Br. J. Pharmac. (1978), **62**, 364–366

EFFECTS OF A NEW SELECTIVE β_1-ADRENOCEPTOR AGONIST ON AMYLASE SECRETION FROM THE RAT PAROTID GLAND

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The effects of a new selective β_1-adrenoceptor agonist, (−)-1-(4-hydroxyphenoxy)-3-isopropyl-amino-propanol-2-hydrochloride (H 133/22), on amylase secretion from the rat parotid gland were investigated in an *in vitro* system. The results were compared to the secretory responses obtained with noradrenaline, adrenaline, methoxyamine and terbutaline. H 133/22 was found to be a potent enzyme secretagogue and appeared even more effective than noradrenaline and adrenaline, particularly at low concentrations. The β_2-adrenoceptor agonist, terbutaline, also stimulated amylase discharge from the parotid gland but was much less potent than H 133/22. Methoxyamine had no effect on enzyme secretion. We suggest that the adrenergic stimulation of amylase secretion from the rat parotid gland is mainly mediated by β_1-receptors.

Introduction Based on response to adrenergic stimulation, amylase secretion from several serous salivary glands has been classified as a β-adrenoceptor effect, modulated by the cyclic adenosine 3′,5′-monophosphate (cyclic AMP) system. However, major differences in responses elicited by the biogenic amines in various organ systems implies that the β-adrenoceptor does not constitute a single receptor population. Evidence has also been presented for a subdivision of β-receptors into two subtypes: one is present mainly in fat tissue and heart (β_1) and the other in lung and blood vessels (β_2) (Lands, Arnold, McAuliff, Luduena & Brown Jr. 1967a; Lands, Luduena & Buzzo, 1967b). In the rat parotid gland, isoprenaline is a potent enzyme secretagogue, whereas salbutamol, a β_2-agonist, is considerably less effective. Accordingly, it has been proposed that the catecholamines act on the parotid secretory cells through a β_1-adrenoceptor mechanism (Butcher, Goldman & Nemerovski, 1975).

In the present investigation a comparative study on amylase discharge from the rat parotid gland was carried out using both synthetic β_1- and β_2-adrenoceptor agonists and the naturally occurring catecholamines, adrenaline and noradrenaline. A new compound, H 133/22 ((−)-1-(4-hydroxyphenoxy)-3-isopropyl-amino-propanol-2-hydrochloride), was employed as a selective β_1-agonist (Carlsson, Dahlöff, Hedberg, Tångstrand & Persson, 1977), and terbutaline was used as a β_2-selective stimulator. The α-adrenoceptor agonist, methoxyamine, was also included in the study.

Methods Female Sprague-Dawley rats, 3 months of age, weighing approximately 200 g, were used for the experiments. Before the animals were killed they were deprived of food for 18 h but had free access to water. All experiments were started between 08 h 00 min and 09 h 00 min to avoid diurnal variations. The parotid glands were rapidly excised and transferred to a Kreb-Henseleit bicarbonate buffer supplemented with pyruvate, glutamate and fumarate (Krebs, 1950). Extraglandular tissues were removed under a dissecting stereomicroscope and the parotid tissue was cut into pieces weighing approximately 5 mg each.

For the incubations the basal medium used was a Krebs-Henseleit bicarbonate buffer (pH 7.4) supplemented with pyruvate, glutamate and fumarate as above; 1 mg/ml bovine serum albumin and 0.6 mg/ml glucose were also included in the medium. All specimens were preincubated for 15 min in 500 μl medium at 37°C in a metabolic shaker. Special plastic vessels designed for continuous equilibration of the medium with 95% O_2 and 5% CO_2 were used (Danielsson, 1974). After preincubation, the medium was removed with the aid of a pipette and the pieces were rinsed once with fresh buffer at a temperature of 37°C. Thereafter, 500 μl of fresh, prewarmed, gassed incubation medium, containing the different secretagogues at three concentrations (10^{-3}, 10^{-5}, 10^{-7} M) was added to the tissue specimens. Control incubations without test substances were included in each experiment. After preincubation and incubation the tissues were homogenized in a sonicator (Branson Inc., 50 W, 15–20 s). Incubation media as well as the homogenates were appropriately diluted with Na-K-phosphate buffer (0.05 M; pH 6.9) and assayed for amylase by a micromodification of the 3,5-dinitrosalicylate (DNS) method, with 2% soluble starch as substrate (Danielsson, 1974). One unit of amylase is defined as the activity liberating reducing groups corresponding to 1 μmol of maltose monohydrate per min at 25°C. The amylase release into the medium was expressed

Table 1 Effects of various adrenoceptor agonists on amylase secretion from the rat parotid gland

Secretagogues	*Non-stimulated controls*	10^{-7} M	10^{-5} M	10^{-3} M
H 133/22	16.77 ± 0.74	33.13 ± 2.16	37.95 ± 4.21	40.02 ± 3.32
(*n*)	(10)	(10)	(6)	(6)
Noradrenaline	14.06 ± 1.33	26.72 ± 2.37	32.76 ± 5.89	36.73 ± 6.04
(*n*)	(9)	(8)	(5)	(5)
Adrenaline	12.68 ± 1.44	19.26 ± 1.69	34.36 ± 2.89	39.18 ± 3.57
(*n*)	(12)	(12)	(8)	(8)
Terbutaline	11.40 ± 1.47	14.50 ± 2.60	18.87 ± 1.96	27.54 ± 1.99
(*n*)	(9)	(9)	(9)	(9)
Methoxyamine	13.79 ± 1.64	8.50 ± 2.13	12.46 ± 2.91	16.67 ± 3.65
(*n*)	(5)	(5)	(5)	(5)

Amylase release is expressed as percentage of the total enzyme activity in tissue and medium. Mean values (%) ± s.e. for number of experiments (in parentheses) are given.

as % of the total amylase activity in medium and tissue homogenate.

In one series of experiments, animals were pretreated with an intraperitoneal injection of reserpine, 10 mg/kg body weight, 18 h before they were killed.

Drugs (−)-Noradrenaline bitartrate and (−)-adrenaline bitartrate were obtained from Sigma Chemical Co, St. Louis, Mo, USA. H 133/22 ((−)-1-(4 hydroxyphenoxy)-3-isopropylamino-2-propanol) was a gift from Hässle AB, Hässle, Sweden and terbutaline sulphate from Draco AB, Lund, Sweden. Reserpine (Serpasil) was from Ciba-Geigy AG, Basel, Switzerland and methoxyamine from Serva Feinbiochemica, D-6900 Heidelberg 1, West Germany.

Results The secretory responses to the secretagogues are summarized in Table 1. At 10^{-3} and 10^{-5} M, H 133/22 evoked an amylase release, on the whole comparable to that obtained with adrenaline and noradrenaline. However, at low concentrations (10^{-7} M) H 133/22 was even more effective than these catecholamines. Terbutaline, at all concentrations, was far less effective than H 133/22, noradrenaline or adrenaline. Methoxyamine did not stimulate amylase secretion.

To determine whether H 133/22 exerts a direct effect on the acinar cells or an indirect effect (e.g. by liberation of noradrenaline from sympathetic nerve endings) rats were pretreated with reserpine 18 h before the experiment to deplete endogenous stores of this amine. After such treatment no difference in effects of H 133/22, noradrenaline or terbutaline on amylase secretion were noted. This indicates a direct action of the secretagogues on the acinar cells of the parotid gland.

Discussion Salbutamol, a β_2-adrenoceptor agonist, has previously been shown to evoke salivation from the submandibular gland of both rats and dogs *in vivo* (Thulin, 1972). However salbutamol is far less potent than isoprenaline, indicating a mainly β_1-adrenoceptor response in these glands (Thulin, 1972). Moreover, Butcher and co-workers (1975) have demonstrated that isoprenaline is considerably more effective than salbutamol in evoking amylase discharge from the rat parotid gland *in vitro*, suggesting a β_1-adrenoceptor mediation also in this gland. In the present study, terbutaline, another effective bronchodilator and a more selective β_2-agonist (Bergman, Persson & Wetterlin, 1969), was found to be less potent than H 133/22 with respect to stimulatory effects on amylase secretion.

H 133/22 has been shown to be a highly potent β_1-selective agonist in the sinus node and myocardium of the cat (Carlsson *et al.*, 1977). Belfrage (1977) has shown that vasodilatation induced by H 133/22 or noradrenaline in dog subcutaneous adipose tissue can be completely blocked by the β_1-antagonist, practolol. Practolol does not antagonize vasodilatation induced by salbutamol (β_2-agonist). Moreover, H 133/22 causes no vasodilatation in skeletal muscle tissue of the dog, where salbutamol is a potent vasodilator (Belfrage, 1977). The above results confirm that H 133/22 is a selective β_1 adrenoceptor-agonist.

In the present investigation it was found that at low concentrations noradrenaline was more potent than adrenaline as an amylase secretagogue. On comparison between the β_1-adrenoceptors of e.g. heart and adipose tissue and the β_2-receptors of the bronchial smooth muscle, it has been found that adrenaline is more effective than noradrenaline in stimulating the β_2-receptors, whereas noradrenaline is equally or even more potent as a β_1-agonist (Fain, 1972). The relatively high secretory potency of noradrenaline in salivary glands suggests that the receptors in these glands are similar to the β-receptors in cardiac and adipose tissue (Robison, Butcher & Sutherland,

1971). The effects of H 133/22 described in the present paper as well as the results obtained with noradrenaline, adrenaline and the β_2-adrenoceptor agonist, terbutaline, provide further support for the concept that amylase discharge from the rat parotid gland in response to sympathomimetic agents should be classified as a β_1-adrenoceptor response. The role of cyclic AMP in this β-adrenergic induction of enzyme secretion is well established. Further studies may elucidate whether, and to what extent, H 133/22 influences the adenylate cyclase activity and cyclic AMP accumulation in the stimulated parotid gland.

This work was supported by grants from the Swedish Medical Research Council (Project No. B77-19X-04992) and the Mångberg Fund, Umeå. The skilful technical assistance of Miss Si W. Domeij is gratefully acknowledged.

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(*Received October 31, 1977.*)