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CHARACTERIZATION OF THE RAT PAROTID β-ADRENOCEPTOR

BENGT CARLSÖÖ, ÅKE DANIELSSON*,
ROGER HENRIKSSON† & LARS-ÅKE IDAHL†

Departments of Otolaryngology, Medicine* and Histology†, University of Umeå,
S-901 87 Umeå, Sweden

1 The effects of various β-adrenoceptor agonists on amylase secretion from the rat parotid gland were studied by means of two different *in vitro* techniques.

2 The dose-response relation for each agonist was established, as also were the ED_{50} values.

3 All drugs appeared to act directly on the acinar cells, as reserpine-treatment did not abolish their secretagogic effects.

4 Two groups of agonists could be distinguished: one group consisting of adrenaline, noradrenaline and the β_1-selective agonist prenalterol (H133/22) with a high enzyme discharge potency and a second group consisting of the β_2-agonists, terbutaline and salbutamol, with a markedly lower effect.

5 The present data further support the theory that rat parotid acinar cells are supplied mainly with β-adrenoceptors of the β_1-subtype, similar to those present in heart and adipose tissue.

Introduction

The β-adrenoceptors can be divided into two subtypes, one present mainly in heart and fat tissue (β_1) and the other in lung and blood vessels (β_2) (Lands, Arnold, McAuliff, Luduena & Brown, 1967a; Lands, Luduena & Buzzo, 1967b). However, the adrenoceptors have not yet been isolated or characterized biochemically, although the recent development of high affinity selective β-adrenoceptor blocking agents as well as of selective β_1- and β_2-receptor agonists has facilitated more specific analyses of the β-receptors present in various tissues, including the salivary glands. Studies employing receptor selective drugs, both *in vivo* and *in vitro*, indicate that salivation in dogs (Thulin, 1972) and parotid amylase release in rats (Butcher, Goldman & Nemerovski, 1975; Derrick, Malbon & Butcher, 1977; Carlsöö, Danielsson & Henriksson, 1978) are mediated mainly via activation of β_1-adrenoceptors.

The present *in vitro* investigation was undertaken in an attempt to obtain further information on the mechanisms of receptor specificity and action in the rat parotid gland, with regard to amylase secretion. For this purpose two quite different sensitive *in vitro* techniques were employed. In one system, enzyme release was studied by use of a multichannel microperifusion technique (Carlsöö, Danielsson, Henriksson & Idahl, 1979), while in the other, amylase discharge was analysed after batch incubation of minute parotid slices according to the method described by Danielsson, Marklund & Stigbrand (1974).

Methods

Animals and tissue preparation

Female Sprague–Dawley rats, 3 to 4 months of age, were deprived of food for 18 h before use but had free access to water. All experiments were started between 08 h 00 min and 09 h 00 min to avoid diurnal variations. In one series of experiments the animals were given an intraperitoneal injection of reserpine, 10 mg/kg body weight, 12 to 18 h before tissue excision. The animals were anaesthetized with ether and the parotid gland from the left side was rapidly excised and transferred to a Krebs–Henseleit bicarbonate buffer supplemented with pyruvate, glutamate and fumarate (Krebs, 1950). Extraglandular tissues were carefully removed under a stereomicroscope.

Batch incubation

Pieces of parotid tissue weighing approximately 5 mg each were transferred to incubation vessels containing basal medium prewarmed to 37°C and equilibrated with 95% O_2 and 5% CO_2. The basal medium was a supplemented Krebs–Henseleit bicarbonate buffer, but 1 g/l bovine serum albumin (BSA) and 0.6 g/l glucose were also added. All specimens were preincubated for 15 min in 500 µl medium at 37°C in a metabolic shaker. Plastic vessels specially designed for continuous equilibration of the medium with 95% O_2 and 5% CO_2 were used (Danielsson, 1974; Danielsson *et al.*, 1974). After preincubation and a rinse in fresh

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buffer, 500 µl of prewarmed and gassed incubation medium containing the different secretagogues was added. Control incubations without added drugs were included in each set of experiments. The incubations were carried out for 30 min and the wet weight of the tissue samples was then recorded. The specimens were sonicated (Branson Inc., 50 W, 15 to 20 s) in a Na–K-phosphate buffer (50 mM, pH 6.9).

Perifusion

A non-recycling, multichannel perifusion system was used (Idahl, 1972; Carlsöö *et al.*, 1979). The microperifusion system was modified to allow perifusion with control and test medium fed to different tissue chambers which were inserted parallel to one another. Pieces of parotid gland were transferred by means of a braking pipette to each of the perifusion chambers (volume 4.5 µl). The tissue pieces were then perifused with gassed (O_2–CO_2) and supplemented Krebs–Ringer–Henseleit bicarbonate buffer (Krebs, 1950) containing BSA and glucose, as for the batch incubations. The perifusion rate was 17 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 secretagogues.

Amylase assay

Incubation media, tissue homogenates and perifusates were analysed for amylase activity by a micromodification of the 3,5-dinitro-salicylate method with 2% soluble starch as substrate (Danielsson, 1974). One unit of amylase is defined as the activity liberating reducing groups corresponding to one µmol of maltose monohydrate per min at 25°C. The amylase release is expressed as the percentage of amylase in medium of the total amylase content in media plus homogenate for the batch incubation experiments. In the perifusions, amylase secretion is expressed as u/min.

Chemicals

Soluble starch and 3,5-dinitro-salicylate were obtained from E. Merck AG, Darmstadt, Germany. (−)-Noradrenaline bitartrate and 5-hydroxydopamine were purchased from Sigma Chemical Co., St. Louis, Mo, U.S.A. Salbutamol was a gift from Glaxo Läkemedel AB, Mölndal, Sweden, and terbutaline sulphate from Draco AB, Lund, Sweden. H133/22 [prenalterol; (−)-1-(4-hydroxy-phenoxy)-3-isopropylaminopropranol-2-hydrochloride] and the racemic compound H80/62 were figts from Hässle AB, Mölndal, Sweden. Reserpine (Serpasil) was from Ciba-Geigy AG, Basle, Switzerland. All chemicals used were of analytical grade. Deionized, sterile filtered water was used throughout the experiments.

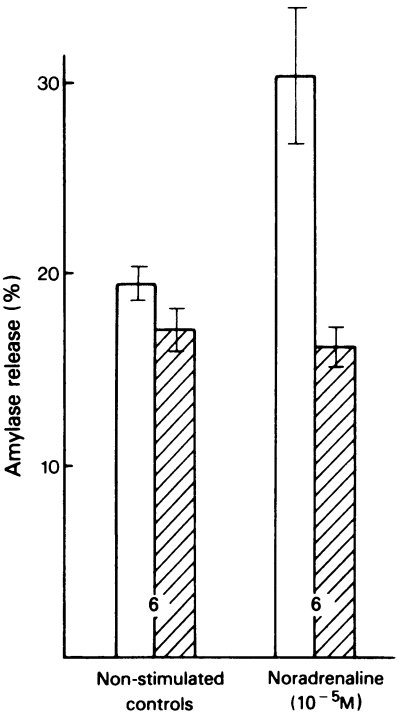

Figure 1 Effect of dinitrophenol (DNP; 10^{-3} M) (hatched columns) on basal and noradrenaline-induced amylase secretion from incubated rat parotid gland. Amylase release is expressed as a percentage. Values are means for indicated number of experiments; vertical lines show s.e. mean.

Results

Uncoupling of oxidative phosphorylation

To study whether non-stimulated amylase release from incubated pieces of parotid gland is an active secretory process of enzymes or merely occurs by passive leakage, dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, was added to the incubation medium. Figure 1 shows how this compound completely abolished noradrenaline-induced amylase secretion, whereas no discernible effect was obtained on the basal secretion values.

Reserpine-treatment

To exclude the possibility that the various β-adrenoceptor agonists might be acting indirectly by liber-

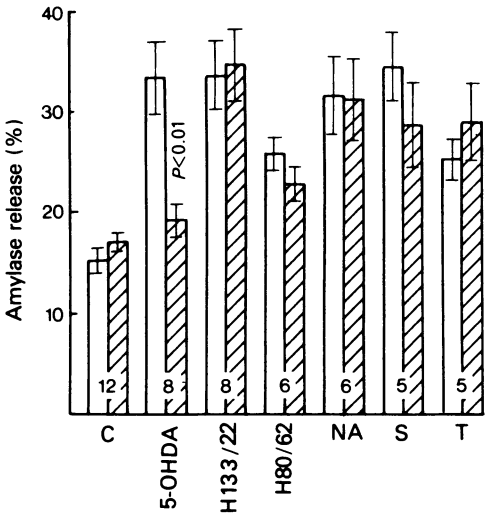

Figure 2 Effect of β-adrenoceptor agonists on amylase secretion from incubated rat parotid gland of reserpine pretreated and control animals. The concentrations used were 10^{-5} M for noradrenaline (NA), H80/62 and prenalterol (H133/22) and 10^{-4} M for 5-hydroxydopamine (5-OHDA), terbutaline (T) and salbutamol (S). Hatched columns indicate reserpine-treated animals and open columns non-treated controls. Amylase release is expressed as a percentage. Mean for indicated number of experiments; vertical lines show s.e. mean.

ating neurotransmitter substances from sympathetic nerve endings present in the gland, one group of rats were pretreated with reserpine in order to deplete the endogenous stores of noradrenaline. Pieces of parotid glands from these animals as well as from non-reserpine-treated rats were incubated in the presence of the secretagogues (Figure 2). 5-Hydroxydopamine (5-OHDA) effectively stimulated amylase release from the parotid gland of the non-reserpine-treated rat but had no secretory effect after reserpine treatment. 5-OHDA is known to exert its secretagogic action by liberating endogenous stores of transmitters. On the other hand, there were no differences in amylase secretion induced by the β-adrenoceptor agonists tested, thus indicating a direct action on the parotid cell membranes.

Dose-response relations

In Figures 3 and 4 the secretory dose-response relations for various β-adrenoceptor agonists with known differing subspecificity (β_1 and β_2) are depicted (noradrenaline, adrenaline, isoprenaline, prenalterol (H133/22), terbutaline and salbutamol). At the plateau level, adrenaline, noradrenaline and isoprenaline caused an almost identical secretory response of roughly 35%, whereas prenalterol tended to give a slightly higher effect of about 40%. Terbutaline, even at the highest concentrations, provoked an amylase secretion of about 27% only.

Table 1 shows the ED_{50} values and relative potencies for the non-selective and selective β-adrenoceptor agonists. Prenalterol, a specific β_1-agonist, was the most effective secretagogue tested, whereas the β_2-agonists, salbutamol and terbutaline, were the least effective. The ED_{50} value for prenalterol was as low as 1.5×10^{-8} M compared with that of terbutaline, 1.2×10^{-5} M. Isoprenaline, noradrenaline, H80/62 (the racemic form of H133/22) and adrenaline constituted an intermediate group with respect to secretagogic action on amylase from rat parotid gland. These data were confirmed in the dynamic perifusion system, in which prenalterol was found to be more effective than terbutaline at a concentration of 10^{-6} M (Figure 5a). However, an increase in the concentration of prenalterol to 10^{-3} M, gave no further increase in amylase secretion. Terbutaline, on the other hand, was more potent at this extremely high concentration (Figure 5b). Furthermore, there was a decrease in prenalterol-induced amylase release after prolonged perifusion with the high concentration of the compound, which could indicate a toxic effect on the acinar cells.

Discussion

In the present study both batch incubations and a multichannel perifusion technique were used to investigate β-adrenoceptor-induced amylase secretion from

Table 1 ED_{50}-values for various non-selective and selective β-adrenoceptor agonists with respect to amylase secretion from pieces of incubated rat parotid gland

Test substance	*ED_{50}-value* (M)	*Potency in relation to:* *Prenalterol (H133/22)*	*Terbutaline*
Prenalterol (H133/22)	1.5×10^{-8}	1	800
Isoprenaline	9.4×10^{-8}	0.16	128
Noradrenaline	1.5×10^{-7}	0.10	80
H80/62	2.6×10^{-7}	0.058	47
Adrenaline	4.0×10^{-7}	0.038	30
Salbutamol	5.6×10^{-6}	0.0027	2
Terbutaline	1.2×10^{-5}	0.0013	1

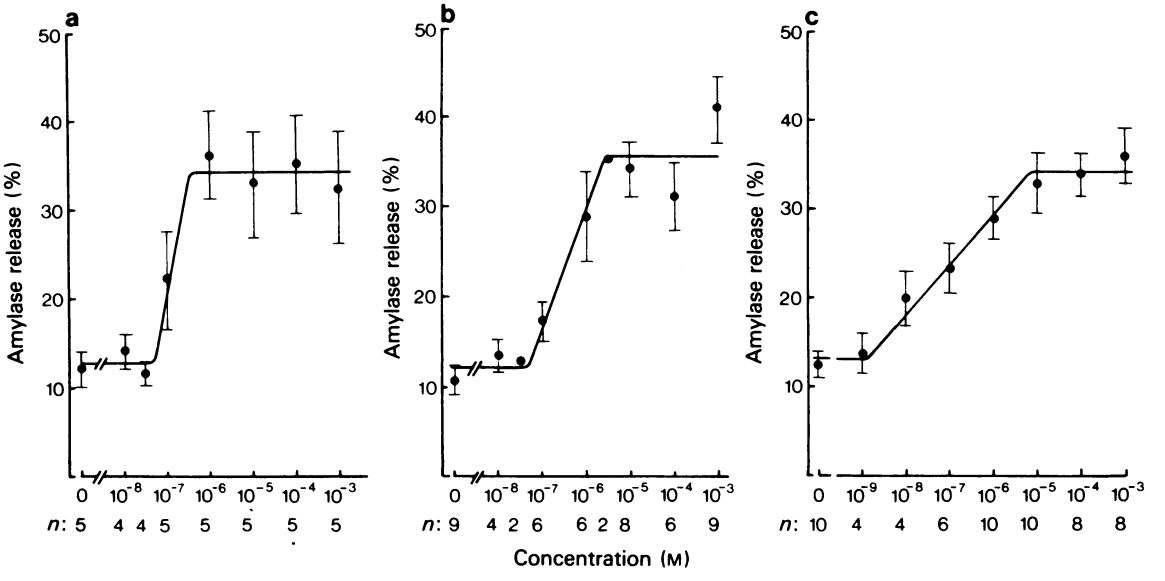

Figure 3 Dose-response curves for (a) noradrenaline, (b) adrenaline and (c) isoprenaline. Amylase release is expressed as a percentage. Each point represents mean for indicated number of observations; vertical lines show s.e. mean.

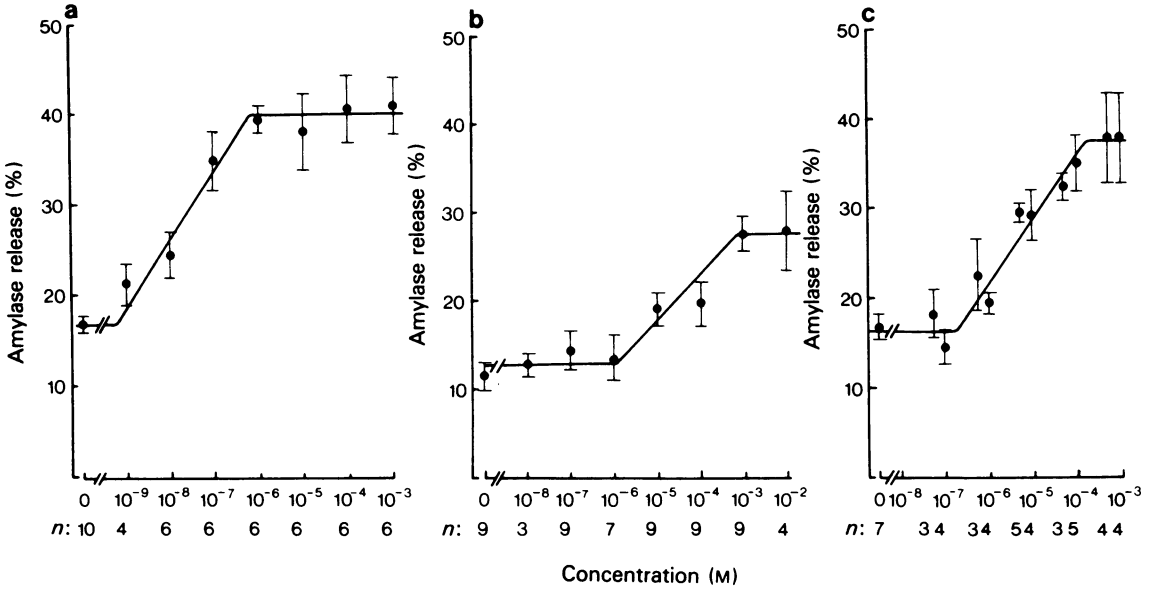

Figure 4 Dose-response curves for (a) prenalterol (H133/22), (b) terbutaline and (c) salbutamol. Amylase release is expressed as a percentage. Each point represents mean for indicated number of observations; vertical lines show s.e. mean.

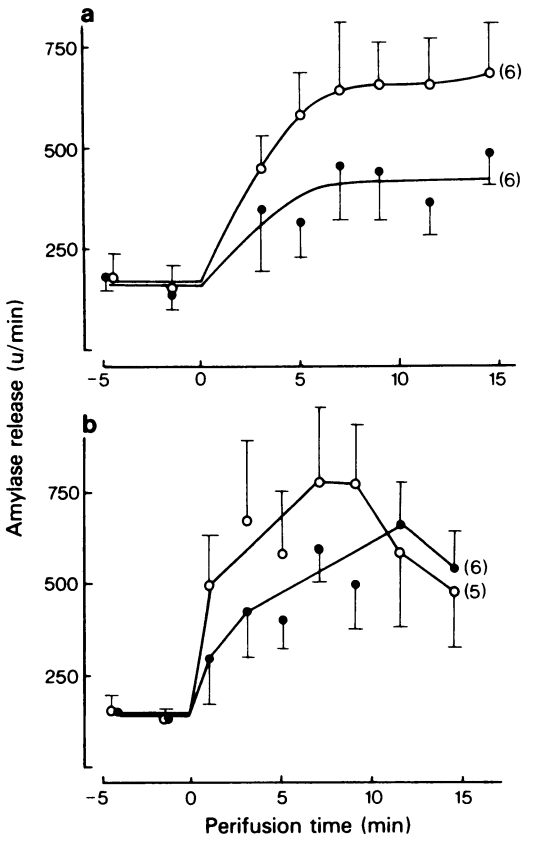

Figure 5 Dynamics of amylase release in response to continuous exposure to selective β_1- and β_2-adrenoceptor agonists at various concentrations. Amylase release is expressed in units/min. Each point represents mean for number of experiments indicated in parentheses. (a) Perifusion with prenalterol (○) or terbutaline (●) at 10^{-6} M; (b) perifusion with prenalterol (○) or terbutaline (●) at 10^{-3} M.

rat parotid gland. The parotid gland of the rat exhibits a relatively high basal enzyme release *in vitro*, which is unaffected by dinitrophenol, indicating that this basal secretion is due mainly to passive diffusion and is unrelated to energy-dependent processes. The same phenomenon has previously been shown to occur also in the guinea-pig submandibular gland (Carlsöö, Danielsson, Marklund & Stigbrand, 1972). The series of experiments with reserpine-treated animals also demonstrated that all tested substances except 5-OHDA appear to exert their secretory effect directly on the acinar cells of the gland. 5-OHDA is known to liberate noradrenaline from the adrenergic nerve terminals by a replacement process (Thoenen & Tranzer, 1971).

Selective β_1-receptor agonists are more potent than the β_2-receptor agonists with regard to salivary amylase discharge. Prenalterol (H133/22), which is an agonist of the β_1 type, is roughly 800 times more potent than the β_2-agonist, terbutaline, in evoking amylase release and about 400 times more potent than salbutamol. Prenalterol is 5 to 10 times more effective than isoprenaline, noradrenaline and adrenaline as well as the racemic form of prenalterol (H80/62). Prenalterol is a highly potent β_1 selective agonist in the sinus node and myocardium of the cat (Carlsson, Dahlöf, Hedberg, Persson & Tångstrand, 1977). Belfrage (1978) has also shown that vasodilatation induced by prenalterol or noradrenaline in canine subcutaneous adipose tissue can be completely blocked by the selective β_1-antagonist practolol, though this latter drug does not counteract vasodilatation in skeletal muscle of the dog, in which salbutamol is a potent vasodilator.

Isoprenaline was also found to be a potent enzyme secretagogue, in agreement with several other studies (e.g. Butcher *et al.*, 1975; 1976). This synthetic catecholamine stimulates β-adrenoceptors in a non-specific manner and does not appear to differentiate between β-receptors of the different subtypes. As regards salbutamol, it is equipotent in its dilator effect on the bronchial smooth muscles, although on the other hand salbutamol exhibits only 5 vs. 1% of the effect of isoprenaline on cardiac muscle and on lipolysis (Fain, 1973), thus indicating a chemical difference in the structure of the β-adrenoceptors of the myocardium and bronchial smooth muscle.

At low concentrations noradrenaline was more potent than adrenaline as an amylase secretagogue from the rat parotid gland. In comparisons between the β_1-adrenoceptors, for instance of heart and adipose tissue, and the β_2-receptors of bronchial smooth muscle, it has been demonstrated that adrenaline is more effective than noradrenaline in stimulating the β_2-receptors, whereas noradrenaline is equally or more potent as a β_1-agonist (Fain, 1973). The superiority of noradrenaline over adrenaline in inducing amylase secretion from rat parotid gland in combination with the high potency of prenalterol further emphasizes that the effect is mediated mainly via the β_1-adrenoceptor subtype. This confirms the previous data of Butcher *et al.* (1975) that isoprenaline is considerably more effective than salbutamol in provoking amylase release from rat parotid gland incubated *in vitro*. The relatively high efficacy of the β_2-selective drugs, salbutamol and terbutaline, at high concentrations, is thought to be due to a non-selective interaction with the β_1-adrenoceptors. Parotid acinar cells are supplied with adrenoceptors of the β_1-subtype, acetylcholine receptors and dopamine receptors

(Bloom, Carlsöö & Danielsson, 1975) involved in the secretion of amylase. Therefore, when used with sensitive micro-incubation techniques, this gland is a suitable model for studies on receptor characterization and mediation of secretory processes.

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Reprint requests to R.H., Dept. of Histology.

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