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DISSOCIATION OF β-ADRENOCEPTOR-INDUCED EFFECTS ON AMYLASE SECRETION AND CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE ACCUMULATION

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1 By using a multi-channel microperifusion system the effects of noradrenaline, the β_1-adrenoceptor agonist prenalterol, and the β_2-selective agonist terbutaline were studied on amylase release and cyclic adenosine 3′,5′-monophosphate (cyclic AMP) content in rat parotid and guinea-pig submandibular glands.

2 Noradrenaline caused significant amylase discharge and cyclic AMP accumulation.

3 Prenalterol was as effective as noradrenaline in causing amylase release but did not significantly affect the cyclic AMP content.

4 Terbutaline stimulated cyclic AMP accumulation, but had little effect on amylase secretion.

5 The present study reveals that there is a dissociation of the β-adrenoceptor-induced amylase release and cyclic AMP formation, and that this dissociation may be due to different β-adrenoceptor subtypes.

Introduction

Since Sutherland and colleagues first suggested that catecholamine binding to the adrenoceptor resulted in adenylate cyclase activation and a subsequent cyclic adenosine, 3′,5′-monophosphate (cyclic AMP) accumulation, several studies have emphasized cyclic AMP's role as second messenger in the β-adrenoceptor-mediated secretory process in salivary glands (*e.g.* Schramm & Selinger, 1975; Leslie, Putney & Sherman, 1976). The correlation between cyclic AMP content and enzyme secretion seems to be especially pronounced during the initial phase of enzyme secretion (Carlsöö, Danielsson, Henriksson & Idahl, 1979). It has been suggested that the adenylate cyclase of salivary glands is identical to the β-adrenoceptor (Robison, Butcher & Sutherland, 1967; Yamamoto, Inoki & Kojima, 1968).

Although numerous investigations support a direct coupling of β-adrenoceptor activation and cyclic AMP formation, data also have been presented suggesting that under certain conditions an increase in glandular cyclic AMP may not be necessary for the induction of β-adrenoceptor-stimulated amylase release (Harfield & Tenenhaus, 1973; Butcher, Goldman & Nemerovski, 1975).

In view of these findings and the large discrepancies in effect on amylase release amongst selective β-adrenoceptor agonists (Carlsöö, Danielsson, Henriksson & Idahl, 1981), it was considered of interest to study further the role of cyclic AMP as a second messenger in signal transmission following stimulation with selective β-adrenoceptor agonists. Because of the known species variation in the degree of cyclic AMP accumulation, the experiments were performed on both rat parotid and guinea-pig submandibular glands. Both glands are serous glands, have a dual innervation and contain large quantities of amylase.

Methods

Animals and tissue preparation

Female Sprague-Dawley rats and male guinea-pigs, age 9–12 weeks, were deprived of food for 18 h before use, but had water *ad libitum*. All experiments were started between 08 h 00 min and 09 h 00 min to avoid diurnal variations. The parotid glands from rats and submandibular glands from guinea-pigs were rapidly removed under pentobarbitone anaesthesia, immersed in basal medium and carefully freed from extraglandular tissue under a stereomicroscope.

Perifusions

A non-recycling, multi-channel perifusion system was used (see Carlsöö *et al.*, 1979). The microperifusion apparatus was modified to allow simultaneous perifusions of individual channels with control or test

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media. Pieces of rat parotid or guinea-pig submandibular glands were transferred by means of a braking pipette to each of the chambers (volume 4.5 μl). The tissue pieces were then perifused with gassed (95% O_2:5% CO_2) Krebs-Henseleit bicarbonate buffer supplemented with pyruvate, glutamate, fumarate and containing bovine serum albumin (1 g/l) and glucose (0.6 g/l). The perifusion rate was 20 μl per min and chamber pressure and oxygen partial pressure were continuously monitored throughout the experiment. A prestimulatory period of 15 min with basal medium preceded perifusion with secretagogue. For cyclic AMP determinations, the perifusion chambers containing tissue pieces were disconnected and plunged into melting isopentane at the intervals indicated in the figures. The chambers were then disconnected at −25°C, the inner tubes containing the frozen tissue were freeze-dried. The dry tissue pieces were then weighed on an ultramicro balance (UM 7, Mettler Instruments AG, Zürich, Switzerland).

Biochemical analyses

The perifusates were analysed for amylase activity using a micromodification of the 3,5-dinitrosalicylate (DNS) method (Danielsson, 1974). One unit of amylase was defined as the activity liberating reducing groups corresponding to 1 μmol of maltose monohydrate per min at 25°C. In the figures all values are expressed in relation to their respective non-stimulated controls.

Cyclic AMP was extracted from the tissue and assayed radioimmunologically with a commercially available test kit (for details see Hellman, Idahl, Lernmark & Taljedal, 1974).

Statistics

The *t* test was applied to the differences between paired control and test values.

Drugs

Soluble starch and 3,5-dinitro-salicylate were purchased from E. Merck AG, Darmstadt, Germany. (−)-Noradrenaline bitartrate was bought from Sigma Chemical Co., St Louis, Mo, USA. Terbutaline sulphate was a gift from Draco AB, Lund, Sweden and prenalterol a gift from Hässle AB, Mölndal, Sweden. ^{125}I-labelled succinyl cyclic AMP tyrosine methyl ester and cyclic AMP antibodies were bought from Becton/Dickinson, Orangeburg, N Y, USA. Bovine serum albumin was obtained from British Drug Houses Ltd, Poole. All reagents were of analytical grade.

Results

The secretagogue concentrations were based on the levels known to cause maximal amylase secretion in rat parotid and guinea-pig submandibular glands (Carlsöö, Danielsson, Marklund & Stigbrand, 1972; Carlsöö *et al.*, 1981; and unpublished results). The absolute values of non-stimulated amylase secretion and glandular content differed considerably between the two species tested (Table 1). There was also a slight species difference in basal content of cyclic AMP. Therefore the results are expressed as percentage change from controls (pre-stimulatory values = 100%).

Figure 1 shows the amylase secretion and cyclic AMP accumulation in rat parotid gland in response to the non-selective agonist noradrenaline, the β_1-selective agonist prenalterol and the β_2-agonist terbutaline. Each drug was given at 10^{-6} M. Prenalterol was almost as potent as noradrenaline in causing amylase secretion, whereas terbutaline induced only a small amount of amylase release (Figure 1a). Noradrenaline increased the tissue content of cyclic AMP by approx. 400%, and terbutaline increased it 250% (Figure 1b). Prenalterol did not significantly

Table 1 Basal amylase content, amylase release, and cyclic AMP content in rat parotid gland and guinea-pig submandibular gland

	n	*Rat parotid gland*	*Guinea-pig submandibular gland*
Amylase (units/g wet weight)	8	17,653 ± 1,357	3,581 ± 568
Basal amylase release (units/min)	8	116.5 ± 13.7	33.9 ± 7.1
Cyclic AMP (pmol/mg dry weight)	6	1.17 ± 0.10	1.67 ± 0.26

Values are means ± s.e.mean.
n = number of animals.

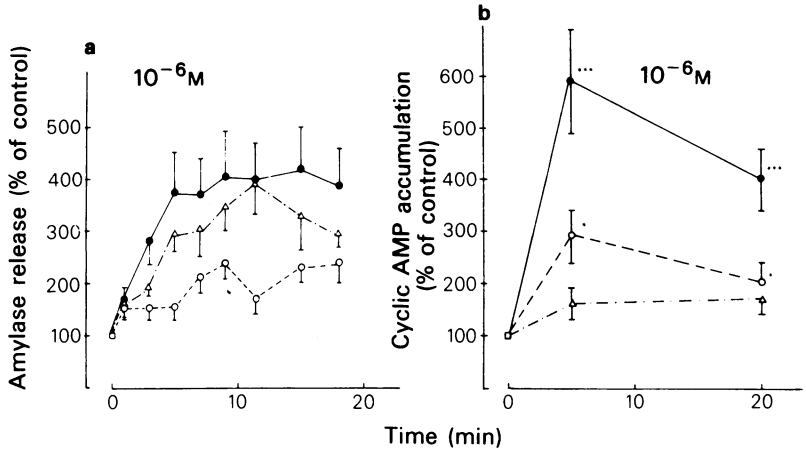

Figure 1 (a) Amylase release and (b) cyclic AMP accumulation in rat parotid gland in response to continuous exposure to 10^{-6} M noradrenaline (●), prenalterol (△), or terbutaline (○). Experimental design described in methods. All values expressed in relation to their non-stimulated, time 0 level (100%). Each point represents mean for 6 separate experiments; vertical lines indicate s.e.mean. $*P<0.05$; $***P<0.001$.

raise the cyclic AMP level. The concentrations of the two selective agonists were increased to 10^{-3} M (Figure 2). While both terbutaline and prenalterol caused marked amylase release, prenalterol failed to increase the cyclic AMP content even at this high concentration.

In the guinea-pig submandibular gland, amylase release was clearly stimulated by all three agonists at a concentration of 10^{-5} M (Figure 3a). Noradrenaline caused a very great increase in tissue cyclic AMP which persisted throughout the perifusion period. As in the rat parotid gland, terbutaline induced cyclic AMP accumulation, whereas prenalterol was without significant effect (Figure 3b). When the drug concentrations were increased to 10^{-3} M, the responses were similar to those in rat parotid gland (i.e. prenalterol still induced no cyclic AMP accumulation).

The secretory response to noradrenaline, terbutaline or prenalterol was not inhibited by perifusion with a Ca^{2+}-free medium, with or without added EGTA (0.5 mM).

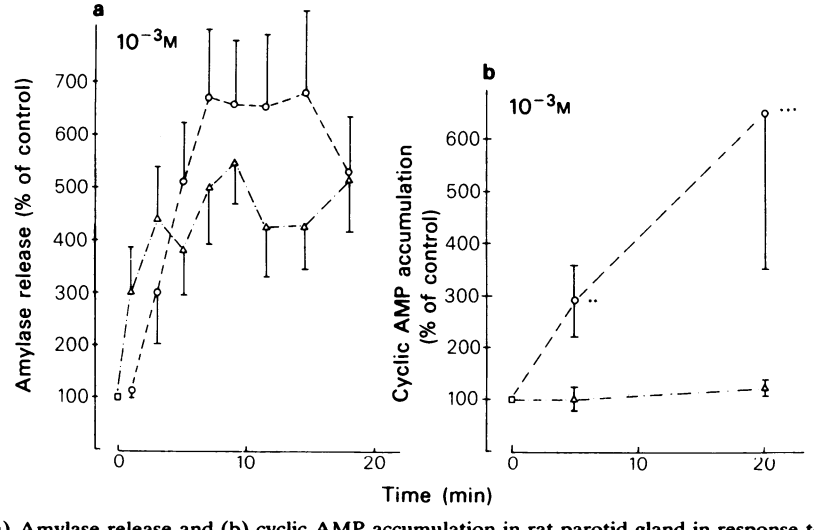

Figure 2 (a) Amylase release and (b) cyclic AMP accumulation in rat parotid gland in response to continuous exposure to 10^{-3} M prenalterol (△) or terbutaline (○). Experimental design described in methods. All values expressed in relation to their non-stimulated, time 0 level (100%). Each point represents mean for 4 separate experiments; vertical lines indicate s.e.mean. $**P<0.01$; $***P<0.001$.

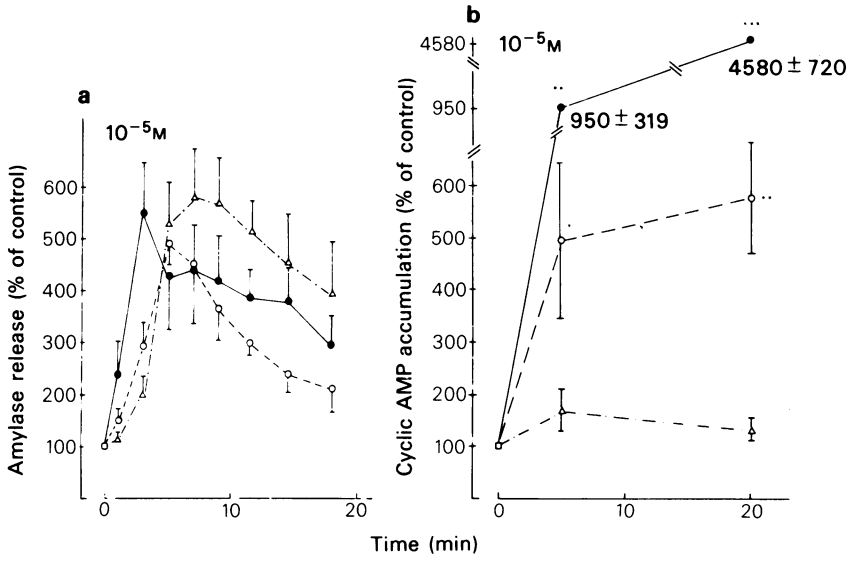

Figure 3 (a) Amylase release and (b) cyclic AMP accumulation in guinea-pig submandibular gland in response to continuous exposure to 10^{-5} M noradrenaline (●), prenalterol (△), or terbutaline (○). Experimental design described in methods. All values expressed in relation to their non-stimulated, time 0 level (100%). Each point represents mean for 4 separate experiments; vertical lines show s.e.mean. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Discussion

In the present study, noradrenaline caused both amylase release and a significant increase in cyclic AMP content in rat parotid and in guinea-pig submandibular glands. The cyclic AMP accumulation was markedly higher in the guinea-pig, whereas amylase release was higher in the rat parotid. However, the percentage of amylase content that was released was similar in both glands.

Prenalterol, characterized as a β_1-agonist (Carlsson, Dahlöf, Hedberg, Persson & Tängstrand, 1977), has been shown to be as effective as noradrenaline and isoprenaline in causing amylase release from parotid gland, while terbutaline, the β_2-agonist, was much less effective (Carlsöö *et al.*, 1981). This dominance of β_1-adrenoceptor agonist in eliciting amylase discharge was further substantiated by the present investigation. On the other hand terbutaline caused a significant increase in cyclic AMP, and prenalterol had no effect. Hence, it can be concluded that secretion of amylase from salivary glands can be induced by a β_1-selective agonist without notable changes in total intracellular content of cyclic AMP. A poor correlation between salivary gland enzyme release and cyclic AMP content is supported by previous studies (Harfield & Tenenhaus, 1973; Butcher *et al.*, 1975).

A lack of significant effect of prenalterol on myocardial adenylate cyclase has previously been shown (Hedberg, Carlsson, Fellenius, & Lundgren, 1981). Moreover, prenalterol produced a dose-dependent inhibition in the isoprenaline-mediated adenylate cyclase activation, whereas this was not observed with the β_2-agonist procaterol. In cat soleus muscle the β_2-agonists were able to stimulate adenylate cyclase activity, while prenalterol was not (Hedberg & Mattsson, 1981).

Ahlqvist (1976) has discussed the possibility that a specific β-adrenoceptor exists for each physiological effect, and the pharmacological specificity of the β-adrenoceptors in different tissue preparations varies (Boisser, Advenier, Giudicelli & Viars, 1971; Harms, Zaagsma & de Vente, 1977). However, it is now well established that there exist no more than two subtypes of the β-adrenoceptor (*e.g.* Barnett, Rugg & Nahorski, 1978; Minneman, Hedberg & Molinoff, 1979). The agonist-stimulated increase in cyclic AMP content could be a parallel phenomenon which is not tightly coupled to the stimulus-secretion process. This would require an alternative mediator for the initiation of the secretion. As shown in this study, extracellular Ca^{2+} does not seem to be a prerequisite for the β-adrenoceptor-stimulated amylase secretion, as is the case for both cholinergic and α-adrenergic stimulation of enzyme and electrolyte output (Schramm & Selinger, 1975; Leslie *et al.*, 1976).

The lack of a correlation between cyclic AMP formation and amylase release may be explained by the presence of spare β-adrenoceptors on the acinar cells. In the heart, prenalterol was found to cause

only a marginal stimulation of adenylate cyclase (Hedberg *et al.*, 1981). Prenalterol seems to be a partial agonist with about 80% intrinsic activity as regards the final physiological effect in the cat heart but only 8% activity on the adenylate cyclase enzyme system. Assuming a direct one-to-one coupling between the β-adrenoceptors and the adenylate cyclase, the results obtained in the heart would fit with the spare receptor theory (Hedberg *et al.*, 1981).

However, in the rat parotid, this theory appears less possible because prenalterol has been shown to be as effective as isoprenaline and noradrenaline in causing amylase secretion (Carlsöö *et al.*, 1981). Conflicting results from different organs could be explained by the fact that some component(s) distal to the receptor rather than the specificity of the β_1/β_2-subtypes determine the relative drug intrinsic effect (Pike *et al.*, 1979).

In conclusion, large amounts of amylase can be released from the rat parotid and guinea-pig submandibular acinar cells despite very small or no increases in cyclic AMP content. These results conflict with the theory that cyclic AMP is the second messenger for all the β-adrenoceptor-induced effects. The results might be explained by assuming that the different β-adrenoceptor subtypes have no parallel effects on amylase release and adenylate cyclase. This raises the question: What is the biological role of large increases in cyclic AMP after stimulation with noradrenaline? Cyclic AMP may serve additional roles within the cell (e.g. stimulation of protein synthesis) directly and independently of its effect on amylase secretion (Grand & Gross, 1969).

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