Supersensitivity of Amylase Secretion from Rat Parotid Tissue— Its Selective Nature for the β_2 -Adrenergic Response

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Abstract—In rat parotid tissue, amylase secretion and accumulation of cyclic AMP were not selective responses to the different β -subtypes, β_1 and β_2 . However, the supersensitivity of the amylase secretory response induced by brief pretreatment with β -agonist was due specifically to a β_2 -adrenergic response.

Our previous studies showed that pretreatment of rat parotid tissues with a β -agonist induced supersensitivity of amylase secretion to the second addition of the same agonist, which was specific to the β -adrenergic system with concomitant quantitative and qualitative alterations in β -adrenoceptors (1). The present report shows that this phenomenon is due selectively to a β_2 -adrenergic subtype response.

Parotid glands were obtained from male Wistar rats (200–300 g), and small pieces of the tissue were prepared as described previously (1). Before experiments, Krebs-Ringer Tris (KRT) solution, consisting of 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 3.0 mM CaCl_2 , 16 mM Tris HCl buffer (pH 7.4) and 10 mM glucose, was aerated with O_2 gas; and the pieces of parotid tissues were equilibrated with the solution for 20 min at 37°C with shaking. About 50 mg of tissue pieces were incubated in 10 ml of KRT solution with an agonist for 10 min (first incubation). The tissue was then washed well with KRT solution, transferred to fresh KRT solution for 10 min at 37°C (resting period), and then challenged by re-incubation with the same agonist for 10 min (second incubation). To measure β -adrenoceptors, tissues suspended in 50 mM-Tris HCl buffer (pH 7.4) with 10 mM- MgCl_2 were homogenized in a polytron and centrifuged twice to remove the cell supernatant. The final suspension contained about 300

μg protein per ml. The binding of [^3H]-dihydroalprenolol (DHA, 51 Ci/mmol), obtained from the Radiochemical Centre (Amersham, England), to the preparation was quantitated by the method described previously (1). Cyclic AMP in tissues was measured by radioimmunoassay (2) with a Yamasa cyclic AMP assay kit (Yamasa Shoyu, Co., Chiba, Japan). Amylase activity was measured as described by Bernfeld (3) with amylose as substrate. Other methods and procedures were as described previously (1). Procaterol hydrochloride was a gift from Otsuka Pharmaceutical Co. (Tokushima, Japan), terbutaline sulfate was from Fujisawa Pharmaceutical Co. (Osaka, Japan), and prenalterol hydrochloride was from Hässle AB (Mölnådal, Sweden).

There is much recent evidence that amylase secretion from the rat parotid gland induced by a β -agonist should be considered as a selective response to the β_1 -adrenergic subtype system. Namely, epinephrine (E) and norepinephrine (NE) had equal efficacies in stimulating amylase secretion (4) and in displacing the binding of an isotope labeled β -antagonist (5, 6), a β_1 -agonist caused greater stimulation than a β_2 -agonist (7, 8), and a β_1 -antagonist strongly inhibited isoproterenol (IPR)-induced amylase secretion (9). Moreover, a recent report (10) showed that the β_1 -agonist prenalterol had a specific effect on the secretion, without causing notable change in the content of cyclic AMP.

which is thought to be a second messenger in stimulus-secretion coupling in the rat parotid gland (11, 12). However, in the present study with selective agonists of different β -subtypes, no selective effects on amylase secretion from the rat parotid tissue were observed. Namely, all tested agonists significantly stimulated the secretion, though dobutamine (β_1) and terbutaline (β_2) were most effective (Fig. 1). A combination of β_1 - and β_2 -antagonists at a submaximal dose of each drug inhibited amylase secretion induced by IPR (for example, a combination of 100 μ M-butoxamine and 10 μ M-

practolol almost completely inhibited the effect of 1 μ M-IPR). Thus, it is not appropriate to classify the secretory response of rat parotid tissues as a selective response of the β_1 -subtype. No selective effects on accumulation of cyclic AMP in the tissue were also observed. Namely, 1) neither procaterol (β_2) nor prenalterol (β_1) affected cyclic AMP accumulation appreciably; 2) significant increase in cyclic AMP accumulation was seen with dobutamine or terbutaline, but a parallel increase in amylase secretion was seen only with dobutamine (Fig. 1). It is noteworthy that all the agonists

Fig. 1. Effects of β -adrenergic agonists on amylase secretion and accumulation of cyclic AMP in rat parotid tissues. Tissues were incubated for 10 min with the indicated concentrations of each agonist in the absence of phosphodiesterase inhibitor, and then amylase secreted into the medium and the cyclic AMP content of the tissues were measured. Values without agonist are means for 22 experiments with standard errors, and other values are those for 4 to 18 experiments.

Fig. 2. Comparison of the stimulatory effects of agonists on amylase secretion and cyclic AMP accumulation during the first and second incubations. Control values are means for 8 to 22 experiments with standard errors. Other values are for 4 to 18 experiments. Significantly different from the value for the first incubation, * $P < 0.01$, ** $P < 0.02$, *** $P < 0.05$.

tested stimulated the secretion as much as NE or IPR, but that they scarcely affected accumulation of cyclic AMP, even at concentrations of 100–200 μM , unlike NE or IPR (for example, 10 μM -IPR stimulated it more than five-fold).

Next we examined whether further incubation (second incubation) with the same agonist as that used in first incubation resulted in supersensitivity of the secretory response. Pretreatment with the β_2 -agonist procaterol or terbutaline at a concentration of 100–250 μM , or with IPR in the presence of the β_1 -antagonist practolol resulted in supersensitivity of the secretory response during the second incubation with same agonist, whereas pretreatment with the β_1 -agonist prenalterol or dobutamine at concentrations of up to 250 μM , or with IPR in the presence of the β_2 -antagonist butoxamine did not (Fig. 2). In every case, cyclic AMP accumulation in the tissue in the second incubation did not exceed that in the first incubation (Fig. 2). Therefore, the present study did not give any evidence for the

essential role of cyclic AMP in the secretory response or the supersensitivity phenomenon.

Since pretreatment of the tissue with 10 μM -IPR for 10 min resulted in 25% increase in the maximal binding sites of [^3H]DHA over the control (1), the effects on [^3H]DHA binding of pretreatment of the parotid tissue with 100 μM concentrations of various selective β -agonists for 10 min at 37°C were studied. Although β_1 -agonists did not have any significant effect, procaterol and terbutaline increased the maximal binding sites from 246 ± 7 fmol/mg protein ($n=5$) to 347 ± 47 ($n=4$; $P < 0.05$) and 371 ± 43 ($n=4$; $P < 0.02$), respectively. Thus it seems likely that supersensitivity of the secretory response of the rat parotid tissue with concomitant change in β -adrenoceptor is induced specifically by the β_2 -adrenergic response, without any concomitant change in cyclic AMP accumulation.

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