Parotid Gland Recovery from an Obstruction—Changes of Amylase Release from the Tissue

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Abstract—The recovery of the parotid gland in the rat was studied by isoproterenol-induced amylase release from the parotid tissue in vitro after the removal of duct ligation following 2 and 7 days of obstruction. The percentage of amylase release did not change, but the activity of amylase in the medium (the released amylase activity) increased gradually after the removal of ligation. When the duct was ligated for 2 days, a complete recovery of the released amylase activity was seen 21 days after the removal of ligature. However, in the case of 7-day-ligation, the recovery was about 70 percent after the same period. An obstruction of a shorter duration produced a more rapid recovery of amylase release. Amylase activity in the parotid tissue increased gradually with time after the removal of ligature, and the recovery rates were very similar to that of the released amylase activity. The present results suggest that the recovery of released amylase activity after the removal of ligature is due to the increase of amylase content in the parotid tissue.

In our previous studies on ligation of the excretory duct, it was demonstrated that monoamine oxidase activity decreased gradually after ligation in the submandibular gland (1), and similarly isoproterenol-induced amylase release reduced progressively after duct ligation in the parotid gland of the rat (2). Leak (3) and Moriya (4) reported on the morphological changes following the ligation of the parotid excretory duct: the number of granules decrease initially and mitochondria, RER and Golgi zones are reduced gradually. These morphological changes caused by duct ligation were, however, reversible (5–7). It is also important, however, to elucidate whether or not the functional changes caused by duct ligation are reversible. Thus, we investigated the relationships between the time course and the recovery of amylase release in the parotid gland after the removal of the obstruction to the free flow of saliva.

Materials and Methods

Male Wistar rats weighing 180 to 250 g given a standard pelleted diet and water ad libitum were used.

Under anesthesia of sodium pentobarbital (40 mg/kg, i.p.), the excretory duct of the parotid gland was carefully isolated from connective tissue and nerve under a surgical microscope. The duct was ligated, and then the ligature was removed according to the procedure of Tamarin (8). On day 2 and 7 after the ligation, the ligature was removed; and after 7, 14 and 21 days, respectively, the rats were killed. In all experiments, the contralateral, unoperated parotid gland was used as the control.

Amylase release from parotid slices induced by isoproterenol was carried out by modifying the method of Leslie et al. (9). The methods used in this study are described in detail elsewhere (2). Briefly, an incubation medium of 5 ml Krebs-Ringer-Tris (KRT) solution, pH 7.4, was used. The parotid glands removed from the rat under sodium pentobarbital anesthesia were cut into small pieces. About 20 mg of parotid slices were aerated with pure oxygen in a KRT solution for 25 min at 37°C, and transferred into a fresh medium and pre-incubated for 10 min. This amount of
Amylase release was expressed as the basal release. The slices were then incubated 3 times consecutively, each for 10 min with fresh medium containing $10^{-5}$, $5 \times 10^{-5}$ and $10^{-4}$ M isoproterenol, respectively. After the final incubation, the slices were weighed and homogenized in 5 ml of fresh medium for 30 sec.

Amylase activity in the media and homogenate was assayed photometrically using blue insoluble starch substrate (Neo-amylase test, Daiichi Pure Chem. Co., Ltd., Japan) (10). The amylase release was expressed as both units of amylase activity released into the medium per 20 mg (wet weight) per 10 min (released amylase activity) and the percentage of amylase in the medium of the total amylase content in media plus the homogenate (percentage of amylase release). The amylase release represents the difference between the basal and the stimulated amylase release. Amylase activity in the parotid tissue was defined as the total amount of amylase activity in the media plus homogenate.

L-Isoproterenol hydrochloride (Sigma Chem.) was used as a solution dissolved with KRT solution.

The data obtained in this study were statistically analyzed by Student's $t$-test.

**Results**

Amylase release from the parotid slices: Changes of amylase release after the removal of ligation following 2 and 7 days of duct ligation are shown in Fig. 1 (released amylase activity) and Fig. 2 (percentage of amylase release).

**Fig. 1.** Changes of the released amylase activity after the removal of ligature. Seven, 14 and 21 days are the period after the removal of ligature. ●—●: control gland, ○ ○: operated gland. Each point represents the mean±S.E. of 5 experiments.
In both studies, the cumulative dose-response curves were linear with $10^{-5}$–$10^{-4}$ M isoproterenol. The released amylase activity increased gradually with time after the removal of ligation. The recovery rate of the released amylase activity after the removal of ligation was calculated as the percentage of that in the control gland, and the rates were same values at each concentration of isoproterenol. When the duct was ligated for 2 days, as shown in Fig. 1(A), the released amylase activity recovered about 55 and 80 percent at 7 and 14 days after the removal of ligation, respectively. The activity of the enzyme recovered completely after 21 days. On the other hand, in the case of the 7-day ligation, as shown in Fig. 1(B), the released amylase activity recovered about 30, 50 and 70 percent at 7, 14 and 21 days after the removal of ligation, respectively.

The changes in the percentage of amylase release after the removal of the obstruction are shown in Fig. 2. No significant differences were observed in comparison with the control gland in all cases, except at the concentration of $10^{-4}$ M isoproterenol on day 7 after the removal of ligation following 7 days of obstruction.

**Amylase activity in the parotid tissue:** As shown in Table 1, amylase activity in the parotid tissue increased progressively after the removal of ligation. When the duct was ligated for 2 days, the activity of the enzyme recovered completely 21 days after removal of the obstruction. The recovery rates of amylase activity were calculated as the percentage of that in the control gland.

![Fig. 2](image-url)

**Fig. 2.** Changes of the percentage of amylase release after the removal of ligature. Seven, 14 and 21 days are the period after the removal of ligature. ●●: control gland, ○ ○: operated gland. Each point represents the mean±S.E. of 5 experiments. *P<0.05, compared with the control gland.
activity in the parotid tissue were similar to those of the released amylase activity.

Discussion

Our previous study demonstrated that isoproterenol-induced amylase release (released amylase activity) in the rat parotid gland decreased progressively after ligation of the excretory duct (2). It was about 35 and 3 percent of that of the control at 2 and 4 days after the ligation, respectively. In the present experiment, the parotid duct was ligated for 2 and 7 days, and when the ligature was removed, the released amylase activity increased gradually. The recovery of the enzyme activity enhanced with time. In the case of 2-day-ligation, a complete recovery of the released amylase activity was seen 21 days after the removal of obstruction, but in the case of the 7-day-ligation, the recovery was about 70 percent for the same period. The shorter duration of the obstruction produced the more rapid recovery of the released amylase activity. The fact suggests that the rate of recovery depends on the duration of the obstruction. On the contrary, the percentage of amylase release after the removal of the ligation was not significantly different in comparison with the control (Fig. 2). Amylase activity in the parotid tissue, as shown in Table 1, increased gradually after the removal of ligature. The recovery rates of amylase activity in the tissue were similar to those of the released amylase activity. Therefore, the recovery of the released amylase activity by removing of the obstruction is due to the increase of amylase activity in the parotid tissue after the ligation was removed.

It is likely that the cellular level of amylase activity correlated with the number of secretory granules present in the acinar cells and reflected the cellular contents of secretory proteins. Two aspects on the recovery of amylase activity in the tissue shown in the present study are presumed: the recovery results from the reactivation in the synthesis of the secretory granules which was stopped by the obstruction to the free flow of saliva or de novo cell differentiation after acinar cells are killed by the obstruction. Tamarin (7) demonstrated that evidence of parenchymal cell death or mitotic activity was extremely rare in morphological observations after the removal of ligature following 31 days of obstruction, and Moriya (4) reported that ribosome, RER and Golgi apparatus concerned with synthesis and transport of secretion products did not change almost 7 days after ligation. Thus, it is presumed that the recovery of amylase activity in the parotid tissue after the removal of the obstruction results from the reactivation in the protein synthesis stopped by the obstruction to the free flow of saliva. It has been reported that the sensitivity of parotid gland to isoproterenol (amylose release) increases with maturity and differentiation (11). If the recovery of amylase activity in the tissue results from de novo cell differentiation, the percentage of amylase release may increase after the removal of the ligature.

Our present study demonstrated that the recovery of isoproterenol-induced amylase release (released amylase activity) after the removal of ligation was dependent on the increase of amylase activity in the parotid tissue. The increase of amylase activity in the

### Table 1. Amylase activity in the rat parotid tissue after the removal of ligature

<table>
<thead>
<tr>
<th>Time ligature (day)</th>
<th>Time removal (day)</th>
<th>Amylase activity (IU/20 mg)</th>
<th>Ligated gland</th>
<th>Control gland</th>
<th>Ligated gland/Control gland ×100</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7</td>
<td>230.1±15.7</td>
<td>414.3±28.1</td>
<td>56±3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>369.5±14.2</td>
<td>485.9±19.8</td>
<td>77±5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>406.4±29.8</td>
<td>398.2±22.3</td>
<td>102±7</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>131.8±10.6</td>
<td>387.0±23.0</td>
<td>34±1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>199.6±13.0</td>
<td>352.6±7.9</td>
<td>57±4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>21</td>
<td>301.8±17.9</td>
<td>425.0±25.7</td>
<td>72±6</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. of 5 experiments.
tissue by removal of the obstruction to the flow of saliva may suggest that amylase biosynthesis interrelates with the secretion of saliva. Thus, the recovery of amylase release after the removal of obstruction may be accelerated by administration of drugs which facilitate secretion of saliva. Further studies are necessary to clarify this problem.

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References