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Dopamine-induced amylase secretion from rat parotid salivary gland *in vitro*: an effect mediated via noradrenergic and cholinergic nerves.

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1 The effect of dopamine on amylase secretion by rat parotid tissue was examined *in vitro*.

2 Dopamine induced marked amylase secretion from the tissue in a dose-dependent manner. Its EC_{50} value was about 4 μM and the maximal response was obtained at a concentration of 100 μM.

3 The dopamine-induced secretion was inhibited by the dopamine-antagonists haloperidol, (+)-butaclamol and spiroperidol.

4 Atropine reduced the dopamine-induced secretion significantly, and physostigmine enhanced the secretion.

5 Parasympathectomy of the gland resulted in a significant decrease in the dopamine-induced secretion, but did not reduce the secretion induced by dopamine with atropine.

6 Dopamine-induced ACh release from parasympathetic nerve terminals in the tissue was studied in tissue preparations that had been loaded with [^{3}H]-choline. Dopamine elicited Ca^{2+}-sensitive tritium release, and dopamine antagonists or parasympathectomy prevented this release.

7 Sympathectomy or reserpine treatment of rats resulted in significant decrease in the dopamine-induced secretion, but increase in noradrenaline (NA)- or isoprenaline-induced secretion.

8 Dopamine-induced NA release was studied by preloading the parotid tissue with [^{3}H]-NA. Dopamine induced Ca^{2+}-sensitive tritium release, and dopamine antagonists or sympathectomy prevented the release.

9 Several lines of circumstantial evidence strongly suggested that dopamine has a specific site for action in the parotid tissue that is independent of NA receptors.

10 In sympathectomized or reserpine-treated glands, atropine completely inhibited the dopamine-induced amylase secretion, suggesting that dopamine did not have a direct effect on postsynapses.

11 These findings indicate that dopamine induces amylase secretion in two indirect ways mediated through ACh and NA released from parasympathetic and sympathetic nerve terminals, respectively.

Introduction

Stimuli mediated through β-adrenoceptors induce secretion from the salivary glands by elevating the intracellular cyclic AMP level, while stimuli mediated through muscarinic cholinoceptors and substance P receptors also induce the secretion by eliciting Ca^{2+} influx into acinar cells (Butcher & Putney, 1980). In rat parotid glands, adrenoceptor and cholinoceptor agonists (Butcher *et al.*, 1975; Leslie *et al.*, 1976) and substance P or a closely related peptide (Rudich & Butcher, 1976; Gallacher, 1983) stimulate amylase secretion, although β-adrenoceptor agonist is the most effective. Numerous results showed that noradrenaline (NA), acetylcholine (ACh) and substance P are the physiological neurotransmitters in the gland. Recently, the levels of α-adrenoceptors (Strittmatter *et*

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al., 1977a, b), β-adrenoceptors (Au et al., 1977), muscarinic cholinoceptors (Talamo et al., 1979) and substance P receptors (Liang & Cascieri, 1980; 1981) of rat parotid gland were determined by binding studies with specific radio-labelled ligands for each receptor.

Recent studies have shown that dopamine activates dopamine-sensitive adenylate cyclase, releases parathyroid hormone from the parathyroid gland and promotes secretion from the exocrine pancreas (Bell, 1982; Lackovic & Neff, 1983). There are also reports that dopamine stimulates the secretions of peroxidase and amylase from slices of guinea-pig submandibular gland (Carlsöö et al., 1974; Bloom et al., 1975) and the secretion of amylase from rat parotid tissue (Sundström et al., 1985) in a different manner from NA, suggesting the existence of dopamine-receptors in the tissue.

The present study was designed to determine whether dopamine induces amylase secretion from rat parotid tissues either via ACh release from parasympathetic nerve terminals or via NA release from sympathetic nerve terminals.

Methods

Preparation of rat parotid tissue

Parotid glands were obtained from male Wistar rats (200–350 g) and small pieces of the tissue were prepared as described previously (Hata et al., 1983). Before experiments, Krebs-Ringer Tris (KRT) solution, consisting of (mM) NaCl 120, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 3.0, Tris HCl buffer (pH 7.4) 16 and glucose 5, was aerated with O_2 and the pieces of parotid tissues were equilibrated with the solution for 20 min at 37°C with shaking. About 30 mg of tissue pieces were incubated in 10 ml of KRT solution for 10 min at 37°C.

Release of tritium-labelled amines

Preloading of [^{3}H]-choline and release of tritium [Methyl-^{3}H]-choline chloride (80 Ci mmol^{-1}) was obtained from New England Nuclear (Boston, U.S.A.). About 60 mg of tissue pieces were put into holder in which they could be transferred easily from medium to medium, and were preincubated with [^{3}H]-choline (15 μCi of original tritiated choline, 19 nM final concentration) for 30 min at 37°C. The tissue was then washed with 100 ml KRT for 10 min and transferred to 5 ml of fresh medium. The amounts of tritium released in 5 min periods were measured by transferring the tissue to fresh medium every 5 min. In tests on the effect of a high K^+ concentration, KRT solution containing 25 mM NaCl and 100 mM KCl instead of the usual concentrations of these ions was used. KRT solution without $CaCl_2$ was used to examine the effect of Ca^{2+}-free conditions. All incubation media contained 10 μM hemicholinium-3 to prevent reuptake of choline formed from ACh (Szerb, 1976; Saito et al., 1984).

Preloading of [^{3}H]-noradrenaline and release of tritium

(−)-[7,8-^{3}H]-noradrenaline (48.4 Ci mmol^{-1}) was obtained from New England Nuclear. About 60 mg of tissue pieces were preincubated with [^{3}H]-NA (10 μCi of original tritiated NA, 21 nM final concentration) for 20 min at 37°C in 10 ml of KRT with 100 μM pargyline. Release of tritium from the tissue in successive 5 min periods was measured as described for ACh release. The effects of a high K^+ concentration and Ca^{2+}-free conditions were studied by the same methods as ACh release. Preliminary experiments by h.p.l.c. showed that almost all the tritium released into the medium on stimulation with dopamine or a high K^+ concentration was due to unmetabolized [^{3}H]-NA.

Denervation procedures

Rats were anaesthetized with pentobarbitone. Unilateral parasympathectomy of the parotid gland was achieved post-ganglionically by cutting the auricular temporal nerve between the masseter and temporalis muscle (Ekström, 1974). Unilateral postganglionic sympathectomy was achieved by excision of the superior cervical ganglion (Arm & Ekström, 1977; De Peusner et al., 1979 a, b). Animals were killed 7 days after these operations and their contralateral innervated gland was used as a control.

Other methods

High performance liquid chromatography (h.p.l.c.) was performed in a Waters liquid chromatograph (model ALC/GPC 244) equipped with a Hitachi u.v. monitor at 250 nm. A 30 cm TSK gel-ODS 120A reverse column was used. The mobile phase was 0.1 μM NaH_2PO_4-H_3PO_4 buffer (pH 2.5). Reserpine was injected daily for 7 days (0.5 mg kg^{-1}, i.p.). Amylase activity was measured as described by Bernfeld (1955) with amylose as substrate. Activity was expressed as maltose liberated into the medium in 100 mg 5 min^{-1} at 20°C. Student's t test was used to evaluate the significance of differences and those giving a value of $P < 0.05$ were regarded as significant.

Drugs

Methoxamine hydrochloride was a gift from Nippon shinyaku Co. (Kyoto, Japan). Reserpine and hemicholinium-3 hydrate were from Aldrich Chemical Co. (Milwaukee, U.S.A.). Amylose was from Wako Pure

Chemical Industries (Osaka, Japan). (−)-Noradrenaline bitartrate, (−)-isoprenaline bitartrate, (±)-propranolol hydrochloride and physostigmine sulphate were from Sigma Chemical Co. (St. Louis, U.S.A.).

Results

Amylase secretion from rat parotid tissue induced by dopamine

Dopamine stimulated amylase secretion from rat parotid tissue in a dose-dependent manner and EC_{50} value was about 4 μM. Though this value was high compared with those of (−)-isoprenaline (Iso; 130 nM) and (−)-NA (1 μM), the maximal secretory response was observed with 100 μM dopamine (Figure 1). The secretory response of the tissue to dopamine was observed within 2 min, and showed a similar time course to the responses to the other catecholamines (Figure 1).

The dopamine-induced secretion was inhibited by the dopamine antagonists haloperidol and spiroperidol. (+)-Butaclamol also inhibited the dopamine-induced secretion, while (−)-butaclamol did not, showing the stereospecificity of the compound. Among the dopamine antagonists tested only sulpiride did not have any significant effect (Figure 2).

The α-adrenoceptor antagonist phentolamine (10 μM) slightly inhibited dopamine-induced secretion and the β-adrenoceptor antagonist (±)-propranolol had a marked inhibitory effect. The inhibitory effect of propranolol was about 100 fold that on Iso-induced secretion (Figure 3).

Effects of atropine and physostigmine on dopamine-induced amylase secretion

The muscarinic ACh antagonist, atropine, inhibited the dopamine-induced secretion, but even at a concentration of 1 mM, it caused only about 50% inhibition (Figure 4a). The anticholinesterase drug, physostigmine potentiated the dopamine-induced secretion (Figure 4b). Atropine (100 μM) and physostigmine (50 μM) did not affect the secretion induced by 1 μM NA; values for amylase secretion induced by NA alone, NA plus atropine and NA plus physostigmine were 236.0 ± 17.4, 242.3 ± 21.0 and 234.8 ± 13.2 mg maltose 100 mg^{-1} tissue 10 min^{-1} (mean ± s.e.mean, $n = 4$), respectively. Physostigmine (100 μM) also did not affect the secretion induced by 30 μM ACh; values for amylase secretion induced by ACh without or with physostigmine were 171.6 ± 9.0 or 184.5 ± 34.7 mg maltose 100 mg^{-1} tissue 10 min^{-1} (mean ± s.e.mean, $n = 4$) respectively.

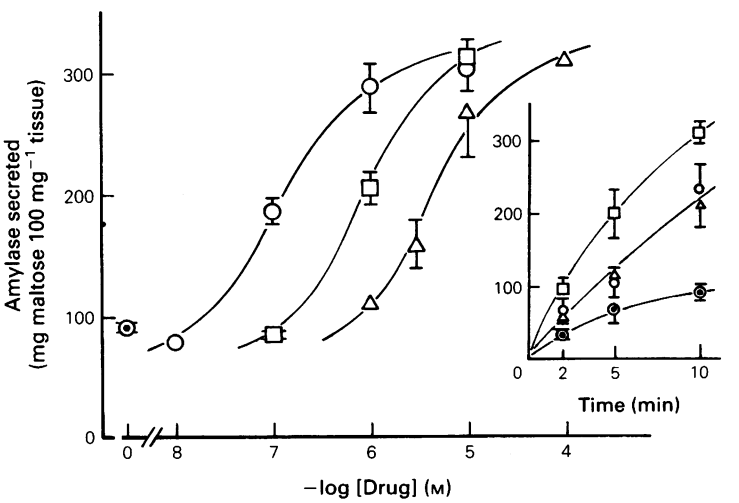

Figure 1 Concentration-response curves of rat parotid tissue to catecholamines. Rat parotid tissues were incubated at 37°C for 10 min without (⊙) or with the indicated concentrations of isoprenaline (Iso) (○), noradrenaline (NA) (□) or dopamine (△). Inset: time course of amylase secretion. Rat parotid tissues were incubated without (⊙) or with 1 μM Iso (○), 10 μM NA (□) or 10 μM dopamine (△). Cumulative amylase secretion into the medium was measured at intervals during incubation. Points and bars are means and standard errors for 4–10 experiments, while single points are means for 2 experiments. Details of experimental conditions are described in the Methods.

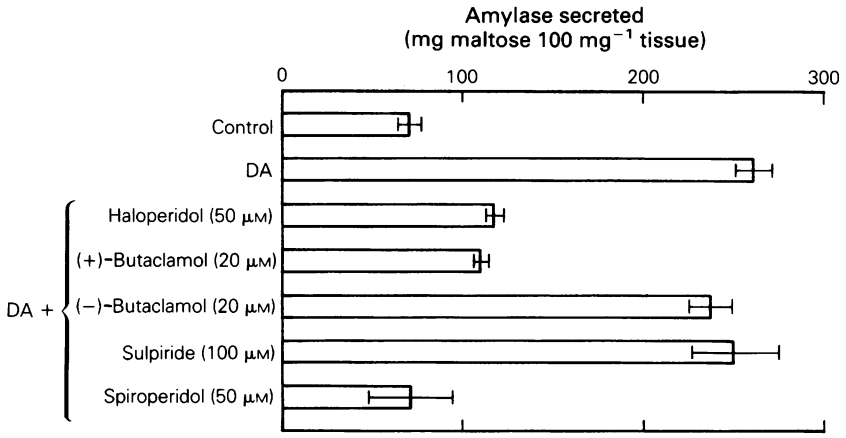

Figure 2 Inhibitory effects of dopamine (DA)-antagonists on dopamine-induced amylase secretion. Parotid tissues were incubated at 37°C for 10 min without (control) or with 10 μM dopamine in the absence or presence of the indicated drugs. Columns and bars are means and standard errors for 6 to 18 experiments.

Influence of parasympathectomy on dopamine-induced amylase secretion

Parasympathectomy of the parotid gland decreased dopamine-induced amylase secretion. On denervation, reduction in the secretion induced by a submaximal dose of dopamine (10 μM) was about 60% of that induced by 100 μM atropine (Figure 5) but denervation did not affect the dopamine-induced secretion in the presence of atropine (Figure 5). In other words, the inhibitory effects of the muscarinic ACh antagonist and denervation of the cholinergic neurone were not additive. Denervation also did not significantly affect the ACh- and NA-induced secretions (Figure 5).

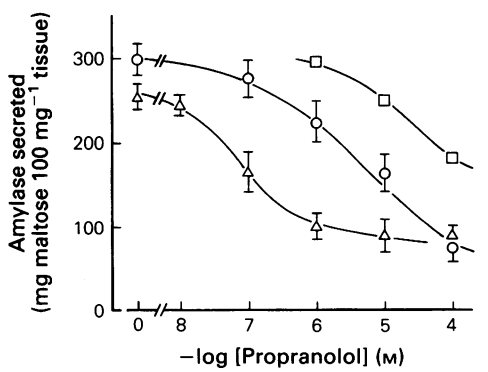

Figure 3 Inhibitory effects of propranolol on catecholamine-induced amylase secretion. Parotid tissues were incubated with 10 μM noradrenaline (□), isoprenaline (○) or dopamine (△) in the presence of the indicated concentrations of propranolol. Points and bars are means and standard errors for 4 to 18 experiments, while single points are means for 2 experiments. For further details, see Figure 1 and the text.

Tritium release from rat parotid tissue preincubated with [^{3}H]-choline

Rat parotid tissue was preincubated with [^{3}H]-choline to label neurotransmitter ACh in parasympathetic nerve terminals with tritium. The amount of tritium released from the preloaded-tissue into the medium in 5 min periods decreased progressively with time (Figure 6a). A high K^+ concentration markedly increased tritium release from the preloaded-tissue, but had little effect in Ca^{2+}-free conditions (Figure 6b). Dopamine (10 ~ 100 μM) also significantly increased tritium release, though the extent of its effect was less than that of a high K^+ concentration. Ca^{2+}-free conditions completely prevented the effect of dopamine (Figure 6c). The dopamine-induced tritium release from parasympathectomized parotid gland tissue preloaded with [^{3}H]-choline was significantly less than that from control tissue (Figure 6d).

Dopamine-induced tritium release from the preloaded-tissue was protected by haloperidol and spiroperidol and also by propranolol, but not by sulpiride (< 100 μM) (Figure 7).

Influences of reserpine-treatment on the catecholamine-induced amylase secretion

Reserpine-treatment of rats resulted in increase in the control level of amylase secretion from parotid tissue in KRT medium. This treatment also resulted in two

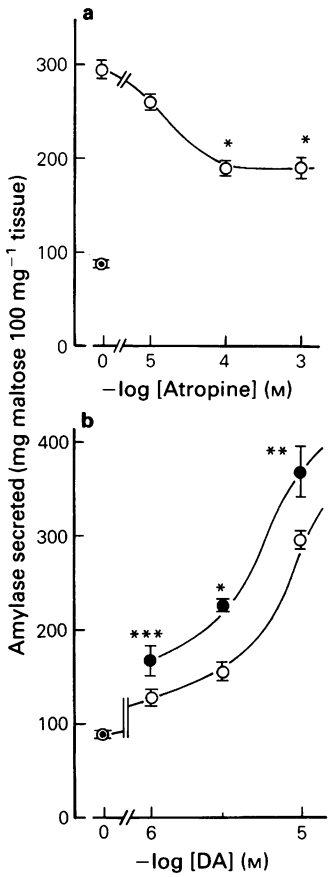

Figure 4 Effects of atropine and physostigmine on dopamine (DA)-induced amylase secretion. (a) Parotid tissues were incubated without (⊙) or with 10 μM dopamine (○) in the presence of the indicated concentrations of atropine. Values are means for 4 to 20 experiments with standard errors. Significance of difference from the value with 10 μM dopamine alone: $*P < 0.01$. (b) Tissues were incubated without (⊙) or with the indicated concentrations of dopamine in the absence (○) or presence (●) of 50 μM physostigmine. Values are means for 4 to 16 experiments with standard errors. Significantly different from the value with each concentration of dopamine alone: $*P < 0.01$; $**P < 0.02$; $***P < 0.05$.

different changes in responsiveness of the tissue to catecholamines. One was increased responses to NA and Iso; the maximal response increased and the EC_{50} value decreased (the EC_{50} values for NA and Iso were 0.3 μM and 34 nM, respectively). The other was decrease in the response to dopamine; even at 100 μM, dopamine did not elicit the maximal response (Figure 8).

Influences of sympathectomy on dopamine and isoprenaline-induced amylase secretion

Sympathectomy of the parotid gland resulted in supersensitivity of the response to Iso and reduced the sensitivity to dopamine, causing changes similar to those after reserpine treatment (Figure 9), but did not change the control level of secretion significantly in KRT medium.

Tritium release from rat parotid tissue preincubated with [^{3}H]-noradrenaline

Rat parotid tissue was preincubated with [^{3}H]-NA to label neurotransmitter NA in sympathetic nerve terminals with tritium. Tritium release from tissue preincubated with [^{3}H]-NA was increased by dopamine and a high K^+ concentration (Figure 10b,c); 10 μM dopamine seemed to be the optimal concentration, 1 μM having little effect, and 100 μM having the same effect as 10 μM. Neither dopamine nor high K^+ had any appreciable effect in Ca^{2+} free conditions (Figure 10b, c). Tissue from sympathetomized parotid gland did not respond to dopamine, in clear contrast to control tissue (Figure 10d). The dopamine-antagonists haloperidol and spiroperidol and propranolol markedly reduced dopamine-induced tritium release from tissue preincubated with [^{3}H]-NA, but sulpiride did not (Figure 11). The α-adrenoceptor agonist methoxamine (100 μM), the β-adrenoceptor agonist Iso (10 μM) and ACh (100 μM) did not have any significant effect on tritium release; values for percentage change in the amount of tritium released after application of methoxamine, Iso and ACh were -5.3 ± 2.8, -18.7 ± 3.4 and $+16.3 \pm 3.2\%$ (mean ± s.e.mean, $n = 3$), respectively.

Prevention of dopamine-induced amylase secretion by a combination of a parasympathethic antagonist and sympathetic denervation

As described above, atropine partly inhibited the dopamine-induced amylase secretion as did sympathetic denervation. Dopamine-induced amylase secretion from sympathectomized tissue was completely prevented by atropine (Figure 12a). Similarly, after reserpine treatment, atropine completely prevented dopamine-induced secretion (Figure 12b).

Discussion

In this study we found that 50% of the dopamine-induced amylase secretion was inhibited by atropine. Thus part of the action of dopamine may be mediated by ACh and this possibility is supported by our finding that physostigmine enhanced the dopamine-induced

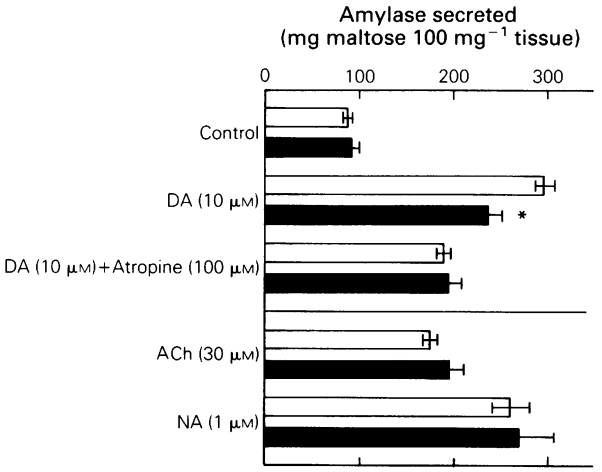

Figure 5 Effects of secretagogues on amylase secretion from parasympathectomized and control parotid tissues. Tissues from parasympathectomized (closed columns) and control (open columns) glands were incubated with the indicated drugs. Values are means for 4 to 20 experiments with standard errors. Significantly different from the value in control gland: $*P < 0.01$. Details of the experiments are described in the Methods.

secretion. The significant decrease in the dopamine-induced secretion observed after parasympathectomy also supports the idea that some of the action of dopamine is mediated through ACh. Since the parotid tissue used in the present study did not include any ganglia (Alm & Ekström, 1976; Talamo *et al.*, 1979), dopamine may act on parasympathetic nerve terminals causing ACh release from them. The extent of decrease in dopamine-induced secretion after parasympathectomy was only about 60% of the inhibition by atropine. This difference may have been due to incomplete denervation in the present study

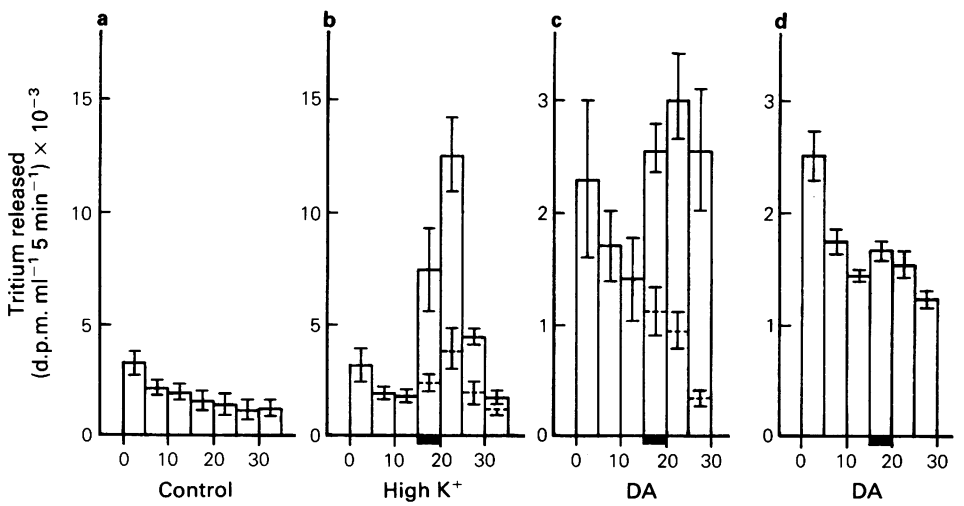

Figure 6 Effects of a high K^+ concentration and dopamine on tritium release from [^{3}H]-choline preloaded tissue. (a, b, c) Tritium release in 5 min periods from [^{3}H]-choline preloaded tissues was measured. A high K^+ concentration and 10 μM dopamine (DA) were applied in the incubation period marked with bold black line. The dashed lines represent results on incubation in Ca^{2+}-free conditions. (d) A similar experiment with a tissue preparation obtained from parasympathectomized gland. Values are means for 3–5 experiments with standard errors. Abscissa scale: incubation period (min). For details, see Methods and the text.

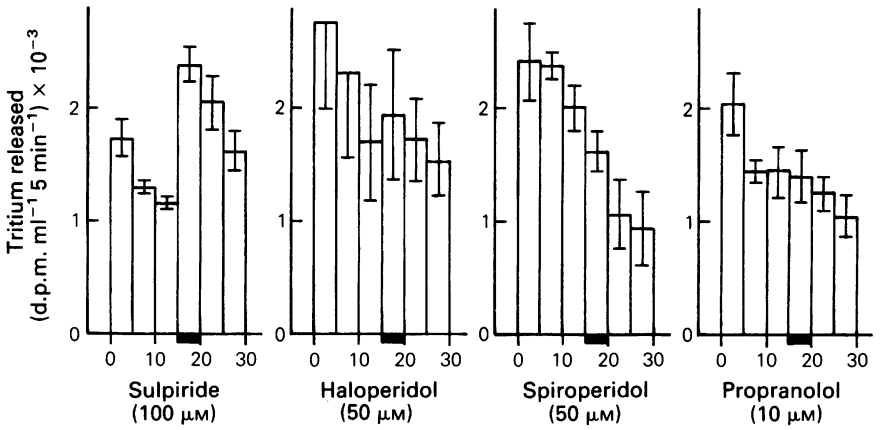

Figure 7 Effects of dopamine antagonists and propranolol on dopamine-induced tritium release from [^{3}H]-choline preloaded-tissue. At the incubation time marked with a bold black line, 10 μM dopamine with the indicated drug was applied. Values are means for three experiments with standard errors. For details, see Figure 6 and Methods.

(Ekström, 1974; Ekström & Emmelin, 1974; Alm & Ekström, 1976). Since the extent of inhibition of dopamine-induced secretion by atropine in parasympathectomized and innervated control gland were the same (Figure 5), the remaining part of the secretory response was probably independent of the cholinergic system. Tritium released by dopamine from tissue that had been preincubated with [^{3}H]-choline could be regarded as due to [^{3}H]-ACh synthesized in the parasympathetic nerve terminals of the tissue, because of the responsiveness of the release to a high K^+ concentration, and its unresponsiveness in Ca^{2+}-free conditions or after parasympathectomy.

These findings indicate that dopamine-induced amylase secretion from rat parotid tissue is partly mediated through ACh released from parasympathetic nerve terminals. The following findings strongly suggest that dopamine has a specific site of action in

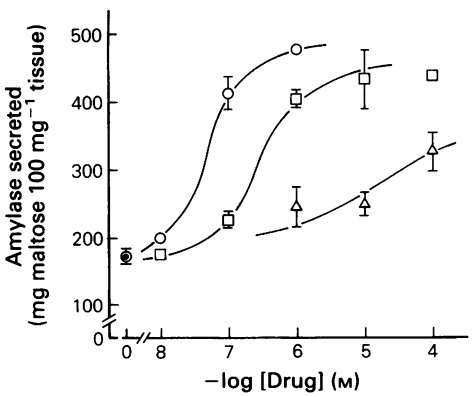

Figure 8 Concentration-response curves to catecholamines of parotid tissue obtained from reserpine-treated rats. Parotid tissues obtained from reserpine-treated rats were incubated without (○) or with the indicated concentration of isoprenaline (○), noradrenaline (□) or dopamine (△). Values are means for 6 to 10 experiments with standard errors, while single points are means for 2 experiments. For details, see legends to Figure 1 and Methods.

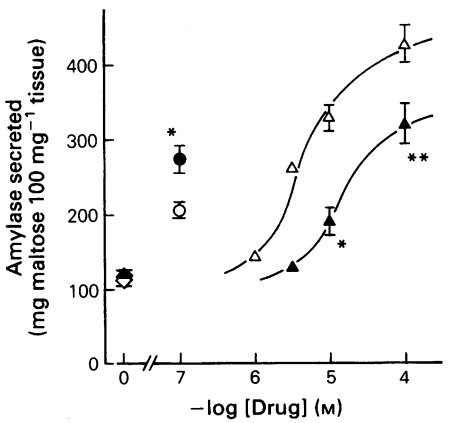

Figure 9 Influences of sympathectomy on the dose-dependence of responses of the parotid tissue to isoprenaline (Iso) and dopamine. Parotid tissues obtained from sympathectomized glands (filled symbols) and controls (open symbols) were incubated without (◇) or with the indicated concentration of Iso (○) or dopamine (△). Values are means for 9 to 20 experiments with standard errors, while single points are means for 2 experiments. For details, see legends to Figure 1 and Methods. Significantly different from secretion of the control: $*P < 0.01$; $**P < 0.02$.

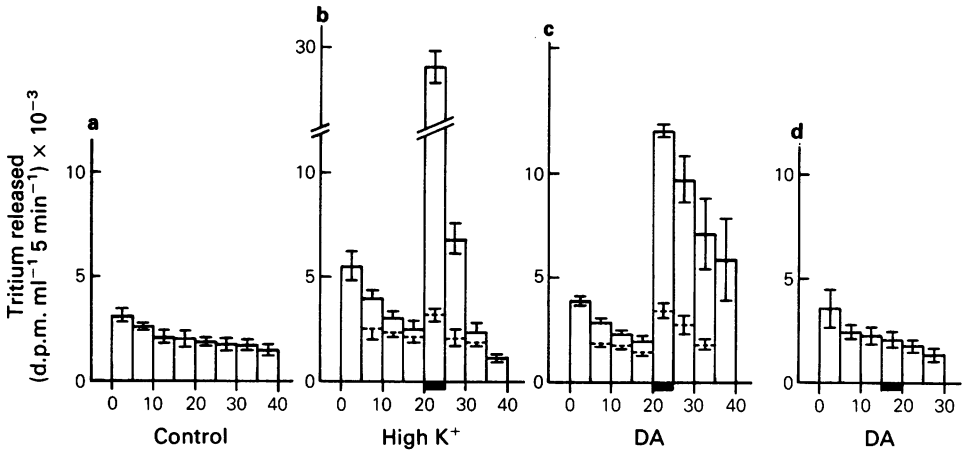

Figure 10 Effects of a high K^+ concentration and dopamine (DA) on tritium release from [^{3}H]-noradrenaline ([^{3}H]-NA)-preloaded tissue. (a, b, c) Tritium release in 5 min periods from [^{3}H]-NA-preloaded tissues were measured. A high K^+ concentration and 10 μM dopamine were applied in the incubation period marked with a bold black line. The dashed lines show values on incubation in Ca^{2+}-free conditions. (d) Similar experiment carried out with tissue from a sympathectomized gland. Values are means for 3–4 experiments with standard errors. Abscissa scale: incubation period (min). For details, see Methods and text.

the tissue, independent of NA receptors; a dopamine-specific antagonist inhibited the dopamine-induced secretion (Figure 2), atropine and parasympathectomy (Figure 5) did not affect NA-induced secretion, and NA did not induce tritium release from tissue preincubated with [^{3}H]-choline. (The amount of tritium released after an application of NA decreased by 8.0 ± 4.1%, mean ± s.e.mean, $n = 3$.)

The β-adrenoceptor antagonist, propranolol, strongly inhibited dopamine-induced amylase secretion (Figure 3). Propranolol also inhibited dopamine-induced [^{3}H]-ACh or [^{3}H]-NA release (Figures 7 and 11). These data suggest that dopamine may have a direct action on β-adrenoceptors, but this is unlikely for the following reasons: (1) sympathectomy and reserpine treatment resulted in supersensitivity of the tissue to Iso and NA, but subsensitivity to dopamine (Figures 8 and 9). (2) A β-adrenoceptor agonist did not elicit release of [^{3}H]-ACh or [^{3}H]-NA from the tissue preparation under the present experimental condi-

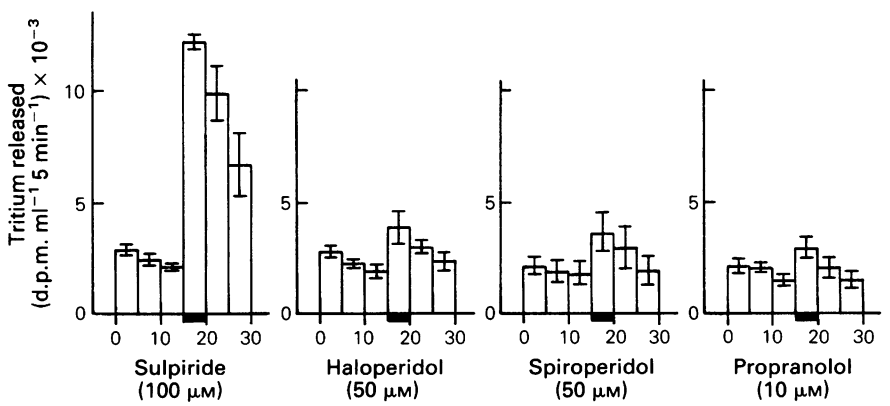

Figure 11 Effects of dopamine antagonists and propranolol on dopamine-induced tritium release from [^{3}H]-noradrenaline-preloaded tissue. Dopamine 10 μM with the indicated drug was applied in the incubation period marked with a bold black line. Values are means for three experiments with standard errors. For details, see Figure 10 and Methods.

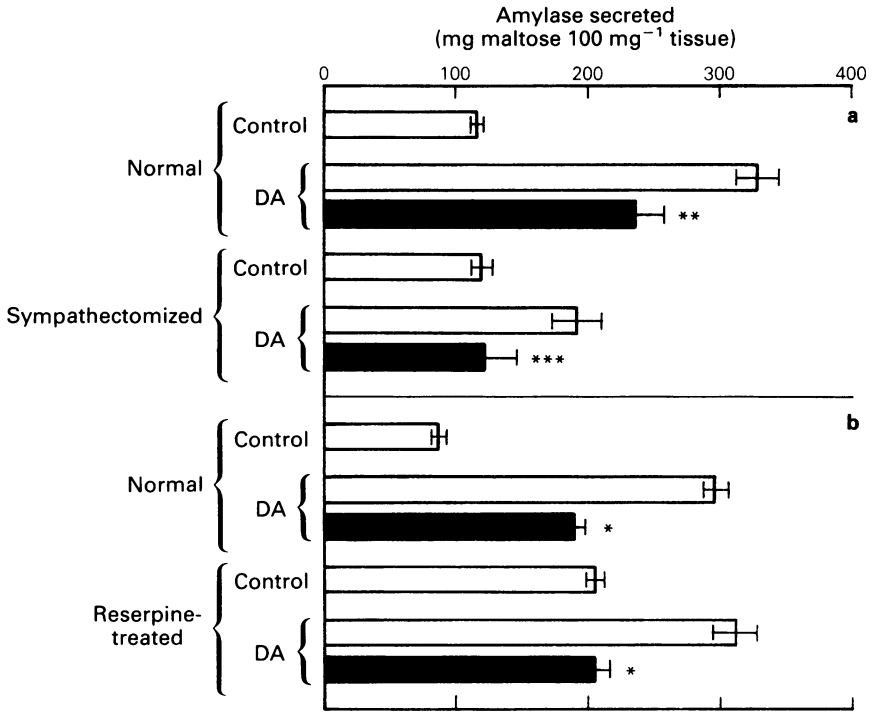

Figure 12 Influences of sympathectomy and reserpine-treatment on the inhibitory effect of atropine on dopamine (DA)-induced amylase secretion. (a) Tissue preparations obtained from sympathectomized and control (Normal) glands were incubated without (control) or with 10 μM dopamine. The effect of 100 μM atropine on dopamine-induced secretion was examined (closed columns). (b) Similar experiments were carried out on tissue preparations obtained from reserpine-treated rats and controls (Normal). Values are means for 5 to 20 experiments with standard errors. Significant difference of the value for dopamine-induced secretion: $*P < 0.01$; $**P < 0.02$; $***P < 0.05$. For details, see Methods.

tions. (3) A combination of sympathectomy and treatment with a parasympathetic antagonist resulted in complete inhibition of the dopamine-induced secretion, indicating the absence of a postsynaptic effect of dopamine, including an effect on postynaptic β-adrenoceptors (Figure 12). Therefore, it seems likely that the marked action of propranolol observed in the present study was due to a direct action on the membrane structure as observed in the central nervous system and many peripheral tissues (Surewicz, 1982; Matthews & Baker, 1982; Drazen *et al.*, 1983; Giacovich & Enero, 1984).

Supersensitivity of the β-adrenergic response of rat parotid gland after sympathetic denervation has been reported (De Peusner *et al.*, 1979a, b; Ekström, 1980). In the present study we observed similar supersensitivity after denervation and also reserpine treatment, but these treatments resulted in subsensitivity of the secretory response to dopamine. These findings clearly indicate that the action of dopamine is different from that of NA and suggest an indirect action of dopamine mediated through NA released from sympathetic nerve terminals. The following evidence indicated the dopamine-induced release of [^{3}H]-NA from sympathetic nerve terminals in tissue preloaded with [^{3}H]-NA. Preloaded tissue released tritium in response to a high K^+ concentration, but not in Ca^{2+}-free conditions or after sympathectomy. Furthermore analysis by h.p.l.c. showed that the tritium released by a high K^+ concentration or dopamine was almost all unmetabolized [^{3}H]-NA. This dopamine-induced [^{3}H]-NA release was inhibited by dopamine-antagonists, and α- and β-adrenoceptor agonists did not elicit the [^{3}H]-NA release. Therefore, dopamine probably has a specific site of action in sympathetic nerve terminals.

These results indicate that the dopamine-induced amylase secretion from rat parotid tissue is partly mediated through NA released from sympathetic nerve terminals.

Parasympathectomy (or application of atropine) resulted in a decrease about 50% in dopamine-induced secretion. Sympathectomy (or reserpine treatment)

also caused a similar decrease in the secretion. However, atropine treatment after sympathectomy or reserpine treatment completely inhibited dopamine-induced amylase secretion. In other words, prevention of the actions of the neurotransmitters ACh and NA released from parasympathetic and sympathetic nerve terminals, respectively, resulted in the disappearance of the dopamine effect. This result leads to the conclusion that dopamine does not act directly on the postjunctional receptor of either autonomic nervous system, but induces amylase secretion from rat parotid tissue in two ways: by eliciting ACh and NA release from the parasympathetic and sympathetic nerve terminals, respectively.

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