient tool for the determination of their origins especially of SE-3, SE-4 and SE-5. Immunological studies using specific antisera to pure amylase isozymes might be helpful in resolving the origins of these serum isozymes.

References