β_1 -Adrenoceptor-Mediated Amylase Release and Cyclic AMP Accumulation in Rat Parotid Gland Tissue

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Abstract—The present study was carried out to determine the relationship of β_1 - and β_2 -subtype to amylase release and cyclic AMP (cAMP) accumulation in rat parotid tissue. In *in vitro* experiments, β -adrenergic agents (isoproterenol and dobutamine)-induced amylase release and cAMP accumulation were all completely inhibited by the β_1 -antagonist metoprolol, but incompletely inhibited by the β_2 -antagonist butoxamine. The β_2 -agonist procaterol caused little or no amylase release or cAMP accumulation. Our results suggest that both amylase release and cAMP accumulation in rat parotid tissue may be selectively induced by β_1 -adrenergic stimulation.

It has been generally accepted that the β -adrenergic signal for amylase release in rat parotid gland is mainly given via β_1 subtype adrenoceptors (1, 2) and that cyclic AMP (cAMP) plays a major role as a second messenger in the regulation of the amylase release by β -adrenergic agonists (3). However, the development of many new β -adrenergic agonists and antagonists has brought about some confusion concerning the functions of β -subtype adrenoceptors. For instance, Carlsöö et al. (4) have recently reported, in a study using some relatively selective β -adrenergic agents, that amylase release and cAMP accumulation may be separately induced by stimulation of β_1 - and β_2 -adrenoceptors. As it has been thought to be necessary to ascertain the subtypes of β -adrenoceptors related to these two β -adrenergic responses, the present study was carried out to determine whether β_1 - or β_2 -adrenergic receptors are responsible for amylase release and cAMP accumulation in rat parotid gland. To accomplish this, several types of β -agonists and antagonists were used.

Male Wistar rats, weighing 180–240 g, were used and deprived of food for 16 hr prior to each experiment. Preparation and incubation of parotid slices were carried out by the procedure described previously (5).

Parotid glands were removed from rats under pentobarbital sodium (50 mg/kg) anesthesia. Pieces of parotid tissue were incubated in 5 ml of Krebs-Ringer-Tris buffer (pH 7.4) bubbled with pure oxygen at 37°C (5). After a 25-min preincubation, the slices were incubated with or without drugs in buffer for 30 min. At the end of the incubation, the slices were transferred and then homogenized in another buffer. Amylase activity in the media and the homogenate was measured by the method of Bernfeld (6). The amount of released enzyme was expressed as a percent of the total content of the enzyme which was initially contained in the slices.

For measurement of cAMP content in the tissue, parotid slices were incubated for 5 min with or without drugs, and after incubation, the slices were rapidly transferred into liquid nitrogen. Cyclic AMP was measured by radioimmunoassay using a commercial assay kit (Yamasa Shoyu). The results are expressed as the ratio of the value in the presence of drugs to the basal value.

Protein concentration of the tissue was measured by the method of Lowry et al. (7).

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

The following drugs were used: (–)-isoproterenol (+)-bitartrate (Sigma), procaterol hydrochloride (Otsuka), dobutamine

hydrochloride (Shionogi), metoprolol tartrate (Fujisawa) and butoxamine hydrochloride (Burrough Wellcome Co.).

The effects of various β -agonists and antagonists on the levels of cAMP in parotid tissue are shown in Fig. 1. Cyclic AMP concentration in the tissue was elevated to about 70-fold of the basal level (5.7 ± 0.55 pmol/mg protein) in a 5-min incubation with isoproterenol (ISP, 10^{-5} M). Metoprolol (10^{-4} M), a β_1 -antagonist (8), completely inhibited the elevation of cAMP level induced by ISP, whereas butoxamine (10^{-4} M), a β_2 -antagonist (9), was not much more effective than metoprolol. A significant increase in cAMP level (2.7-fold of the basal level) was caused by the β_1 -agonist dobutamine (10), and this increase was completely blocked by metoprolol. However, a β_2 -agonist (11), procaterol (10^{-5} M), had no effect on the cAMP level in the tissue, even with a higher dose (10^{-3} M, data not shown).

Fig. 1. Effect of β -agonists and antagonists on cyclic AMP levels in rat parotid tissues. The tissues were incubated with agonists, isoproterenol (ISP, 10^{-5} M), dobutamine (DBT, 10^{-5} M) and procaterol (PTL, 10^{-5} M) in 37°C Krebs-Ringer-Tris buffer (pH 7.4) for 5 min. The antagonists, metoprolol (MTP, 10^{-4} M) and butoxamine (BTX, 10^{-4} M) were added to the medium 2 min before the agonists. Each column shows the ratio of the value in the presence of drugs to the basal values (unchallenged) and indicates the mean $\pm$ S.E. The basal cAMP content is 5.7 ± 0.55 pmol/mg protein. n: The number of experiments. ** $P < 0.01$ for the difference from the control (unchallenged) value. § $P < 0.01$ for difference from the value of ISP stimulation.

Figure 2 shows the effects of various β -agonists and antagonists on amylase release in parotid tissue. More than 40% of the total amylase was released by ISP (10^{-5} M) during a 30-min incubation. Metoprolol (10^{-4} M) clearly inhibited amylase release induced by ISP, whereas butoxamine (10^{-4} M) only slightly inhibited the effect of ISP. The enzyme secretion was markedly increased by dobutamine (10^{-5} M, $38.4 \pm 2.4\%$), and metoprolol completely inhibited this effect. On the other hand, procaterol was much less effective on the enzyme secretion ($9.6 \pm 1.3\%$), and butoxamine had no effect on release induced by procaterol. Throughout the present experiments, neither metoprolol (10^{-4} M) nor butoxamine (10^{-4} M) had any effect on the basal amylase release or cAMP level in the tissue.

The present results show that both amylase release and cAMP accumulation are induced by β_1 -type agonists. In addition, the results in experiments using β_1 - and β_2 -type antagonists are compatible with the above findings. However, there is a discrepancy

Fig. 2. Effect of β -agonists and antagonists on amylase release from rat parotid tissues. The agonists isoproterenol (ISP, 10^{-5} M), dobutamine (DBT, 10^{-5} M) and procaterol (PTL, 10^{-5} M) were incubated with the tissues for 30 min in 37°C Krebs-Ringer-Tris buffer (pH 7.4) and the antagonists metoprolol (MTP, 10^{-4} M) and butoxamine (BTX, 10^{-4} M) were added to the medium 2 min before the agonists. Each column shows the percentage of released amylase in the tissue before incubation and indicates the mean $\pm$ S.E. n: The number of experiments. § $P < 0.01$ for the difference from the value of ISP stimulation, ¶ $P < 0.01$ for the difference from the value of DBT stimulation.

between our results and those of Carlsöö et al. (4) on the subtype of β -adrenoceptors related to cAMP accumulation in the parotid tissue. Based on their results from experiments using terbutaline as a β_2 -agonist, they argue that cAMP accumulation induced by β -agonists is mediated via β_2 -type adrenoceptors. Despite the fact that terbutaline is classified as a β_2 -agonist, it induces the accumulation of cAMP in a dose-dependent manner, just as dobutamine (a β_1 -agonist), and it causes marked amylase release (Y. Suzuki and H. Ohshika, unpublished data). However, procaterol (a β_2 -agonist) did not cause elevation of the cAMP level in parotid gland even with a high dose (10^{-3} M). It is possible that terbutaline has a β_1 -adrenergic action together with the primary β_2 -adrenergic action in rat parotid gland. Our results show that both amylase release and cAMP accumulation are induced by a common β_1 -adrenergic stimulation, and these results are compatible with the concept that cAMP is a second messenger in β -adrenergic-stimulated amylase release. Amylase release induced by β -adrenergic stimulation may be dependent on the β_1 -adrenergic stimulation of the cAMP accumulation. The effect of dibutyl cyclic AMP on the enzyme secretion supports this hypothesis. The β -adrenoceptors in rat parotid gland may nearly all be classified into the β_1 -subtype because of the identical potency order of β -adrenergic agonists on adenylate cyclase activation, cAMP accumulation and amylase release (1), and by the specific radioligand binding (12, 13). The present results suggest that the release of amylase is induced by the increase of the parotid cAMP that is caused by stimulation of β_1 -subtype adrenoceptors in the tissue.

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