Forskolin Induces Supersensitivity of the Amylase Secretory Response of Rat Parotid Tissue

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Abstract—The stimulatory effect of forskolin on amylase secretion was studied by pretreating rat parotid tissue with forskolin for 10 min, incubating it in medium without forskolin for 10 min, and then treating it with forskolin again. Pretreatment with 10 nM forskolin for 10 min resulted in increased amylase secretion and enhanced accumulation of cyclic AMP in the tissue during the second incubation with forskolin. In the presence of colchicine or vinblastine, the enhancement in cyclic AMP accumulation during the second incubation with forskolin was prevented, but the increased amylase secretion remained unchanged. The increased amylase secretion was counteracted only in the presence of concanavalin A. On the other hand, increased amylase secretion induced by isoproterenol (IPR) pretreatment was counteracted by colchicine, vinblastine, concanavalin A or strychnine. These data suggest that the total amount of cyclic AMP in the tissue does not have any essential role in the supersensitivity of the amylase secretory response, and that the supersensitivity induced by forskolin differs from that induced by IPR.

Forskolin, a diterpene that activates adenylate cyclase by acting directly on its catalytic subunit (1, 2), stimulates amylase secretion from mouse parotid tissue with concomitant elevation of the cyclic AMP content and activation of protein kinase in the tissue (3). In the parotid gland, activation of $\beta$-adrenoceptors by $\beta$-adrenergic agonist results in an increase in amylase secretion. The importance of cyclic AMP in the secretory response has been widely accepted (4-7), but some recent findings are incompatible with this idea (5, 8-11), and in previous studies, we also found that brief pretreatment of the tissue with $\beta$-agonist resulted in supersensitivity of the secretory response during further incubation with the same agonist without any concomitant change in the level of cyclic AMP (12, 13). The present paper reports that forskolin induces supersensitivity of the amylase secretory response of rat parotid tissue with concomitant elevation of its cyclic AMP content. The mechanism of this effect is discussed in relation to the role of cyclic AMP in amylase secretion.

Materials and Methods

Drugs: Forskolin was obtained from Calbiochem (San Diego, U.S.A.). Colchicine, vinblastine sulfate and concanavalin A were purchased from Sigma (St. Louis, U.S.A.). Strychnine nitrate was from Wako Pure Chemicals (Osaka, Japan). Tritiated dihydralprenolol was from Amersham (England).

Preparation of rat parotid tissue: Parotid glands were obtained from male Wistar rats (200-300 g), and small pieces of the tissue were prepared as previously described (12). Before the experiment, the Krebs Ringer Tris (KRT) solution was aerated with O$_2$ gas, and the pieces of parotid tissue were equilibrated in it for 20 min at 37°C with shaking.

Treatment with forskolin or isoproterenol: About 30 mg (wet wt.) of pieces of tissue were incubated in 10 ml of KRT solution containing forskolin or isoproterenol (IRP) for 10 min (first incubation or pretreatment).
The tissue was then washed well with KRT solution and transferred to fresh KRT solution for 10 min at 37°C (rest period) and then re-incubated in KRT solution with forskolin or IPR for 10 min (second incubation).

Other methods: Cyclic AMP in tissue was measured by radioimmunoassay (14) with a Yamasa cyclic AMP assay Kit (Yamasa Shoyu, Co., Chiba, Japan) and expressed as pmole/mg protein. Amylase activity was measured as described by Bernfeld (15) with amylase as the substrate. Activity was expressed as the amount of maltose liberated into the medium in mg/5 min/100 mg tissue at 20°C. Binding of [3H]dihydroalprenolol to parotid tissue was measured as described previously (12). Statistical significance was evaluated by Student's t-test.

Results

Effects of forskolin on the amylase secretory response and cyclic AMP accumulation by parotid tissue: Forskolin at a concentration of over 1 μM significantly stimulated amylase secretion from rat parotid tissue. The secretory response of the tissue became supersensitive to forskolin after pretreatment (first incubation) with the stimulant at a concentration of over 10 μM (Fig. 1A). Forskolin also increased the cyclic AMP content in the tissue, especially at a concentration of 10 μM, and further incubation (second incubation) resulted in more accumulation of cyclic AMP than that during the first incubation (Fig. 1B). Forskolin-treatment in the presence of 1 μM-
IPR also resulted in supersensitivity of the secretory response and increased accumulation of cyclic AMP, but only the effects of the two drugs on cyclic AMP accumulation were synergistic (Fig. 1).

**Effects of colchicine, vinblastine, concanavalin A and strychnine on the supersensitivities induced by forskolin and isoproterenol:** The presence of 100 μM-colchicine (Fig. 2) or 10 μM-vinblastine (data not shown) counteracted the accumulation of cyclic AMP induced by pretreatment with forskolin, but did not affect the amylase secretory response clearly, indicating the dissociation of these two parameters. In another experiment, the cyclic AMP content was determined 5 min after the beginning of the first and second

![Fig. 2](image-url). Effect of colchicine on the supersensitivity and enhanced accumulation of cyclic AMP induced by forskolin-pretreatment. Amylase secretion (A) and cyclic AMP accumulation (B) induced by forskolin in the absence or presence of colchicine were measured. Values are means for 8 experiments with standard errors. Significantly different from the value for the first incubation: **P<0.02, ***P<0.05. For further details, see Fig. 1 and the Methods.

![Fig. 3](image-url). Effect of vinblastine, concanavalin A and strychnine on the supersensitivity induced by forskolin-pretreatment. Values are means and standard errors for 6 experiments on the effects of concanavalin A (Con-A) and strychnine and for 8 experiments in other cases. Significantly different from the value for the first incubation: *P<0.01, **P<0.02, ***P<0.05. For further details, see Fig. 1 and the Methods.
incubations. The values obtained showed a similar tendency of change to those obtained after 10 min. Among the drugs tested, only concanavalin A (0.1 mg/ml) counteracted the supersensitivity of the secretory response induced by forskolin (Fig. 3). As previously shown, rat parotid tissue became supersensitive to IPR after pretreatment with IPR, but the extent of cyclic AMP accumulation after the second incubation did not exceed that after the first incubation (12). All the drugs tested, except concanavalin A, when added at the same concentrations as in tests with forskolin, prevented IPR-induced supersensitivity of the secretory response (Fig. 4), indicating a difference in their effects on the secretory responses induced by forskolin and IPR (Figs. 2A and 3).

Discussion

Previously, we found that supersensitization of the secretory response of rat parotid tissue to IPR or β2-agonist, induced by a short period of pretreatment with these agonists, was not accompanied by concomitant increase in cyclic AMP accumulation in the tissue (12, 13). In contrast, in this study, we found that the supersensitivity induced by pretreatment with 10 μM forskolin was associated with increased cyclic AMP accumulation. However, the extent of this increase was significantly greater than that of the secretory response, and the effects of forskolin and IPR had synergistic effects only on cyclic AMP accumulation (Fig. 1). Furthermore, colchicine inhibited the enhanced accumulation of cyclic AMP, but not the increased secretory response (Fig. 2). These results suggest that the increase in level of cyclic AMP in the parotid tissue is not related with the supersensitivity of amylase secretion induced by forskolin. Synergistic effects of forskolin and IPR on cyclic AMP accumulation were also observed with rat adipocytes (16, 17). Thus, rather than having a simple action on the catalytic site, forskolin may act on some other step in the coupling of β-adrenoceptors with adenylate cyclase.

Antimitotic drugs inhibit amylase secretion from rat parotid gland under some experimental conditions in vitro (18, 19) as well as in vivo (20), but the effects of only long periods of treatment with these drugs (1–6 hr) have been examined. In the present study, colchicine (≤100 μM) and vinblastine (≤10 μM) did not have any significant effect on amylase secretion during incubation with or without forskolin, but they specifically counteracted the enhanced accumulation of cyclic AMP by forskolin. Since forskolin is thought to act on a site in the membrane, the sites of action of antimitotic drugs may also be in the membrane.
Strychnine precipitates microtubule protein (21), affects microfilaments in the erythrocyte membrane (22) and causes expansion of the membrane (23). It was also recently suggested to affect the membrane structure of rat vas deferens (24). Concanavalin A, a plant lectin, affects the mobility of many receptors on the membrane surface by binding to cell membrane-specific glycoproteins (25); it inhibits the desensitization of various receptors (26–31), and it also regulates insulin receptors under some experimental conditions (32). On the other hand, supersensitization induced by β-agonist was followed by quantitative and qualitative changes in β-adrenoceptors (12). Thus, it seemed interesting to study the effects of strychnine and concanavalin A on the supersensitivities induced by IPR and forskolin. Results showed that concanavalin A counteracted induction of supersensitivity by pretreatment with forskolin, while strychnine did not (Fig. 3). In contrast, both drugs, as well as antimitic drugs, counteracted the supersensitivity induced by IPR (Fig. 4). Thus, there may be some difference between the effects of these drugs on the actions of IPR and forskolin. It seems unlikely that these drugs interfere with the binding of IPR to β-adrenoceptors, because they did not affect amylase secretion during the first incubation with IPR, and they had little effect on the binding by parotid tissue of [3H]dihydroalprenolol, which binds to β-adrenoceptors (data not shown). Accordingly they might act on the membrane structure and affect some step in the mechanism inducing the supersensitivity. The present study showed that forskolin induces supersensitization of the amylase secretory response, that the mechanism of this induction is different from that of induction of supersensitivity by IPR, and that change in the total amount of cyclic AMP in the tissue does not have any marked role in the induction of supersensitivity by forskolin or IPR.

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