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DOPAMINE-INDUCED AMYLASE SECRETION FROM GUINEA-PIG SUBMANDIBULAR GLAND

G.D. BLOOM, B. CARLSÖÖ & Å. DANIELSSON

Department of Histology, University of Umeå, S-901 87 Umeå, Sweden

1 The effects of dopamine, 5-hydroxydopamine (5-OHDA) and noradrenaline on amylase secretion from the guinea-pig submandibular gland were investigated under *in vitro* conditions.

2 All three amines greatly enhanced amylase secretion. Blockade of dopamine β-hydroxylase did not inhibit the response to dopamine.

3 Noradrenaline and dopamine stimulated amylase release from salivary glands of reserpine-treated animals, whereas 5-OHDA had no stimulatory effect on secretion in guinea-pigs pretreated with reserpine.

4 Haloperidol completely inhibited dopamine-induced enzyme discharge, but did not affect noradrenaline-initiated secretion.

5 Apomorphine caused a slight enzyme release by itself; it diminished the dopamine secretory effect, but did not modify that of noradrenaline.

6 Pimozide and fluspirilene attenuated the dopamine-induced enzyme discharge, but compared with haloperidol they were less effective.

7 It is concluded that dopamine exerts a secretagogic action different from that of noradrenaline. The possible presence of a specific dopamine receptor in salivary glands is discussed.

Introduction

Catecholamines, by stimulating the adenylate cyclase system, are most potent inducers of enzyme secretion in salivary glands. Parasympathomimetic agents, although potent, are less effective (Schramm, 1967; Carlsöö, Danielsson, Marklund & Stigbrand, 1972). Noradrenaline-induced enzyme secretion from salivary glands is inhibited by β-adrenoceptor blocking agents but not by α-adrenoceptor blockers. Dopamine, a precursor substance in the biosynthesis of noradrenaline, is also a potent inducer of amylase and peroxidase discharge from the guinea-pig submandibular gland. However, the dopamine initiated secretion is inhibited not only by β-adrenoceptor blockers but also by α-adrenoceptor blockers (Carlsöö, Danielsson, Marklund & Stigbrand, 1974). This difference in the pattern of inhibition would seem to indicate that the action of dopamine differs from that of noradrenaline and adrenaline. A specific dopamine receptor has recently been postulated for the exocrine pancreas of the dog (Hashimoto, Satoh & Takeuchi, 1971: Furuta, Hashimoto, Iwatsuki & Takeuchi, 1973). The different effects of dopamine on the one hand and noradrenaline and adrenaline on the other hand on enzyme secretion from guinea-pig submandibular gland, could be due to a specific dopamine receptor in this gland as well.

The present study was designed to investigate in more detail the effects of dopamine on enzyme secretion from salivary glands. In an effort to establish the possible existence of a specific dopamine receptor, different drugs known to antagonize the effect of this biogenic amine were used. The experiments were performed on incubated guinea-pig submandibular gland slices.

Methods

Animal preparations

Male guinea-pigs, three months of age, weighing roughly 300 g were used for the investigation. The animals were starved overnight before being killed. In one series of experiments, nine animals were given an intraperitoneal injection of reserpine (10 mg/kg body weight) 12 h before killing them. Nine animals not given reserpine were used as controls. In all the experiments the submandibular glands of both sides were rapidly excised under sodium pentobarbitone anaesthesia. Under a

stereomicroscope extraglandular fat and connective tissue were removed. In all experiments except those involving reserpine pretreatment the glands from two guinea-pigs were pooled. In the study on the effects of pretreatment with reserpine, glands from one control and one treated animal were used for each experiment. They were cut into small fragments, which were distributed among the incubating vessels. All specimens were preincubated for 30 min in 3 ml of bicarbonate buffer (pH 7.4) supplemented with pyruvate, glutamate and fumarate (Krebs, 1950) and also containing albumin (5 mg/ml) and glucose (0.6 mg/ml). After preincubation the medium was replaced by 3 ml of fresh incubation buffer containing different secretagogues and test-substances. Control incubations without additives were included in each experiment. The dopamine receptor blocking agents were present during both the preincubation and the incubation periods.

Preincubation as well as incubation was carried out at 37° C under continuous equilibration of the medium with 95% O_2 and 5% CO_2 in a metabolic shaker (Danielsson, 1974). After 60 min of incubation the specimens were removed by filtration through a nylon net and were homogenized in 3 ml of 50 mM phosphate buffer (pH 6.9), using an Ultra-Turrax homogenizer (Janke und Kunkel K.G., Staufen, Germany) run at a speed of 9,600 rev/min for 45-60 s at 4° C. The homogenates were centrifuged at about 3000 *g* for 5 minutes. The amylase activities of the crude homogenate and supernatant did not differ significantly. Both incubation media and supernatants were assayed for amylase.

Amylase assay

Samples of tissue extracts (supernatants) or incubation media were appropriately diluted with 50 mM phosphate buffer and assayed for amylase by a micro-modification of the 3,5-dinitrosalicylate (DNS) method with 2% soluble starch as substrate (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. The amylase release was expressed both as units/g tissue wet weight recorded in the medium and as percentage of the total amylase activity in tissue and medium.

Chemicals and drugs

Soluble starch and 3,5-dinitrosalicylic acid were obtained from E. Merck A.G., Darmstadt, Germany. All chemicals were of analytical grade. (−)-Noradrenaline bitartrate was purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks. 3-Hydroxytyramine hydrochloride (dopamine) was from Sigma Chemical Co., St. Louis, Mo., U.S.A. Reserpine (Serpasil) was from Ciba-Geigy A.G., Basel, Switzerland and apomorphine hydrochloride from Sandoz A.G., Basel, Switzerland. Sodium diethyl-dithiocarbamate (DDTC) was from Fluka A.G., Buchs, Switzerland, and sodium pentobarbitone (Mebumal) from ACO, Sweden. Haloperidol, fluspirilene and pimozide were kindly donated by Janssen Division, Beerse, Belgium through A.B. Leo, Helsingborg, Sweden. 5-Hydroxydopamine was a gift from A.B. Hässle, Mölndal, Sweden.

Statistical evaluation

The statistical probability that the effect of an additive to the incubation medium was a chance effect, was ruled out from the mean differences between test and control incubations in a series of identical but separate experiments.

Results

Dopamine and noradrenaline elicited amylase secretion of about equal magnitude from guinea-pig submandibular gland slices (Table 1). DDTC, a dopamine-β-hydroxylase inhibitor, neither influenced basal enzyme release nor the secretory response to dopamine or noradrenaline (Table 1). L-DOPA (10^{-4} M) the precursor of dopamine, was found to be without significant stimulatory effect on amylase secretion (% amylase release 3.9 ± 0.64 after treatment as compared with 6.1 ± 2.98 in controls; $n = 4$).

To investigate whether dopamine exerts a direct action on the acinar cells or an indirect one by liberating noradrenaline from sympathetic nerve endings, guinea-pigs were pretreated with reserpine to deplete the endogenous stores of noradrenaline. The submandibular glands of these animals were then incubated with the secretagogues listed in Table 2. The results were compared to those obtained from non-reserpine-treated control animals (Table 2). Injection of reserpine 12 h before the *in vitro* incubations, resulted in an increase in total amylase content of the glands as compared to the glands of non-reserpine-treated animals (Table 2). Amylase secretion from submandibular glands of control animals was enhanced by dopamine, 5-hydroxydopamine (5-OHDA) and noradrenaline. 5-OHDA was without secretory effect on reserpine-treated animals, whereas dopamine and noradrenaline were still most effective secretagogues.

In Table 3 apomorphine and three neuroleptic

drugs (haloperidol, pimozide and fluspirilene), which interact with dopamine receptors in the CNS, were tested on dopamine- and noradrenaline-induced amylase secretion. Apomorphine itself, displayed a slight statistically significant, enhancement of the enzyme release. On the other hand haloperidol (a butyrophenone), pimozide and fluspirilene (diphenylbutylamines) did not influence the basal secretion. Dopamine-stimulated enzyme release was antagonized by all these compounds. Haloperidol was the most efficient inhibitor and reduced amylase secretion almost to the basal level. Apomorphine, haloperidol and fluspirilene did not affect the noradrenaline-initiated amylase discharge. On the other hand, pimozide, besides attenuating amylase secretion induced by dopamine, also reduced the amylase discharge caused by noradrenaline.

Table 1 Effect of sodium diethyl-dithiocarbamate (DDTC) on noradrenaline- and dopamine-induced amylase secretion

Test-substance	n	*Amylase activity in medium*	*Amylase activity in homogenate*	*% amylase release*
Control	5	34 ± 16	414 ± 125	7.1 ± 1.47
DDTC (2×10^{-4} M)	5	25 ± 12	417 ± 84	5.0 ± 1.43
Noradrenaline (10^{-5} M)	5	273 ± 38	447 ± 130	40.9 ± 3.58
Noradrenaline (10^{-5} M) + DDTC (2×10^{-4} M)	5	210 ± 54	356 ± 74	36.7 ± 2.11
Dopamine (10^{-4}M)	5	211 ± 25	385 ± 148	41.9 ± 8.06
Dopamine (10^{-4} M) + DDTC (2×10^{-4} M)	5	214 ± 21	326 ± 88	43.8 ± 7.03

After a preincubation period of 30 min at 37° C the specimens were incubated in a supplemented Krebs-Ringer bicarbonate buffer for 60 min with listed concentrations of noradrenaline and dopamine. The dopamine-β-hydroxylase inhibitor (DDTC; 2×10^{-4} M) was present during both preincubation and incubation periods. Amylase activity is expressed as u/g tissue wet weight and amylase release as % of the total amylase activity in tissue and medium. Mean values ± s.e. for indicated number of experiments.

Table 2 Effects of dopamine, 5-hydroxydopamine and noradrenaline on amylase secretion in reserpine-treated guinea-pigs

Secretagogue	n	*Amylase activity in medium*	*Amylase activity in homogenate*	*% amylase release*
Non-pretreated animals				
Control	9	46 ± 4	466 ± 47	9.8 ± 1.36
Dopamine (10^{-4} M)	6	263 ± 36**	406 ± 28	38.6 ± 3.05***
5-Hydroxydopamine (10^{-4} M)	6	135 ± 24*	407 ± 63	25.5 ± 3.19**
Noradrenaline (10^{-5} M)	8	286 ± 26***	408 ± 42	41.4 ± 3.32***
Reserpine-pretreated animals				
Control	9	56 ± 4	838 ± 95	6.6 ± 0.61
Dopamine (10^{-4} M)	6	237 ± 36**	810 ± 110	22.6 ± 1.19***
5-Hydroxydopamine (10^{-4} M)	6	60 ± 9	752 ± 92	7.8 ± 1.34
Noradrenaline (10^{-5} M)	8	420 ± 53***	814 ± 59	33.4 ± 2.27***

Nine guinea-pigs received an injection of reserpine (10 mg/kg body weight, i.p.) and nine control animals the same volume of 0.9% w/v NaCl solution 12 h before being killed. Pieces of submandibular glands from one reserpine-treated and one control animal were incubated simultaneously for 60 min at 37° C in a supplemented Krebs-Ringer bicarbonate buffer with listed concentrations of secretagogues. Amylase activity is expressed as u/g tissue wet weight and amylase release as % of the total amylase activity in tissue and medium. Mean values ± s.e. for listed number of observations.
*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Table 3 Effects of dopamine receptor blocking agents on noradrenaline- and dopamine-induced amylase secretion

	% amylase release			
Normal Control	7.8 ± 1.44 (7)	6.3 ± 2.50 (6)	5.3 ± 1.48 (8)	4.9 ± 0.53 (6)
Noradrenaline (10^{-5} M)	43.7 ± 3.85 (7)	34.9 ± 2.44 (6)	33.6 ± 1.27 (8)	36.8 ± 2.24 (6)
Dopamine (10^{-4} M)	46.8 ± 1.99 (9)	31.8 ± 3.17 (6)	32.0 ± 1.52 (7)	33.7 ± 1.56 (6)
Inhibitors	*Apomorphine (2×10^{-4} M)*	*Haloperidol (2×10^{-4} M)*	*Pimozide (2×10^{-4} M)*	*Fluspirilene (2×10^{-4} M)*
	% amylase release			
Inhibitor alone	12.8 ± 0.91** (7)	8.2 ± 2.55 (4)	4.4 ± 0.75 (5)	11.1 ± 3.35 (4)
Noradrenaline + inhibitor	38.3 ± 1.65 (7)	31.0 ± 1.47 (6)	26.5 ± 2.61* (8)	30.9 ± 2.85 (6)
Dopamine + inhibitor	32.6 ± 2.09** (9)	12.1 ± 2.81*** (6)	21.6 ± 2.17*** (7)	20.2 ± 3.46* (6)

After a preincubation period of 30 min at 37°C the specimens were incubated in a supplemented Krebs-Ringer bicarbonate buffer for 60 min with 2×10^{-4} M apomorphine, haloperidol, pimozide or fluspirilene. The dopamine receptor blockers were present during both preincubation and incubation periods. Amylase release is expressed as % of the total amylase activity in tissue and medium. Mean values (%) ± s.e. for indicated number of observations (*n* within brackets).
*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Discussion

L-Dihydroxyphenylalanine (L-DOPA) and 3-hydroxytyramine (dopamine) are biochemical precursors in the biosynthesis of noradrenaline. However, it has been suggested that these precursor substances may have important physiological functions of their own. The presence of a specific dopamine receptor in the CNS is well established (e.g. Hornykiewicz, 1966), and such a receptor has also been suggested for the renal and mesenteric vascular beds (Yeh, McNay & Goldberg, 1969). Dopamine greatly enhances amylase and peroxidase secretion from incubated guinea-pig submandibular gland with a maximal effect at a concentration of 10^{-4} M (Carlsöö *et al.*, 1974). Dopamine at this concentration is about as potent as noradrenaline in inducing amylase release. This effect of dopamine is inhibited by both propranolol and phenoxybenzamine (β- and α-adrenoceptor blocking agents respectively) (Carlsöö *et al.*, 1974) whereas noradrenaline action is exclusively inhibited by β-blockers. This difference in action between dopamine and noradrenaline raises the question of the possible existence of a specific dopamine receptor in salivary glands.

Dopamine does not seem to exert its effect by being converted to noradrenaline, since the presence in the incubation medium of a dopamine-β-hydroxylase inhibitor, DDTC (Molinoff & Axelrod, 1971; Tjälve, 1971) did not influence the dopamine-induced amylase release.

A 'false' neurotransmitter substance, 5-OHDA is taken up by autonomic nerve endings and replaces the physiological transmitter(s) (Thoenen & Tranzer, 1971). This amine was found to stimulate amylase secretion in the present study. Its secretagogic action does not seem to be due to a direct effect on the acinar cells, as glands from reserpine-treated animals are not stimulated. However, dopamine and noradrenaline induce amylase secretion from the submandibular gland irrespective of preceding reserpine-treatment. The percentage of amylase release in response to dopamine and noradrenaline is somewhat lower in the reserpine-treated group as compared to controls. This is partly explained by the increased

total amylase content of the glands after reserpine treatment. The experiments indicate that 5-OHDA acts by liberating endogenous stores of noradrenaline, whereas dopamine seems to exert its main effect directly on the acinar cells.

Apomorphine, a known agonist for dopamine receptors in the CNS (Ernst, 1967; Andén, Rubenson, Fuxe & Hökfelt, 1967), slightly increases amylase discharge from the submandibular gland. This effect, however, is not of the same magnitude as that obtained with dopamine. On the other hand, dopamine-induced secretion is reduced by roughly 30% in the presence of apomorphine, whereas noradrenaline-stimulated release is unaffected. Some neuroleptic drugs seem to act mainly by blocking the CNS dopamine receptors (e.g. Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970). Haloperidol inhibits dopamine-induced renal, mesenteric and coronary vasodilatation (see e.g. Goldberg, 1972). In our experiments, haloperidol almost completely inhibited the dopamine-initiated amylase discharge without affecting secretion induced by noradrenaline.

Two new potent neuroleptic drugs, pimozide and fluspirilene, which interact with the dopamine receptors of the CNS (Janssen, Niemegeers, Schellekens, Dresse, Lenaerts, Pinchard, Schaper, van Nueten & Verbruggen, 1968; Andén *et al.*, 1970; Ojeda, Harms & McCann, 1974) were also studied with respect to their effects on dopamine-stimulated secretion. Neither drug had any effect on its own on secretion but both inhibited amylase release caused by dopamine. They were less effective inhibitors than haloperidol. Furthermore, pimozide also reduced amylase release caused by noradrenaline, whereas fluspirilene did not significantly alter that secretory response.

Salivary gland activity in response to dopamine differs from that in response to noradrenaline in that it can be inhibited by both α- and β-adrenoceptor blocking agents, whereas the latter is inhibited solely by agents which block the β-adrenoceptor (Carlsöö *et al.*, 1974). In addition, the action of dopamine but not that of noradrenaline is inhibited by haloperidol, fluspirilene and apomorphine. Though the pattern of blockade is not identical with the pattern in the CNS the characteristics of the salivary gland's response to dopamine seem to be sufficiently different from noradrenaline to postulate a specific receptor for dopamine in the gland. However, there is as yet, no evidence of the presence of dopaminergic neurones in these glands.

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