Effect of Duct Ligation on Amylase Release from Rat Parotid Slices

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Abstract—The effect of parotid duct ligation on amylase release from the rat parotid gland by isoproterenol was investigated in vitro. Unilateral ligation of the excretory duct progressively reduced amylase activity in the medium (the released amylase activity), but did not change the percentage of amylase release. Amylase activity in the parotid tissue decreased progressively after duct ligation, and the decrease rates of the amylase activity were very similar to that of the released amylase activity. Accordingly, the decrease of the released amylase activity after the ligation may be not due to an alteration in the amylase release mechanism, but due to the decrease of amylase content in the parotid tissue.

Ligation of the excretory duct of the salivary gland causes marked morphological changes (1, 2). It is important to study the influences of the obstruction to the free flow of saliva not only on the cell structure but on the function. Our previous studies showed that duct ligation of the rat submandibular, sublingual and parotid glands reduced the activity of monoamine oxidase, which is known as a mitochondrial enzyme (3). Amylase release is a very important function in the parotid gland. The present experiment was undertaken to study the influences of duct ligation on the functional capacity in the parotid gland. Thus, we investigated the relationships between the time course and the alterations of amylase release in the parotid gland after ligation.

Materials and Methods

Male Wistar rats weighing 200 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 parotid excretory duct was carefully dissected and ligated unilaterally at two sites about 1 cm from its opening into the mouth. In all experiments, the contralateral, non-ligated parotid gland was used as the control. The rats were subsequently studied at 3 and 12 hr and at 1, 2, 3, 4 and 7 days after duct ligation.

Amylase release from parotid slices was induced by isoproterenol by modifying the method of Leslie et al. (4). An incubation medium of 5 ml Krebs-Ringer-Tris (KRT) solution, pH 7.4, was used. The parotid gland removed from the rat under sodium pentobarbital anesthesia was cut into small pieces, and about 20 mg of parotid slices was placed in a nylon net basket and glass test tube. The slices were equilibrated in KRT solution, aerated with pure oxygen for 25 min at 37°C. The slices were transferred into fresh medium and preincubated for 10 min. This amount of amylase release was expressed as the basal release. Then the slices were incubated 3 times consecutively, each for 10 min with fresh medium containing 10^{-5}, 5\times10^{-5} and 10^{-4} M isoproterenol, respectively. After the final incubation the slices were weighed and homogenized in 5 ml of fresh medium. The KRT solution had the following composition (mM): NaCl, 120.0; MgCl_2, 1.2; CaCl_2, 3.0; β-hydroxybutyrate Na, 5.0; Tris (hydroxymethyl)-aminomethane, 20.0; KCl, 5.0; and buffered with HCl.

Amylase activity in the incubation media and homogenate was assayed photometrically using blue insoluble starch sub-
strate (Neo-amylase test, Daiichi Pure Chem. Co., Ltd., Japan) (5). 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 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 the homogenate.

The following drugs were used: L-isoproterenol hydrochloride (Sigma Chem.) and DL-propranolol hydrochloride (Sigma Chem.). Isoproterenol and propranolol were used as a solution dissolved with KRT solution.

Statistical significance was evaluated by Student’s t-test.

Results

1. A cumulative dose-response curve to isoproterenol: As shown in Fig. 1(a), the cumulative dose-response curve of amylase release from the rat parotid slices was linear with 10^{-5}-10^{-4} M isoproterenol. Therefore, the response to isoproterenol was dose-dependent under the present experimental conditions. In the experiments using an inhibitor, as shown in Fig. 1(b, c), the response was inhibited by the β-blocker propranolol.

2. Amylase release from the parotid slices:

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Fig. 1. Stimulation of amylase release from rat parotid tissue by isoproterenol in the presence of propranolol. Amylase release is expressed as the percentage of amylase released into the medium. Isoproterenol alone (a) or in combination with the β-blocker propranolol: 10^{-5} M (b) and 10^{-4} M (c). Each point represents the mean±S.E. of 4 experiments.
Changes of amylase release after excretory duct ligation are shown in Fig. 2 (released amylase activity) and Fig. 3 (percentage of amylase release). The cumulative dose-response curves of released amylase activity and percentage of amylase release were linear with $10^{-5}-10^{-4}$ M isoproterenol in both the ligated and the control glands.

The released amylase activity did not vary significantly between 3 and 12 hr after the ligation. However, one day after the duct ligation, the enzyme activity in the ligated gland decreased so rapidly that the ratio of the activity to the control gland was about 62 percent. Two days after the ligation, the activity of amylase released from the ligated gland was about 38 percent of that released from the control gland. Thereafter the released amylase activity in the ligated gland continued to decrease for 3 and 4 days after the ligation and became 6 and only 2 percent of those in the control gland, respectively. One week after the duct ligation, the activity in media was undetectable.

The percentage of amylase release, as shown in Fig. 3, did not change significantly after the duct ligation, although the slope of the cumulative dose-response curve in the ligated gland 4 days after the ligation became higher than that of the control gland.

3. Amylase activity in the parotid tissue: Changes of amylase activity in the tissue after excretory duct ligation are shown in Table 1. Although the activity of amylase had not changed significantly in the early stage after the duct ligation, it began to decrease

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Fig. 2. Effect of duct ligation on amylase release from rat parotid tissue by isoproterenol. Amylase release is expressed as units of amylase activity released into the medium. □—□ control gland, ○—○: ligated gland. Each point represents the mean±S.E. of 4 to 6 experiments.
one day after the ligation. Thereafter, further reductions of the enzyme activity occurred in the ligated tissue for one week after the ligation. Changes of amylase activity in the tissue after the ligation were similar to those seen in the case of the released amylase activity.

Fig. 3. Effect of duct ligation on amylase release from rat parotid tissue by isoproterenol. Amylase release is expressed as the percentage of amylase released into the medium. ●—●: control gland, ○—○: ligated gland. Each points represents the mean±S.E. of 4 to 6 experiments.

Table 1. Amylase activity in rat parotid tissue after duct ligation

| Time ligated | Amylase activity (IU/20 mg tissue) |  |
|--------------|-----------------------------------|---|
|              | Ligated gland                      | Control gland | Ligated gland/Control gland×100 |
| 3 hr         | 529.8±35.6                        | 503.5±20.7    | 106±8                              |
| 12 hr        | 520.9±70.5                        | 500.5±33.6    | 103±7                              |
| 1 day        | 317.3±25.0*                       | 498.4±23.1    | 64±7                               |
| 2 day        | 166.6±27.3*                       | 490.3±29.5    | 36±7                               |
| 3 day        | 20.1±2.3*                         | 445.6±40.4    | 5±1                                |
| 4 day        | 11.5±1.4*                         | 467.0±26.7    | 3±1                                |
| 7 day        | 2.6±0.5*                          | 513.5±49.0    | 1±0                                |

Each value represents the mean±S.E. of 4 to 6 experiments. Significant difference from control gland: *P<0.01.
Discussion

It has been reported that amylase release from the rat parotid slices was the response via \( \beta \)-adrenergic receptor (6). In our present experiments, as shown in Fig. 1(a), amylase release increased dose-dependently to \( 10^{-6} - 10^{-4} \) M isoproterenol. In the inhibition experiments using a \( \beta \)-blocker, propranolol, amylase release was inhibited (Fig. 1(b, c)). These results were in agreement with previous observations (6).

The cumulative dose-response curve will shift to the left or right side, respectively, according to increase or decrease of the response induced by isoproterenol. However, when the slope of the curve changes, for example as in the case of the ligated gland 4 days after the ligation (Fig. 3), it may indicate that amylase is releasable for the isoproterenol stimulation, because it was shown that the secretory granules in acinar cells localized near the lumina after duct ligation (7).

In our present experiments, amylase release was expressed as both the released amylase activity and the percentage of amylase release. After the ligation of the excretory duct, the released amylase activity decreased progressively with time (Fig. 2), whereas the percentage of amylase release did not vary significantly after the ligation (Fig. 3). This may suggest that the duct ligation hardly influences the mechanism of amylase release, particularly the receptor of the cell membrane. Amylase activity in the parotid tissue, as shown in Table 1, decreased progressively after the duct ligation, and the changes of the enzyme activity interrelated with the changes of the released amylase activity approximately. Therefore, the progressive reduction of the released amylase activity caused by the duct ligation may not be due to the alterations of the receptor in the cell membrane, but due to the decrease of amylase content in the parotid tissue after the ligation. Martinez et al. (8) reported that the numbers of both the cholinergic and adrenergic receptors were significantly reduced 2 weeks after the duct ligation. In our present study, however, sympathetic (\( \beta \)-adrenergic) dysfunction was not apparent at least until the 4th post-ligation day. If \( \beta \)-adrenergic receptors decreased in number after the ligation, the percentage of amylase release in the ligated gland will be reduced.

Since amylase is contained in secretory granules, zymogen granules in the parotid gland, the decrease of amylase activity in the parotid tissue after the duct ligation is probably due to the disappearance of secretory granules caused in the early stage after the ligation. It is well-known that monoamine oxidase localizes in the mitochondria. Our previous study demonstrated that about 30 percent of the enzyme activity remained 14 days after the ligation in the parotid gland (manuscript in preparation). In the present experiments, 35 percent of the amylase activity remained in the ligated tissue 2 days after the ligation, and only 3 percent was present 4 days after the ligation. Based on the results of monoamine oxidase and amylase after the duct ligation, it seems that the duct ligation in the parotid gland has a stronger influence on the enzyme in secretory granules than the mitochondrial enzyme. This possibility is supported by the following histochemical observation by Moriya (7): He reported that secretory granules disappeared earlier than the Golgi apparatus, mitochondria and granula endoplasmic reticula after excretory duct ligation in the rabbit parotid gland.

Since the obstruction to the free flow of saliva by excretory duct ligation, as shown in Fig. 2, caused a reduction of amylase release (released amylase activity), it may be interesting to investigate whether or not this reduction of amylase release is reversibly by removing the obstruction.

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