Abstract—The present study was carried out to determine the relationship of \( \beta_1 \)- and \( \beta_2 \)-subtype to amylase release and cyclic AMP (cAMP) accumulation in rat parotid tissue. In in vitro experiments, \( \beta \)-adrenergic agents (isoproterenol and dobutamine)-induced amylase release and cAMP accumulation were all completely inhibited by the \( \beta_1 \)-antagonist metoprolol, but incompletely inhibited by the \( \beta_2 \)-antagonist butoxamine. The \( \beta_2 \)-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 \( \beta_1 \)-adrenergic stimulation.

It has been generally accepted that the \( \beta \)-adrenergic signal for amylase release in rat parotid gland is mainly given via \( \beta_1 \) 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 \( \beta \)-adrenergic agonists (3). However, the development of many new \( \beta \)-adrenergic agonists and antagonists has brought about some confusion concerning the functions of \( \beta \)-subtype adrenoceptors. For instance, Carlsoo et al. (4) have recently reported, in a study using some relatively selective \( \beta \)-adrenergic agents, that amylase release and cAMP accumulation may be separately induced by stimulation of \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors. As it has been thought to be necessary to ascertain the subtypes of \( \beta \)-adrenoceptors related to these two \( \beta \)-adrenergic responses, the present study was carried out to determine whether \( \beta_1 \)- or \( \beta_2 \)-adrenergic receptors are responsible for amylase release and cAMP accumulation in rat parotid gland. To accomplish this, several types of \( \beta \)-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), pro-caterol 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 β₁-antagonist (8), completely inhibited the elevation of cAMP level induced by ISP, whereas butoxamine (10⁻⁴ M), a β₂-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 β₁-agonist dobutamine (10), and this increase was completely blocked by metoprolol. However, a β₂-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).

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±2.4%), and metoprolol completely inhibited this effect. On the other hand, procaterol was much less effective on the enzyme secretion (9.6±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 β₁-type agonists. In addition, the results in experiments using β₁- and β₂-type antagonists are compatible with the above findings. However, there is a discrepancy.
between our results and those of Carlsoo 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 β2-agonist, they argue that cAMP accumulation induced by β-agonists is mediated via β2-type adrenoceptors. Despite the fact that terbutaline is classified as a β2-agonist, it induces the accumulation of cAMP in a dose-dependent manner, just as dobutamine (a β1-agonist), and it causes marked amylase release (Y. Suzuki and H. Ohshika, unpublished data). However, procaterol (a β2-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 β1-adrenergic action together with the primary β2-adrenergic action in rat parotid gland. Our results show that both amylase release and cAMP accumulation are induced by a common β1-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 β1-adrenergic stimulation of the cAMP accumulation. The effect of dibutyryl cyclic AMP on the enzyme secretion supports this hypothesis. The β-adrenoceptors in rat parotid gland may nearly all be classified into the β1-subtype because of the identical potency order of β-adrenergic agonists on adenylyt 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 β1-subtype adrenoceptors in the tissue.

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