EFFECT OF THEOPHYLLINE AND 3', 5'-CYCLIC ADENOSINE MONOPHOSPHATE ON THE SALIVARY AMYLASE SECRETION FROM RABBIT PAROTID GLAND

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The salivary glands are innervated by both the sympathetic and parasympathetic nerves, receiving a main secretory innervation from the parasympathetic nervous system (1). It was reported that the sympathetic nervous system played an important role in regulation of secretion of amylase, a main component of parotid saliva, from the rabbit parotid gland and that the β receptors were the ones involved in this mechanism (2–5). It is known that catecholamine stimulates adenyl cyclase which may exist in the cell membrane and catalyzes the formation of cyclic AMP in many tissues (6). Robinson et al. (7) postulated that in most and perhaps all tissues the β receptors and adenyl cyclase were probably identical. Bdolah and Shramm (8) reported that theophylline and butyryl derivatives of cyclic AMP induced amylase release from rat parotid slice.

The present investigation was undertaken to clarify the probable function of cyclic AMP as an inducer of amylase secretion from the rabbit parotid gland in vivo.

MATERIALS AND METHODS

Male rabbits weighing 2 to 3.5 kg were anesthetized by the intraperitoneal injection of urethane (1.5 g/kg). Additional small doses of urethane were given when necessary. The trachea was exposed and cannulated, and the superior cervical ganglion was removed.

The parotid duct was cannulated using a short piece of stainless steel tubing which was connected to a fine polyethylene tubing with a dead space of approximate 25 µl. The peripheral cut end of auriculotemporal nerve was electrically stimulated using platinum bipolar electrodes with 2 msec pulses at 6 to 10 Hz. The voltage and frequency were adjusted to maintain the salivary flow at 100 to 200 mg of the saliva per min.

Each experiment was started after the amylase activity and salivary flow reached a steady state. Each drop of the saliva was counted and collected separately to determine amylase activity and protein concentration (single drop analysis) (2). Amylase activity was determined by the modification of the Bernfeld's method (9). One unit of amylase activity was defined as the amount which catalyzes the formation of reducing sugar equivalent to 1 mg of maltose hydrate in 6 minutes at 30°C. Protein was determined by the method of Lowry et al. (10).

Drugs used were 1-adrenaline hydrochloride, propranolol hydrochloride, dichloro-...
isoprenaline hydrochloride (DCI), theophylline, 3', 5'-cyclic adenosine monophosphate (cyclic AMP), N'-2'O-dibutyryl 3', 5'-cyclic adenosine monophosphate (dibutyryl cyclic AMP) and adenosine 5'-monophosphate disodium (5'-AMP). DCI was administered by infusion for 10 minutes. The intervals of injection of theophylline were 60 to 90 minutes. Drugs were administered intravenously via femoral vein.

Intraarterial injections were made via common carotid artery, during occlusion of internal carotid artery and inferior alveolar artery, so as to divert much injected substance to the parotid gland.

Each result was obtained from 3 to 5 rabbits and each figure illustrated is an example from typical experiments.

RESULTS

Effect of theophylline on amylase secretion induced by auriculotemporal nerve stimulation

The intravenous injection of theophylline (3-30 mg/kg) caused increases in amylase activities and protein concentrations in the saliva induced by auriculotemporal nerve stimulation, but did not produce constant and remarkable effect in the flow rate. The increases in amylase and protein secretion revealed at 30 to 40 seconds after the administration of theophylline and reached a maximum at about one and a half minutes. In a case of adrenaline (1 μg/kg), the increases in amylase and protein secretion started at about 15 seconds.

![Graph](image)

**Fig. 1.** Effects of theophylline and adrenaline on amylase and protein secretion induced by auriculotemporal nerve stimulation in rabbit parotid gland. Ordinate: Amylase activity (×10⁴ units/g, ○) Protein concentration (mg/g, ○) and salivary flow rate (mg/min, dotted line). Adrenaline (1 μg/kg) and theophylline (10 mg/kg) were intravenously injected at time ↑. Effect of theophylline on amylase secretion was revealed later than that of adrenaline.
FIG. 2. Dose response curves of theophylline on amylase and protein secretion induced by auriculotemporal nerve stimulation in rabbit parotid gland. Increasing per cent was obtained from the formula:

\[
\text{Increasing per cent} = \frac{\text{the average of three maximum values with theophylline}}{\text{the average of three values before theophylline}} \times 100
\]

Vertical lines indicate the S.E. of the mean. The means are based on at least three animals at each point. Increasing actions of theophylline on amylase and protein secretion were dose dependent.

FIG. 3. Combined effect of theophylline and adrenaline on amylase secretion induced by auriculotemporal nerve stimulation in rabbit parotid gland. Ad: Adrenaline 1 mg/kg i.v., THEO: Theophylline 3 mg/kg i.v., THEO + Ad: Theophylline 3 mg/kg i.v. + Adrenaline 1 mg/kg i.v. Theophylline was injected about 20 seconds before adrenaline. Adrenaline effect on amylase secretion was potentiated by theophylline.
after the administration and reached a maximum at 45 to 50 seconds. The responses to theophylline in amylase and protein secretion revealed later than those to adrenaline and continued longer. In all experiments, the change in amylase activities of the saliva was roughly parallel to that in protein concentrations (Fig. 1). The increases in amylase and protein secretion were proportional to the dose of theophylline administered (Fig. 2).

**Combined effect of theophylline and adrenaline on amylase secretion**

Adrenaline (1 μg/kg) was injected 20 seconds after injection of theophylline (3 mg/kg), so the maximum responses to adrenaline and theophylline in amylase and protein secretion as to reveal at the same time.

The increases in amylase and protein secretion produced by adrenaline were potentiated by theophylline, and the duration of adrenaline effect was prolonged (Fig. 3). The combined effects of adrenaline (1 μg/kg) and theophylline (3 mg/kg) on amylase and protein secretion were greater than the sum of individual their effect (Table 1).

**Table 1. Combined effects of adrenaline and theophylline on amylase and protein secretion induced by auriculotemporal nerve stimulation in rabbit parotid gland.**

| Drugs     | Increasing per cent* |
|-----------|----------------------|
| Ad (1 μg/kg i.v.) | 333 ± 92‡ |
| THEO (3 mg/kg i.v.) | 45 ± 17 |
| Ad + THEO | 604 ± 45 |

Ad : Adrenaline, THEO : Theophylline
‡ Values are mean ± S.E. of increasing per cent.
The means are based on at least three animals.
* Increasing per cent was obtained from the formula:

\[
\text{Increasing per cent} = \left( \frac{\text{the average of three maximum values after theophylline}}{\text{the average of three values before theophylline}} \right) \times 100
\]

**Influence of β receptor blocking agents on theophylline effect on amylase secretion**

After the treatment with propranolol (300 μg/kg), amylase activities and protein concentrations of the saliva in the steady state were decreased to about a half. The increases in amylase and protein secretion produced by theophylline were decreased to 30 per cent of control by pretreatment with 300 μg/kg of propranolol 5 minute beforehand, but the rates of increases to levels in steady state in amylase and protein secretion produced by theophylline were not remarkably changed with propranolol (Fig. 4). In contrast, amylase activities and protein concentrations of the saliva in the steady state were increased 1.5 to 2 fold after the treatment with DCI. The increases in amylase and protein secretion produced by theophylline were decreased to about 70 per cent of control by pretreatment with 5 mg/kg of DCI 5 minute beforehand. The maximum values of amylase activities and protein concentrations after theophylline were not so much changed by pretreatment with DCI (Fig. 5).
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FIG. 4. Influence of propranolol on the effects of theophylline on amylase and protein secretion induced by auriculotemporal nerve stimulation in rabbit parotid gland. Abscissa: Time scale was plotted arbitrarily so that there are equal intervals between drops. Theophylline (10 mg/kg) was intravenously injected at time ↑.

FIG. 5. Influence of DCI on the effects of theophylline on amylase and protein secretion induced by auriculotemporal nerve stimulation in rabbit parotid gland. Abscissa: Time scale was plotted arbitrarily so that there are equal intervals between drops. Theophylline (10 mg/kg) was intravenously injected at time ↑.

Effect of dibutyryl cyclic AMP on amylase secretion induced by auriculotemporal nerve stimulation

Significant increases in amylase activities and protein concentrations in the saliva
were not observed following intravenous injection of cyclic AMP and dibutyryl cyclic AMP in doses of 2 to 10 mg/kg.

After intraarterial injection of 2 to 5 mg/kg of dibutyryl cyclic AMP, amylase activities and protein concentrations in the saliva were slightly increased in 5 out of 7 experiments, but the flow rate was not changed. Effect of dibutyryl cyclic AMP on amylase secretion determined under infusion of theophylline. Under infusion of theophylline (1.5 mg/kg/min), amylase activities and protein concentrations in the saliva were increased gradually and reached a steady state about 15 minutes after the start of the infusion. Fifteen minutes after the start of the infusion, the intraarterial injection of dibutyryl cyclic AMP (5 mg/kg) caused increases in amylase and protein secretion in 3 out of 4 experiments (Fig. 6). While, after the intraarterial injection of 5'-AMP (5 mg/kg), a slight increases in amylase and protein secretion were observed in 2 out of 4 experiments.

**DISCUSSION**

Secretion of amylase, a typical secretory digestive enzyme and main component of parotid saliva, is regulated by both the sympathetic and parasympathetic nervous systems (2, 4, 11, 12). In previous reports (2-5, 11), it was reported that stimulation of the cervical sympathetic nerve and administration of various sympathomimetic amines markedly increased the amylase and protein secretion induced by parasympathetic nerve stimulation, and that the order of potency in producing an increase in amylase secretion was isoprenaline, adrenaline and noradrenaline, and that β receptor blocking agents supressed the increase in amylase secretion produced by various adrenergic stimuli, while α receptor
blooming agents did not. It was proposed that the sympathetic nervous system was important in the regulation of amylase secretion and that the β receptors were the ones involved in this mechanism. Effects of theophylline and various agents on amylase activities, protein concentrations and salivary flow rate in the steady state during the electric stimulation of auriculotemporal nerve, parasympathetic nerve, were observed by means of single drop analysis (2).

As well as adrenaline, theophylline also caused increases in amylase and protein secretion which were dose dependent. The responses to theophylline in amylase and protein secretion revealed later than those to adrenaline, and continued longer. Theophylline is known to inhibit phosphodiesterase, the enzyme which converts cyclic AMP to 5'-AMP (13). Since theophylline could increase the level of cyclic AMP by preventing its destruction, it may be considered that the increases in amylase and protein secretion produced by theophylline are mediated via cyclic AMP. The time lag of theophylline response may reflect the time for accumulation of cyclic AMP by preventing its destruction. It has been suggested that Ca++] plays an important role in the amylase secretion process at the salivary gland (14). It has been proposed that the release of Ca++] from intracellular stores might be involved in the inotropic activity of methylxanthines (15, 16). A work on Ca++] mobilizing effect of methylxanthines in the salivary glands required to discuss a possibility that Ca++] may play a role in the theophylline effect on amylase secretion. On the other hand, theophylline stimulates the central nervous system (CNS) and cardiac muscle. In the present experiments, section of the parasympathetic nerve and removal of the superior cervical ganglion were done, amylase levels are not modified by blood flow (17). Therefore, the actions of theophylline on the CNS and circulatory system were considered to be ruled out in a possible mechanism of increasing effect on amylase secretion. The peak of increase in amylase secretion produced with 10 mg/kg of theophylline was a little lower than that produced with 1 μg/kg of adrenaline. Schramm et al. (8, 18) showed that 10 mM of theophylline acted as a stimulant of amylase secretion from rat parotid slice equal in potency to 0.01 mM of adrenaline. Relative potency of theophylline to adrenaline in stimulating action on amylase secretion in vivo was like to a little weaker than that in vitro. Sutherland et al. (19) proposed four desirable criteria to establish that hormones acted by stimulating adenyl cyclase as mechanism by which given response was produced. The combined effect of adrenaline and theophylline was greater than the sum of individual their effect. This results are satisfied third criterion proposed by Sutherland et al. (19).

The increases in amylase and protein secretion produced by theophylline were decreased to about 30 and 70 per cent of control by pretreatment with 300 μg/kg of propranolol and 5 mg/kg of DCI respectively. As mentioned in the other reports (3, 5), these doses of propranolol and DCI almost completely inhibited catecholamine induced increase in amylase secretion. It was reported that inhibitory actions of DCI on inotropic action, phosphorylase activation and lipolysis induced by theophylline were much weaker than on those induced by catecholamines (20, 21). The present results suggest that theophylline
does not act primarily on $\beta$ receptors. It has been demonstrated that part of the chronotropic effect of methylxanthine in the dog heart-lung preparation is due to noradrenaline release since it is reduced by both propranolol and reserpine (22). Part of the theophylline-induced increase in amylase secretion which was depressed with $\beta$ receptor blocking agents may be due to noradrenaline release. The difference of inhibition degree by DCI and propranolol may also be explained by considering that DCI itself have sympathomimetic properties but propranolol have not.

Tsujimoto et al. (23) demonstrated that when injected into portal vein of dog, exogenous cyclic AMP (2 mg/kg) mimicked the action of adrenaline in causing the increase in concentrations of blood glucose and serum potassium, and the hepatic phosphorylase activation in vivo. Dibutyryl cyclic AMP is known to be more resistant than cyclic AMP to the action of phosphodiesterase and to penetrate cell membranes more readily, at least in some tissues (24). In this experiment, a significant increase in amylase secretion could not be observed after the intravenous and intraarterial injection of cyclic AMP and dibutyryl cyclic AMP. Cyclic AMP and dibutyryl cyclic AMP may penetrate into cell of intact salivary gland much more slowly than into intact hepatic cell. Schramm et al. (8, 18) described that initial lag period, longer than 5 minutes, was apparently due to the slow penetration of dibutyryl cyclic AMP into the cell in rat parotid gland. The present results in vivo is not agreement with those of Schramm et al. (8, 18) in vitro. A possible reason for this difference is that if injected substance would be distributed nonselectively, tissue concentration of dibutyryl cyclic AMP might be about one-hundredth of those in medium for incubation. Another reason is that the permeability into cell of intact salivary gland for dibutyryl cyclic AMP may differ from that into cell of salivary tissue sliced. It has not been demonstrated that cyclic AMP or dibutyryl cyclic AMP mimicked the action of catecholamines in causing inotropic action which is mediated through $\beta$ receptors in numerous studies (19).

From present experiments, it can not be concluded yet whether cyclic AMP may play a probable role in the regulation of amylase secretion.

**SUMMARY**

The present experiment was carried out to determine the effect of theophylline, cyclic AMP and dibutyryl cyclic AMP on amylase secretion induced by the auriculotemporal nerve stimulation in the rabbit parotid gland.

1. Theophylline showed increase in amylase secretion which revealed later and continued longer than that produced by adrenaline.

2. The combined effect of adrenaline and theophylline on amylase secretion was greater than the sum of individual their effect.

3. The increasing action of theophylline on amylase secretion was partially inhibited by pretreatment with propranolol and DCI.

4. A significant increase in amylase secretion could not be observed after cyclic AMP and dibutyryl cyclic AMP.
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