Hypertrophy and Dysfunction of Parotid Gland Induced by Chronic Stimulation of $\beta_1$-Adrenergic Receptors

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Abstract—The present study was carried out to determine the $\beta$-receptor subtype responsible for the hypertrophy and dysfunction of parotid glands in rats chronically treated with isoproterenol (ISP). Treatment with dobutamine (DBT), a $\beta_1$-agonist, (30 mg/kg twice a day for 4 days) produced a significant enlargement of the parotid gland just as in the ISP-treated rats. The effects of secretagogues on amylase release and ISP-induced cyclic AMP accumulation were markedly decreased in the parotid tissue of the treated rats. Co-administration of the $\beta_1$-antagonists metoprolol and acebutolol clearly inhibited the development of parotid enlargement and its secretory dysfunction induced by ISP or DBT. Procaterol treatment (30 mg/kg, twice a day for 4 days) did not cause any dysfunction or any increase in the gland weights. These results indicate that both hypertrophy and dysfunction of the rat parotid gland, which were observed in chronic treatment with ISP, may result from chronic stimulation of $\beta_1$-adrenoceptors.

Chronic administration of the nonselective $\beta$-adrenergic agonist isoproterenol (ISP) results in the enlargement of salivary glands in rodents (1, 2). Some investigators take interest in this phenomenon as a model of general proliferation and hypertrophy of cells accompanied by DNA synthesis. Recently, we found decreased responses to secretagogues in amylase release from the tissue slices of hypertrophied parotid glands (3). However, there are many reports as to the presence of subtypes of $\beta$-adrenergic receptors, the so-called $\beta_1$- and $\beta_2$-subtypes. Butcher et al. (4) suggested that ISP-induced amylase release accompanied by elevation of cyclic AMP (cAMP) is mediated by stimulation of $\beta_1$-subtype receptors, while the role of $\beta_2$-subtype receptors is still obscure in rat parotid tissue. Cyclic AMP, accepted as a second messenger for amylase release, is also increased by stimulation of $\beta_2$-subtype receptors in parotid gland (5). smooth muscles or lung (6) and a $\beta_2$-agonist induced enlargement of the parotid gland (7). Therefore, the role of these receptors in the parotid tissue is not yet clarified.

The aim of this study is to determine the receptor subtype which is responsible for hypertrophy and the decreased response to secretagogues in the rat parotid gland.

Materials and Methods
Animals and pretreatments: Male Wistar rats weighing 180–240 g, were used. They were deprived of food for 16 hr prior to each experiment, and animals were used 24 hr after the last injection. $\beta$-Adrenergic agonists and antagonists were administered intraperitoneally, twice a day for 4 days to rats which were divided into 9 groups as regards to the respective drugs of pretreatment. These animal groups were given isoproterenol (8 mg/kg/day), dobutamine (60 mg/kg/day), procaterol (60 mg/kg/day), theophylline (100 mg/kg/day), and 0.5% Tween 80 (2 ml/kg/day), as a vehicle. Metoprolol (80 mg/kg/day) and acebutolol (80 mg/kg/day) were administered 30 min before isoproterenol or dobutamine injection.

Measurement of tissue weight: Parotid and submaxillary glands were removed from rats under anesthesia with sodium pentobarbital (50 mg/kg, i.p.). The wet weights of the right glands were rapidly determined after removal
of excess fat and duct tissue. The isolated heart was also weighed after the blood was washed out with saline. Results were expressed as mg wet weight of tissue/g of body weight.

Amylase release from parotid slices: Preparation and incubation of the parotid slices were carried out by the procedure described previously (3). The parotid glands were removed from rats under anesthesia, and pieces of the tissue which together weighed approximately 10 mg were incubated in 5 ml of Krebs-Ringer-Tris solution (pH 7.4) with the following composition (in mM): NaCl, 120; KCl, 15; MgCl₂, 1.2; CaCl₂, 3.0; Tris (hydroxymethyl)-aminomethane, 20; hydroxy-n-butyric acid, 5.0; bubbled with pure oxygen at 37°C. All the slices were preincubated for 25 min, and then the slices were incubated for 30 min in fresh buffer with or without drugs. At the end of the incubation, the slices were removed and homogenized in 5 ml of fresh buffer. Amylase activities in media and homogenates were measured by the method of Bernfeld (8). The amount of released enzyme was expressed as the percent of the total amount of the enzyme which was initially contained in the slices.

Cyclic AMP measurement: The cyclic AMP (cAMP) content in the parotid tissue of the pretreated rats was measured at 5 min of incubation with or without 10⁻⁵ M isoproterenol. Cyclic AMP was assayed by a commercial assay kit (Yamasa Shoyu, Choshi), and results were expressed as cAMP pmol/mg tissue protein.

Tissue protein concentration was measured by the method of Lowry et al. (9).

**Drugs and reagents:** (-)-Isoproterenol(+)-bitartrate (Sigma), dobutamine hydrochloride (Shionogi), procaterol hydrochloride (Otsuka), metoprolol tartrate (Fujisawa), acebutolol hydrochloride (Kanebo), theophylline (Katayama Kagaku) and dibutryl-3',5'-cyclic AMP-sodium (Yamasa Shoyu).

**Statistical analysis:** Levels of significance were calculated using Student's t-test.

**Results**

**Hypertrophic effects of β-agonists on salivary glands and hearts:** As shown in Table 1, chronic administration of isoproterenol (ISP) increased the weights of parotid and submaxillary glands 4.5 and 1.5 fold, respectively, compared with control animals. Similarly, dobutamine (DBT) pretreatment induced a significant enlargement of both glands. In the animals treated with ISP and DBT, hearts were larger than in control animals. Parotid and submaxillary glands were slightly, but significantly, enlarged by theophylline pretreatment.

Unlike the β₁-agonists, the β₂-agonist procaterol (PTL) did not result in any alteration in size of the salivary glands.

**Amylase release from parotid tissue of β-agonist pretreated rats:** In the parotid tissue from control rats (control tissue) which were injected with 0.5% Tween according to the same schedule as for β-agonist administration, amylase release at 10⁻⁵ M ISP was 47.1 ± 2.8% of total amylase content during 30 min incubation. There was no difference

| Treatment         | Parotid | Submaxillary | Heart |
|-------------------|---------|--------------|-------|
|                   | (mg wet weight/g body weight) | (mg wet weight/g body weight) | (mg wet weight/g body weight) |
| Vehicle (Control) | 0.65±0.04 | 0.93±0.04 | 3.08±0.04 |
| Isoproterenol     | 2.22±0.17** | 1.44±0.08** | 4.30±0.11** |
| Dobutamine        | 1.71±0.10** | 1.21±0.03** | 3.31±1.42** |
| Procaterol        | 0.67±0.06  | 0.87±0.04  | 2.52±0.15  |
| Theophylline      | 0.78±0.06*  | 1.28±0.06** | 2.89±0.12  |

Right parotid and right submaxillary glands and hearts of the treated rats were removed 24 hr after the last injection. Isoproterenol (4 mg/kg), dobutamine (30 mg/kg), procaterol (30 mg/kg) and theophylline (50 mg/kg) were intraperitoneally injected twice a day for 4 days. Values are the mean±S.E. for 5–7 experiments. *P<0.05, **P<0.01, compared with vehicle-treated rats.
between the values obtained in tissues from Tween-treated and intact animals. The basal (non-challenged) release of amylase in intact tissue (3.4±0.5% per 30 min) was similar to that in the Tween-treated tissue (3.23±0.3%).

In parotid tissue from ISP-treated rats (ISP-treated tissue), the enzyme secretion induced by ISP, DBT, PTL and dibutyryl cyclic AMP (db-cAMP) was remarkably decreased as compared with the corresponding secretagogue responses in control tissue (Fig. 1A). In the tissue slices from DBT-treated rats (DBT-treated tissue), the responses to the above secretagogues were also diminished (Fig. 1B). However, the basal release of the enzyme was higher in both ISP- and DBT-treated tissues (6.52±0.7%, 7.07±0.8%).

ISP-induced cAMP accumulation in parotid tissue of β-agonist-treated rats: The stimulatory effects of ISP on cAMP accumulation in rat parotid tissue were examined in vitro using the tissue from pretreated animals. The results are presented in Fig. 2. The levels of ISP (10⁻⁵ M)-stimulated cAMP in control tissue (289.2±22 pmol/mg protein) was not different from those in the tissue of intact rats (322.0±24 pmol/mg protein). In the ISP- and DBT-treated tissue, the effects of 10⁻⁵ M ISP decreased to about 1/3 and 1/2 of the effect in the control tissue, respectively. In contrast, ISP-induced elevation of cAMP levels in the isolated tissue was not altered by PTL pretreatment.

Co-administration of the β-antagonists metoprolol (MTP, 80 mg/kg/day) or acebutolol (ABT, 80 mg/kg/day) with ISP (8 mg/kg/day) or DBT (60 mg/kg/day) reversed the decreased response to the challenging dose of ISP, which was observed...
in the tissue of ISP- or DBT-treated rats.

Pretreatment with MTP or ABT alone had no effect on ISP-induced cAMP accumulation in isolated parotid tissue. The basal levels of cAMP in all of the treated tissues were almost the same or slightly lower than the control or the intact tissue (6.9±0.7 and 7.2±0.7 pmol/mg protein, respectively).

Table 2. Preventive effects of $\beta_1$-antagonists on decreased amylase release or enlargement of parotid gland induced by pretreatment of isoproterenol or dobutamine

| Pretreatment (i.p.) | Tissue weight (g) | Amylase release (10^−5 M) |
|---------------------|-------------------|--------------------------|
| Vehicle             | 100(%)            | ISP 100(%)                |
| ISP                 | 341.1             | 100(%)                   |
| MTP+ISP             | 101.0             | 82.2                     |
| ABT+ISP             | 91.1              | 88.6                     |
| DBT                 | 258.9             | 35.2                     |
| MTP+DBT             | 112.6             | 106.3                    |
| ABT+DBT             | 99.9              | 98.3                     |

Amylase release from parotid tissue of treated rats was calculated as a percentage of the release induced by isoproterenol (ISP) or dobutamine (DBT) in the tissue from the vehicle-treated rats. ISP (4 mg/kg) or DBT (30 mg/kg) was intraperitoneally injected twice a day for 4 days. Metoprolol (MTP, 40 mg/kg) or acebutolol (ABT, 40 mg/kg) was injected 30 min before the injection of ISP or DBT.

Inhibitory effects of $\beta_1$-antagonists on ISP-induced dysfunction and enlargement of parotid gland: As shown in Table 2, the $\beta_1$-antagonists MTP and ABT blocked the development of ISP- and DBT-induced secretory dysfunction and enlargement of the parotid gland.

Discussion

In the present study, chronic administration of ISP to rats induced an enlargement of parotid and submaxillary glands, and this change was prevented by combined administration of the $\beta_1$-adrenergic blocking agents, ABT or MTP (Tables 1 and 2). The results suggest that these enlargements of the glands may be caused by repeated stimulation of $\beta_1$-receptors with ISP. This assumption is further supported by the facts that a selective $\beta_1$-agonist, DBT, and phosphodiesterase inhibiting methylxanthines (10) caused enlargement of the glands which were blocked by $\beta_1$-antagonists as in the case of ISP (Table 2). These results are in accord with the findings of Brenner and Wulf (11) using rat submaxillary gland. In addition, the chronic administration of both ISP and DBT increased the weight of rat hearts (Table 1). It is considered that the hypertrophy of parotid gland and heart may be associated with the elevation of cAMP levels in these tissues as a result of $\beta_1$-adrenoceptor stimulation, even if the mechanisms of hypertrophy are different in each tissue (12–14). In parotid tissue, the increased level of cAMP may initiate a sequence of biochemical changes to hyperplasia and hypertrophy of acinar cells (12, 15, 16).

On the other hand, Henriksson (17) suggested that despite the fact that the development of the enlargement and the amylase release from parotid tissue are mediated by $\beta_1$-adrenoceptor, the elevation of cAMP level in the same tissue, an important factor for amylase release, is regulated through activation of $\beta_2$-adrenoceptors. However, Butcher et al. (4) and we (18, 19) showed that the increase of the cAMP level in rat parotid gland is mediated by stimulation of $\beta_1$-adrenoceptors as in the case of amylase release. This discrepancy may result from differences in the $\beta$-agonists used for classification of $\beta$-adrenoceptor subtypes. Some investigators used terbutaline in their experiments as a $\beta_2$-selective agonist and found that this agent potentially elevated cAMP in the cells (5, 17) and caused enlargement of parotid glands (7). However, they did not examine the inhibitory effects of the $\beta_1$- and $\beta_2$-selective antagonist which have only
recently become available. Ekström and Malmberg (20) showed that terbutaline-induced enlargement of parotid gland was inhibited by the $\beta_1$-adrenergic blocking agent MTP as shown in the present study. These results suggest that terbutaline probably has stimulating effects on both $\beta_1$- and $\beta_2$-adrenoceptors.

In the present study, PTL caused a slight release of amylase from both control and enlarged tissue. The effect was smaller in DBT ($\beta_1$-selective agonist)-treated tissue than control tissue. Furthermore, in our preliminary experiment using intact parotid tissue, PTL-induced amylase release was completely antagonized by MTP and ABT (Y. Suzuki and H. Ohshika, unpublished data). These results suggest that PTL may have weak $\beta_1$-stimulating action (minor) in addition to $\beta_2$-stimulating action (major effect). It is considered that the $\beta_1$-stimulating action of PTL used for pretreatment may be too weak to alter the size and function of parotid glands.

Amylase release induced by ISP in rat parotid tissue is mediated via $\beta_1$-adrenoceptors according to the elevation of cAMP levels (4, 18). The present results show that ISP-induced elevation of cellular cAMP and amylase release were lower in the pretreated glands than the intact glands (Fig. 2). These decreased responses to $\beta$-agonists might be explained as an impairment of the $\beta_1$-adrenoceptor and adenylate cyclase coupling system, because the occurrence of the decreased responses was completely blocked by co-administration of $\beta_1$-adrenergic blocking agents (Fig. 2).

Obviously, the decreased number of $\beta_2$-adrenergic binding sites (21) and lower elevation of cAMP can be reasonably regarded as one of the causes of the impaired secretory function in the enlarged parotid gland. However, the decreased amylase release was observed in experiments using not only $\beta_2$-adrenergic agonists but also other secretagogues, for instance, carbachol and db-cAMP, in the previous study (3). Considering these results, it seems possible that repeated administration of $\beta_1$-adrenergic agonists, in other words, repeated elevation of cAMP concentration, might change the secretory function both structurally and biochemically (3, 22–24).

It is concluded that the repeated stimulation of $\beta_1$-adrenergic receptors in rat parotid gland causes an enlargement of the gland and decreased responses to $\beta_1$-adrenergic drugs in it and that the development of these changes is completely blocked with $\beta_1$-adrenergic antagonists.

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