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COMPARISON OF THE SECRETORY PROCESSES IN THE PAROTID AND SUBLINGUAL GLANDS OF THE MOUSE

I. REGULATION OF THE SECRETORY PROCESSES

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SUMMARY

1. Secretion from the mucous sublingual gland of the mouse has been investigated and compared with the serous parotid gland The influence of acetylcholine, noradrenalin and adrenalin on the secretion of glycoproteins (e g. mucins) and proteins (e.g. amylase) from these glands in vitro, and the involvement of cyclic AMP and Ca^{2+} has been studied.

2. Secretion from the parotid gland could be stimulated by both acetylcholine and the catecholamines. It appears that cyclic AMP plays an important role in the adrenergic secretory process, but not in the cholinergic-induced secretion. In the latter case, exogenous Ca^{2+} strongly increased the secretion

3. Mucin secretion from the sublingual gland could be affected by acetylcholine in the presence of exogenous Ca^{2+}. Noradrenalin and adrenalin induced only a slow mucin secretion and, for this secretory process, exogenous Ca^{2+} is also required. Though cyclic AMP is present in the sublingual gland, no influence on its level could be detected in this gland after stimulation of the adrenergic β-receptor, whereas, in contrast to the parotid gland, dibutyryl cyclic AMP induced only a slow secretion. Because it was observed that the sublingual gland of the mouse is not innervated sympathetically, it seems reasonable to suppose that the catecholamines stimulate the mucin secretion from this gland via hormonal receptors and not via the adrenergic β-receptor.

4. The protein secretion from the sublingual gland could be stimulated by both acetylcholine and the catecholamines An involvement of cyclic AMP in this process was not observed. Addition of exogenous Ca^{2+} is less important, as was found for the mucin secretion. So it has been concluded that protein and mucin secretion from the sublingual gland are regulated via different pathways.

INTRODUCTION

The organic and inorganic composition of the saliva is dependent on the activity of the different salivary glands, which are stimulated predominantly by the

autonomic nervous system [1] Several aspects of the secretory process of the serous parotid gland have been investigated already Schramm et al [2] reported that noradrenalin stimulates an adenylate cyclase, enhancing the concentration of cyclic AMP in the parotid gland of the rat This enzyme is related to the adrenergic β-receptor [3] A cyclic AMP-dependent protein kinase appears to be of critical importance for the secretion of amylase from the parotid gland [4] Butcher et al [5] indicated that microfilaments are involved in this secretory process, in which the membranes of the zymogen granules fuse with each other and with the plasma membrane, releasing the secretory product [6]

A model for the biochemical events occurring in secretion has already been given by Rasmussen [7] (Fig 1) Within this framework, secretion can be realized in different ways (a) the same secretory process can be regulated by two transmitters, or one transmitter can stimulate (b) different secretory processes within the same gland or (c) comparable processes in different glands. This can be illustrated by the following examples (a) The secretory cells of the parotid glands are innervated both sympathetically and parasympathetically [8, 9] Both parts of the autonomic nervous system appear to be involved in the secretion of amylase [1, 10] There is good evidence that in the adrenergic secretory process cyclic AMP possesses a key position. However, cyclic AMP is not involved in the cholinergic secretory process [11] (b) For the parotid gland of the rat, it has been demonstrated that the catecholamines stimulate

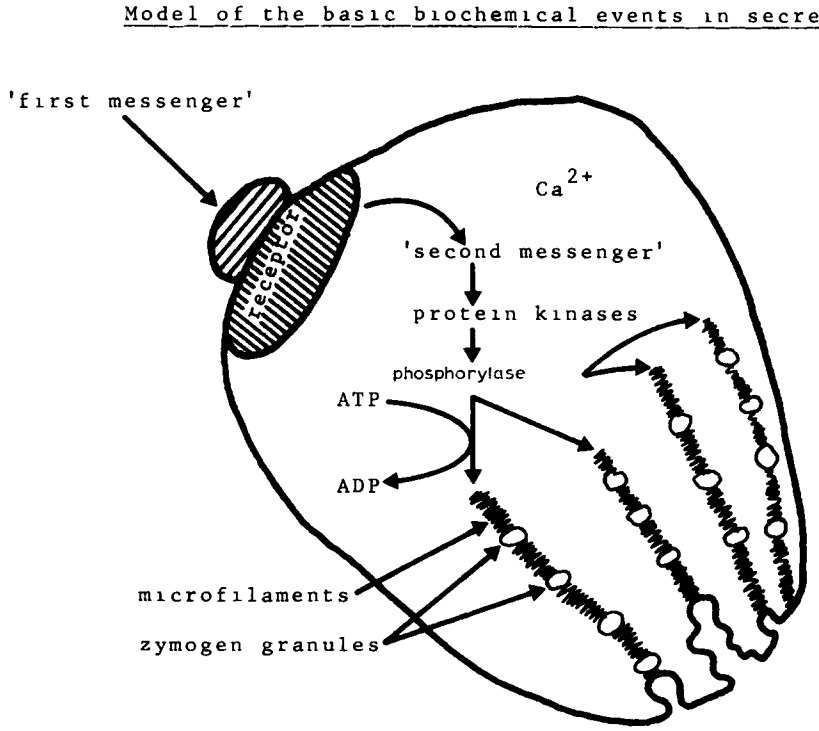

Fig 1 Model of the basic biochemical events in secretion (modified from Rasmussen [7])

both amylase and electrolyte secretion [1, 3, 12]. The amylase secretion is stimulated via the adrenergic β-receptor, while the secretion of electrolytes is regulated via the adrenergic α-receptor [3]. Both processes can also be stimulated by acetylcholine, but in that case probably via one and the same muscarinic receptor, as atropine inhibits both processes in the parotid gland [10, 13]. (c) Moreover, there are differences in the secretory processes of the serous and mucous cells of the salivary glands. For example, the fusion process of the membranes is different for the serous and mucous cells in the sublingual gland [14], whereas electron microscopically the serous secretory process in the sublingual gland seems to be similar to that in the parotid gland. Therefore, the intracellular reactions leading to the release of the secretory product are probably not the same for the serous and mucous cells.

For this reason, the secretion from the mucous sublingual gland of the mouse has been investigated and compared with the secretion induction of the serous parotid gland. The approach has been to study in vitro the effect of adrenalin, noradrenalin and acetylcholine on the secretion of glycoproteins (e.g. mucins) and proteins (e g. amylase), and in vivo the effect of isoproterenol and pilocarpine on the cyclic AMP level in the glands. Parts of this work have already been presented in a preliminary form [15].

MATERIALS

Acetylcholine iodide was purchased from Koch-Light, L-noradrenalin tartrate from Merck-Schuchardt, pilocarpine hydrochloride, D, L-isoproterenol hydrochloride, theophylline, cyclic AMP, cyclic GMP and N^2-O^2-dibutyryl cyclic GMP from Sigma, N^6-2′-O-dibutyryl cyclic AMP from Boehringer Mannheim, adrenalin from Nutritional Biochemical Corporation and cyclic [^{3}H]AMP from the Radiochemical Centre (Amersham).

METHODS

Incubation procedure

Female mice (Swiss-random) of 20–22 g were used for the investigation. The mice were decapitated, and the salivary glands were removed rapidly and cleaned in a Krebs-Ringer bicarbonate solution containing 5 mM β-hydroxybutyric acid, 10 mM nicotinamide, 10 mM inosine and 0.5 mM adenine (designated as Krebs-Ringer solution) [12].

The parotid glands were cut into five and the sublingual glands into two slices and, until all glands had been cleaned, they were preincubated in 3 and 2 ml Krebs-Ringer solution, respectively, and gassed with water-saturated carbogen (95 %, O_2, 5 % CO_2). Slices of six parotid or sublingual glands, respectively about 150 and 50 mg, were used per incubation vessel. After preincubation, the medium was decanted and the glands were incubated in Warburg vessels containing, respectively, 3 and 2 ml fresh Krebs-Ringer solution. The vessels were shaken continuously and gassed with carbogen. When $CaCl_2$ was omitted from the Krebs-Ringer solution, NaCl replaced it in equal molarity. In that case this medium was used throughout the whole experiment. All work was carried out in a room kept at a constant temperature of 37 °C.

During the incubation, 300-μl samples of the incubation fluid were removed at

periodic intervals and immediately placed in ice These samples were replaced by an equal aliquot of fresh incubation medium containing all additions. For the calculation of the percentages of secretion, a correction was made for the replacement of the samples.

Addition of stimulating agents occurred after a control incubation of 30 min, as a test on the normal behaviour of the salivary gland slices. In order to compensate for the oxidation of adrenalin and noradrenalin by the oxygen, a fresh dose of these secretagogues was added each half hour.

After incubation the vessels were placed in ice, which appeared to be effective for terminating the secretion. Within 5 min the fluid was decanted and the gland slices were weighed and homogenized in fresh Krebs-Ringer solution (with or without exogenous Ca^{2+}, because Mermall [16] has demonstrated that the activity of amylase can be influenced by Ca^{2+}). The homogenates were centrifuged at $100\,000 \times g$ for 1 h Homogenization and centrifugation were performed at 4 °C. The supernatant fraction and the samples from the incubation medium were assayed for amylase, sialic acid and protein. The total activity, or amount of these compounds in the supernatant plus the final incubation fluid, represents the material initially present in the supernatant fraction of the homogenized gland slices. The secretory percentages have been calculated relative to this initial amount

Sialic acid was assayed as marker for the glycoproteins in the serous parotid gland. In the mucous sublingual gland over 90 % of sialic acid is present in the mucins (unpublished observation) Therefore, in this gland, sialic acid may serve as a marker for the mucins

The results indicated in the figures are representative experiments, each of which was repeated at least three times.

Analytical procedures

Amylase was determined by the dyed amylopectine method as described by Voorhorst et al. [17], with a slight modification consisting of diluting the substrate 1 : 1 (v/v) with Caraway buffer without addition of glycogen Protein was determined according to the method of Lowry et al. [18], using horse serum as a reference Sialic acid was assayed by the thiobarbituric acid assay of Warren [19], after hydrolysis of the samples in 0 05 M H_2SO_4 at 80 °C, for 1 h. The values were expressed as *N*-acetylneuraminic acid (AcNeu). All determinations were performed on a microscale.

Ca was determined by atomic absorption (A_{423}) with an air-acetylene flame, using the Ca-standard for atomic absorption of Merck. Lanthanum chloride was added to prevent interference of phosphate.

Measurement of cyclic AMP in vivo

Female mice (Swiss-random) of 20–22 g were injected intraperitoneally with a solution of saline, isoproterenol (5 or 10 mg/kg body weight) or pilocarpine (3 or 5 mg/kg body weight). At various time intervals after the injection the mice were decapitated The glands were removed rapidly, cleaned in a cold saline solution and immediately frozen in liquid N_2. The glands were weighed and homogenized in 6 % (w/v) trichloroacetic acid with addition of cyclic [^{3}H]AMP ($\pm$20 nCi) as tracer. The cyclic AMP was extracted and washed with diethyl ether according to the method of Brown et al. [20]. The extracts were dried and dissolved in a 50 mM Tris · HCl buffer

(pH 7.4) with 8 mM theophylline and 6 mM 2-mercaptoethanol, and cyclic AMP was determined according to the method of Brown et al. [20]. The recovery of the cyclic AMP after the extraction procedure was 80 % or more. The cyclic AMP acceptor protein was isolated from bovine adrenals and stored in liquid N_2. The standard curve for cyclic AMP (0.2–20 pmol) was not influenced by cyclic GMP (100 pmol).

Fluorescence technique

For the histochemical demonstration of catecholamines by fluorescence microscopy, the glands were freeze-dried, using diphosphorous pentoxide as a water vapour trap. The histochemical reaction was performed according to Falck and Owman [21], by treating the dried tissues with formaldehyde vapour at 80 °C for 1 h, as described by Tilders et al. [22].

RESULTS

Incubation conditions

Table I shows that using the incubation medium according to Batzri et al. [12], it was possible to induce a high secretion from the parotid gland in vitro. All amylase in the parotid gland is soluble and secretable. The amounts of sialic acid and protein of the 100 000 × *g* supernatant fraction of the gland slices comprise both secretory material and non-secretory, soluble material of the gland slices. For this reason the calculated secretory percentages of these products will always be lower than 100

Secretion from the sublingual gland could be induced in the same incubation medium. However, under the experimental conditions used, only about 45 % of the protein-bound sialic acid and about 25 % of the protein was secreted (maximal incubation time was 3 h). Like Batzri et al. [12], no correlation was observed between the concentration of secretory products in the gland slices and the secretion rate or maximal secretory percentages, although their concentration varied considerably in the different groups of mice (Table I).

TABLE I

SECRETORY PRODUCTS IN THE GLAND SLICES

The amounts of secretory substances in the parotid and sublingual gland slices after preincubation are calculated as described in Methods Amylase is expressed as units/g wet weight and sialic acid and protein are given as mg/g wet weight. Used secretagogues. 12 μM adrenalin for the parotid gland, 10 μM acetylcholine for the protein-bound sialic acid secretion; 12 μM noradrenalin for the protein secretion from the sublingual gland

Gland	Secretory product	Present after preincubation	Soluble after preincubation	Maximal secretion observed after incubation of 3 h (%)
Parotid	amylase	15–30 10^6	15–30 · 10^6	90–100
	sialic acid	1 5–2 0	0 5–1 0	75– 85
	protein	70–100	40–60	50– 60
Sublingual	sialic acid	7–10	5–9	40– 50
	protein	20–50	10–30	20– 30

Because the energy supply of the gland slices might be a limiting factor for the secretory process [12], the glands have also been incubated in a Krebs-Ringer solution with a double concentration to adenine, nicotinamide, inosine and β-hydroxybutyric acid. The only effect, however, was a higher spontaneous secretion from the gland slices during the control incubation As $(Na^+ + K^+)$-ATPase may interfere with the energy supply, addition of 1 mM ouabain, to inhibit this enzyme, was also studied It had no effect on the acetylcholine-induced overall secretion from the parotid gland and the protein secretion from the sublingual gland. On the other hand, the mucin secretion from the latter gland was observed to be inhibited to a large extend (about 50 %) by the addition of ouabain (results not shown) Apparently the energy supply is not the limiting factor in the experiments.

Although the sublingual glands of the mouse contained some amylase, as has also been observed by Shear [23] with a starch substrate film method for histochemical localization, the secretion of amylase from this gland could not be measured, as most of the amylase leaked from the slices during the preincubation In contrast, the parotid gland slices lost maximally 10 % of their amylase content during this period.

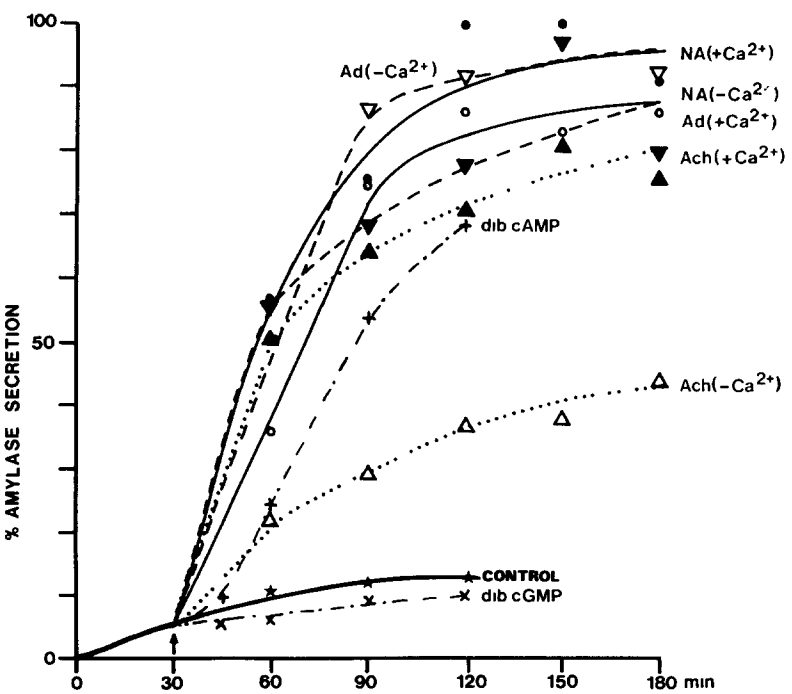

Fig. 2. Amylase secretion from the parotid gland in vitro Addition of secretagogues after a control incubation of 30 min (as indicated by the arrow). The 'spontaneous' secretion after 30 min was about the same in the different experiments △, 10 μM acetylcholine, ▽, 12 μM adrenalin, ○, 12 μM noradrenalin, closed symbols in the presence of exogenous Ca^{2+}, open symbols in the absence of this component, +, 1 mM dibutyryl cyclic AMP, ×, 1 mM dibutyryl cyclic GMP, ★, control incubation (with or without exogenous Ca^{2+}) All percentages obtained under influence of one and the same secretagogue are derived from the same experiment

Secretion induction and Ca^{2+} requirement

The Krebs-Ringer solution contained 2.5 mM $CaCl_2$ (designated exogenous Ca^{2+}). When $CaCl_2$ was omitted, the Krebs-Ringer solution contained only 0.005 mM Ca^{2+}, as determined by atomic absorption photometry. After 30 min of control incubation (the moment of addition of a secretagogue) the Ca^{2+} concentration of the Ca^{2+}-free incubation medium was less than 0 025 mM. After a control incubation of 3 h, taking samples every 30 min, the Ca^{2+} concentration was not enhanced for either the parotid or the sublingual gland slices.

Protein-bound sialic acid and protein (e.g. amylase) secretion from the parotid gland Both the catecholamines (noradrenalin and adrenalin) and acetylcholine induced a high secretion of protein-bound sialic acid, protein and amylase from the parotid gland For an effective acetylcholine-induced secretion the $CaCl_2$ in the incubation medium was required. In contrast, for the catecholamine-induced secretion exogenous Ca^{2+} was not necessary, but, if present, the initial secretion appeared to be increased somewhat, i.e in the case of noradrenalin stimulation of the overall secretion and the adrenalin stimulation of the protein-bound sialic acid secretion (Figs 2, 3 and 4 and Table II).

Protein-bound sialic acid secretion from the sublingual gland. Acetylcholine stimulated the protein-bound sialic acid secretion from the sublingual gland in the

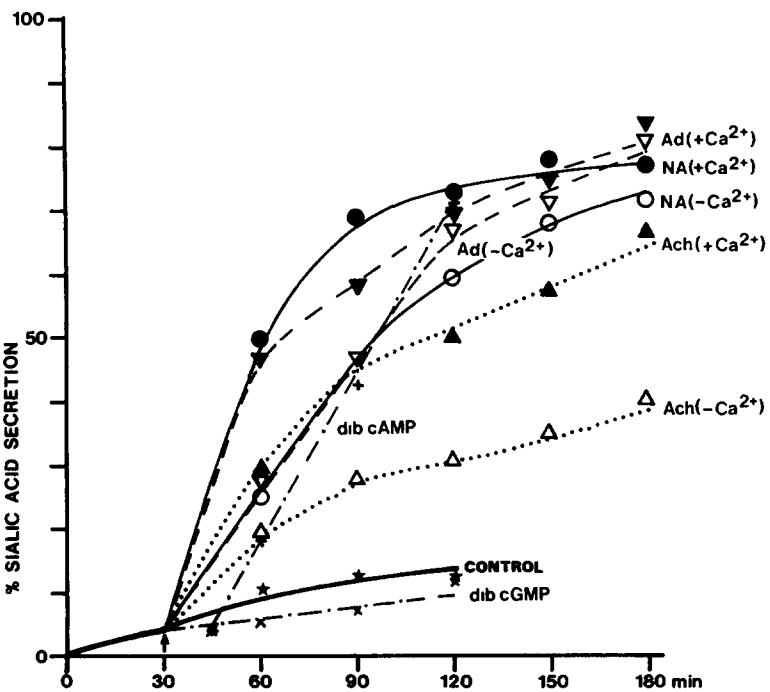

Fig. 3. Protein-bound sialic acid secretion from the parotid gland in vitro Addition of secretagogues after a control incubation of 30 min (as indicated by the arrow). The 'spontaneous' secretion after 30 min was about the same in the different experiments. For explanation of symbols, see legend to Fig 2 All percentages obtained under the influence of the same secretagogue are derived from the same experiment

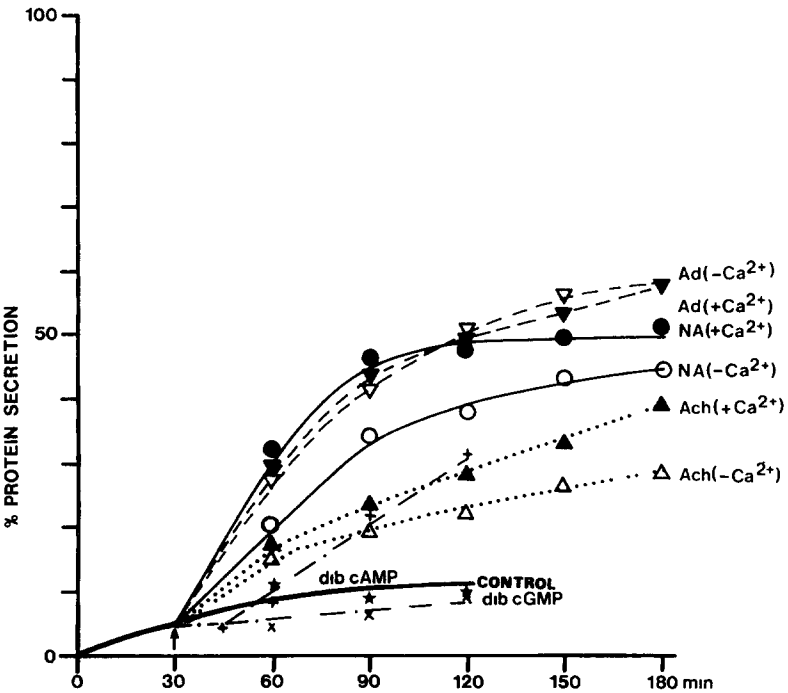

Fig 4 Protein secretion from the parotid gland in vitro Addition of secretagogues after a control incubation of 30 min (as indicated by the arrow) The 'spontaneous' secretion after 30 min was about the same in the different experiments For explanation of symbols, see legend to Fig 2 All percentages obtained under the influence of the same secretagogue are derived from the same experiment

TABLE II

INFLUENCE OF EXOGENOUS Ca^{2+} ON THE IN VITRO SECRETION

Influence of 2 5 mM $CaCl_2$ on the secretion from the parotid and sublingual gland slices, relative to the secretion in the absence of this component

$$\frac{\text{secretory percentage in the presence of exogenous } Ca^{2+}}{\text{secretory percentage in the absence of exogenous } Ca^{2+}} \times 100\ \%)$$

The figures are mean values of 3 or 4 experiments

Stimulant	Incubation time (min)	Parotid gland		Protein-bound sialic acid	Sublingual gland	
		Amylase	Protein		Protein	Protein-bound sialic acid
Noradrenalin	30	157	144	160	102	153
(12 μM)	150	104	107	107	107	160
Adrenalin	30	118	107	173	68	110
(12 μM)	150	87	104	103	80	132
Acetylcholine	30	162	176	187	97	268
(10 μM)	150	135	177	140	101	150

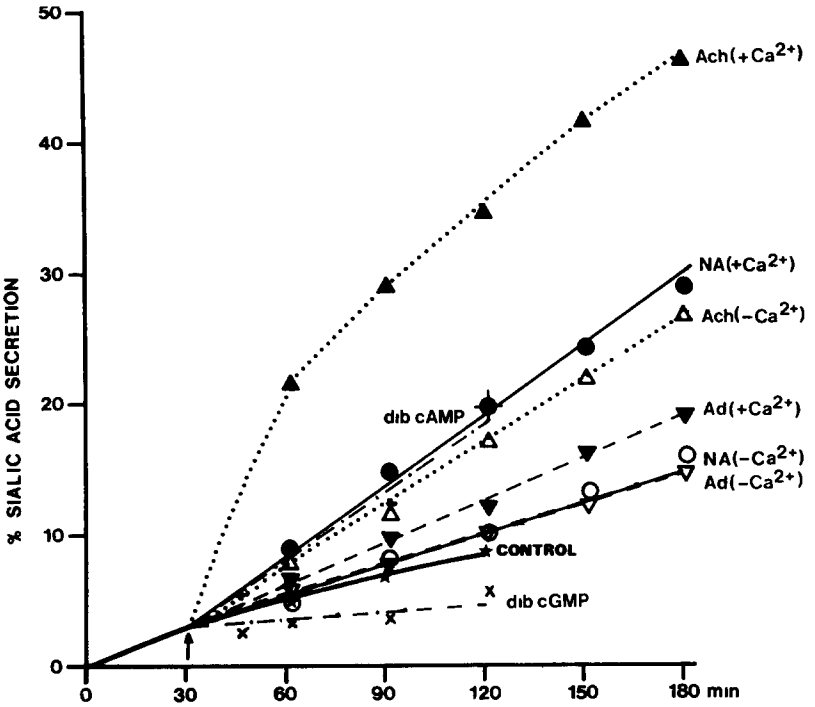

Fig 5 Protein-bound sialic acid secretion from the sublingual gland in vitro Addition of secretagogues after a control incubation of 30 min (see arrow) The 'spontaneous' secretion was about the same in the different experiments at this moment △, 10 μM acetylcholine; ▽, 12 μM adrenalin; ○, 12 μM noradrenalin, closed symbols, in the presence of exogenous Ca^{2+}, open symbols, in the absence of this component, +, 1 mM dibutyryl cyclic AMP, ×, 1 mM dibutyryl cyclic GMP, ★, control incubation (with or without exogenous Ca^{2+}) All percentages obtained under the influence of the same secretagogue are derived from the same experiment.

presence of exogenous Ca^{2+}. The catecholamines induced only a slow secretion from this gland and, in contrast to the parotid gland, in this case exogenous Ca^{2+} was necessary as in the acetylcholine-induced secretory process (Fig. 5 and Table II).

Protein secretion from the sublingual gland. The protein secretion from the sublingual gland was stimulated slightly by both acetylcholine and the catecholamines. In this case no clear effect of exogenous Ca^{2+} on the secretory process was observed (Fig. 6 and Table II).

Innervation

In the predominantly mucous sublingual gland, acetylcholine stimulated the protein-bound sialic acid secretion more effectively than noradrenalin. On the contrary, in the serous parotid gland the secretion was stimulated by both neurotransmitter substances.

By catecholamine-fluorescence microscopy it has been demonstrated that the serous acini of the parotid gland are surrounded by sympathetic nerve endings. In the sublingual gland only arteries and veins are innervated sympathetically. The mucous acini of this gland did not appear to be innervated in this way (Plates 1 and 2).

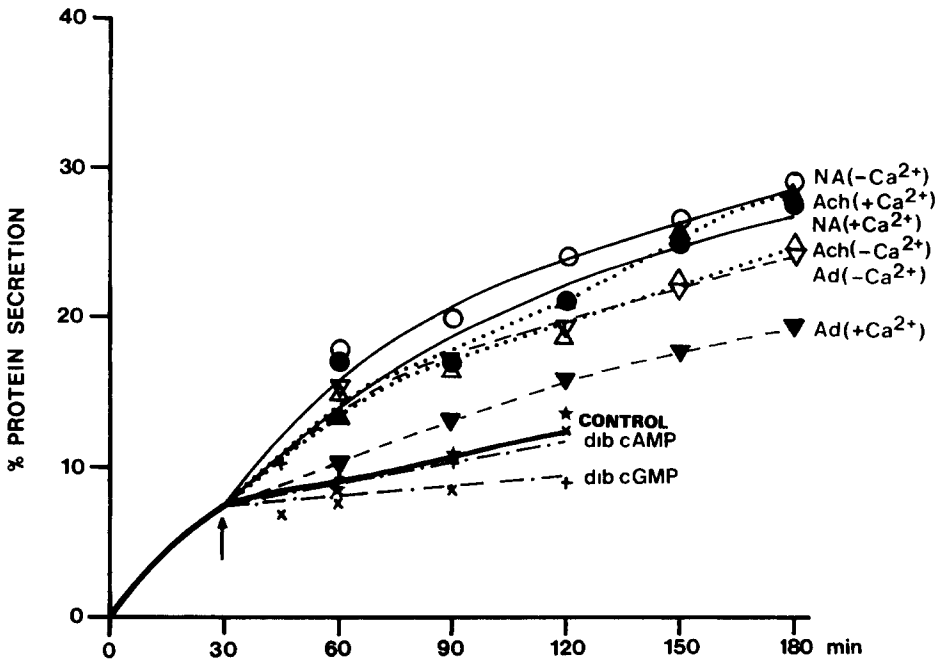

Fig 6 Protein secretion from the sublingual gland in vitro Addition of secretagogues after a control incubation of 30 min (see arrow) The 'spontaneous' secretion was about the same in the different experiments at this moment For explanation of symbols, see legend to Fig 5 All percentages obtained under the influence of the same secretagogue are derived from the same experiment

Cyclic AMP involvement in the secretory process

Cyclic AMP level in vivo. As mentioned in the introduction, cyclic AMP plays an important role in the process of secretion of amylase from the parotid gland. When mice were injected with the adrenergic β-stimulator isoproterenol, the cyclic AMP level in the parotid gland was strongly elevated 10 min after the intraperitoneal injection The cholinergic muscarinic stimulator pilocarpine had no effect on the cyclic AMP level in this gland (Fig. 7).

In the sublingual gland the cyclic AMP content was about the same as in the parotid gland. Injection of isoproterenol, however, did not increase the cyclic AMP level in this gland, while pilocarpine seemed to enhance this level only very slightly (Fig 8)

In vitro secretion under influence of dibutyryl cyclic AMP. After a short latent period, 1 mM dibutyryl cyclic AMP induced protein-bound sialic acid, amylase and protein secretion from the parotid gland slices, comparable with that under influence of the transmitter substances (Figs 2–4) From the sublingual gland only a slow protein-bound sialic acid secretion was induced by this drug (Fig. 5), and the protein secretion from this gland was not stimulated at all in this way (Fig 6). Dibutyryl cyclic GMP had no stimulating influence on the secretion either in the parotid gland or in the sublingual gland (Figs 2–6)

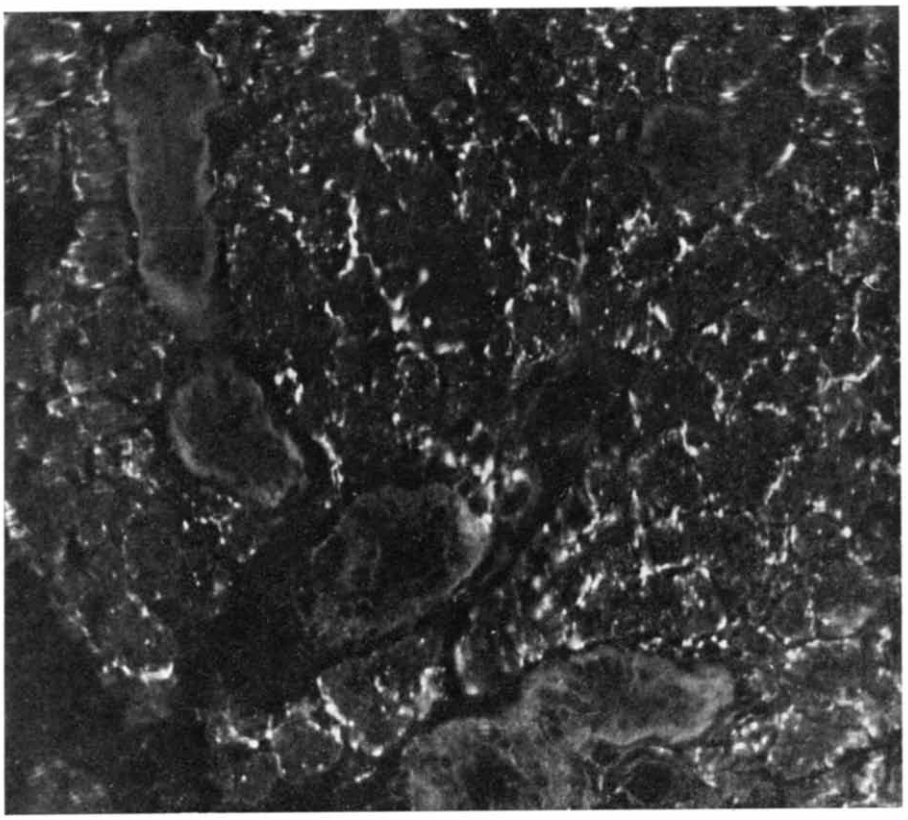

Plate 1 Parotid gland of the mouse catecholamine fluorescence of sympathetic nerve endings. All acini appear to be innervated sympathetically. However, the duct cells are not innervated in this way (film, Ilford PAN F, exposure time, 60 s, magnification, 158×)

DISCUSSION

In the literature, the secretion of saliva in an in vitro slice system has been described only for the parotid and the submandibular glands of some species. In this way the amylase, overall protein and K^+ secretion from the parotid gland of the rat [3, 10, 12, 24], and the amylase and glycoprotein (mucin) secretion from the submandibular gland [10, 25, 26] have been reported. However, up to now the secretory process in the predominantly mucous sublingual gland has not been described. Under our experimental conditions, stimulation of this gland in the same incubation medium as used for the parotid gland leads to a secretion of protein-bound sialic acid (mucin), which is readily measurable, though not maximal (Table I). Apparently, the energy supply of the gland slices is not the limiting factor. Addition of more substances which increase the ATP level in the cells or which inhibit the ATP-consuming enzyme, $(Na^+ + K^+)$-ATPase [12], did not enhance the secretion. The mucous secretion is probably more difficult than the serous secretion, because the viscous material is retained in the ducts. Inhibition of the ion (water) transport in the gland slices, by

Plate 2. Sublingual gland of the mouse catecholamine fluorescence of sympathetic nerve endings Only the blood vessels are innervated sympathetically, not the acini and the duct cells (film, Ilford PAN F, exposure time, 60 s, magnification, 158×)

ouabain, makes the secretion of the viscous mucins even more difficult (unpublished observation). Apparently the $(Na^+ + K^+)$-ATPase activity is of fundamental importance for the mucous secretory process. This observation can support the view that water secretion sustains the mucin secretion by washing out the ducts [27, 28] An other possibility is that the ion transport itself possesses a key position in this secretory process.

Regulation of secretion from the parotid gland by the autonomic nervous system

The secretion of glycoprotein and protein (e.g. amylase) from the parotid gland is stimulated by both catecholamines and acetylcholine The level of the secretion of amylase and protein under influence of adrenalin (Figs 2 and 4) is in good agreement with the experiments of Batzri et al. [12] and Bdolah et al. [29] with the parotid gland of the rat, whereas after addition of acetylcholine amylase is secreted to a much higher degree, as was found by Bdolah et al. [29] with carbamylcholine However, a high amylase or protein secretion from the parotid gland of the rabbit and the rat under influence of carbamylcholine or acetylcholine in the presence of an inhibitor of

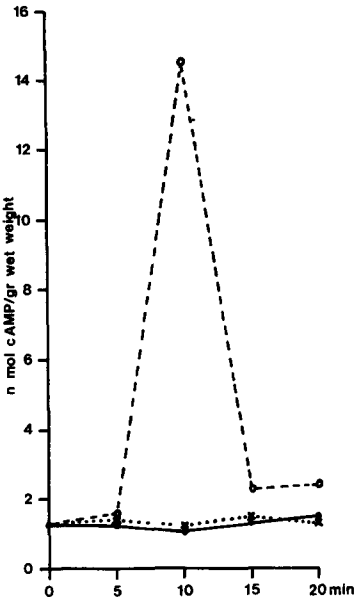

Fig 7. Cyclic AMP level in the parotid gland of the mouse after an intraperitoneal injection of 0.9 % NaCl (●), isoproterenol, 5 mg/kg (○) or pilocarpine, 3 mg/kg (×)

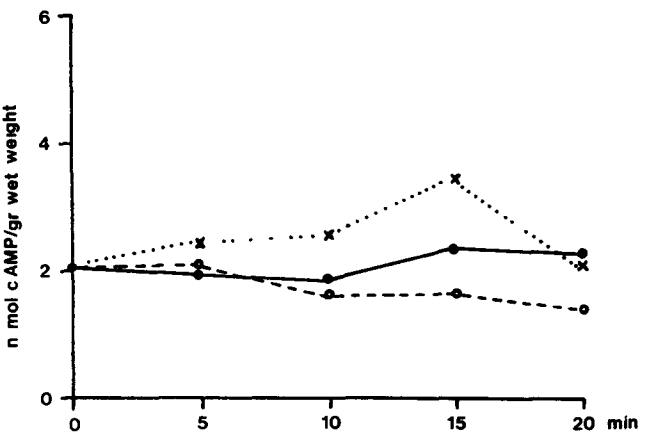

Fig. 8. Cyclic AMP level in the sublingual gland of the mouse after an intraperitoneal injection of 0 9 % NaCl (●), isoproterenol, 5 mg/kg (○) or pilocarpine, 3 mg/kg (×).

cholinesterase is reported by several authors [10, 24, 30]. Though we did not use a cholinesterase inhibitor, we found in our experiments an effective stimulation of the secretion from the salivary glands of the mouse under influence of acetylcholine. A possible explanation for this fact is that the samples were replaced by fresh incubation medium containing acetylcholine. It is reported by Hokin et al. [10] that very low concentrations of cholinergic drugs are sufficient to stimulate the secretion from the parotid glands of the guinea pig and the rabbit.

There are differences in the regulation of the parotid secretion by catecholamines and acetylcholine with regard to (a) the involvement of cyclic AMP and (b) the Ca^{2+} requirement In agreement with the experiments of Malamud [31], the cyclic AMP level in the parotid gland is enhanced by stimulation of the adrenergic β-receptor, and dibutyryl cyclic AMP is capable of inducing secretion from this gland (Figs 2–4) [32] On the other hand, acetylcholine has no effect on the cyclic AMP level of the parotid gland, but needs, in contrast to noradrenalin, exogenous Ca^{2+} in the incubation medium for an effective stimulation of the secretion Rossignol et al. [24] have shown that the ionophore A 23187, which enhances the permeability of the cell membrane for Ca^{2+}, induces protein secretion from the salivary glands of the rat In our experiments dibutyryl cyclic AMP has the same effect.

In analogy to the scheme proposed by Gross et al [33] for the activation of phosphorylase *b* in muscles, acetylcholine may activate an inactive phosphorylase kinase via Ca^{2+}, which accumulate in the gland under influence of this drug In this way the same phosphorylase in the salivary glands may be activated by catecholamines via cyclic AMP and by acetylcholine via Ca^{2+} (Fig. 9)

On the other hand, there is good evidence that intracellular Ca^{2+} has a function in the noradrenalin-induced secretory process, which can not be said about exogenous Ca^{2+} For instance, Ishida et al. [30] have reported that the noradrenalin-induced amylase secretion from the parotid gland of the rat, just as the cholinergic secretion, is Ca^{2+}-dependent. However, on the contrary, Schneyer et al. [1] asserted that exogenous Ca^{2+} is not necessary for the adrenalin-induced amylase secretion from the parotid gland of the rat, and Batzri et al [12] showed in his experiments that exogenous Ca^{2+} inhibits the adrenalin-induced amylase secretion from this gland Under our experimental conditions, exogenous Ca^{2+} is not required for the secretion induction by catecholamines, although it has been observed that the secretory process in the parotid gland of the mouse can occur somewhat more rapidly when these ions

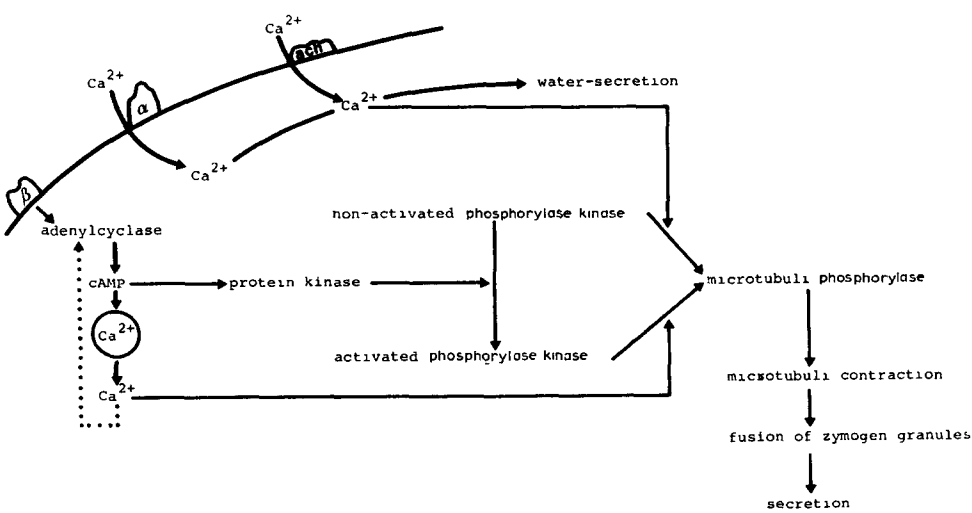

Fig 9 Hypothetical scheme of the regulation of the secretory processes in the parotid gland of the mouse (modified from Gross et al. [33])

are added (Figs 2–4 and Table II).

Dormer et al. [34] reported that adrenalin induces a release of Ca^{2+} from the mitochondria and microsomes in the parotid gland of the rat. Ca^{2+} plays an important role in the secretory process [35] and the intracellular Ca^{2+}-pool appears to be sufficient for the secretion induction. However, when catecholamines stimulate the gland slices in the presence of exogenous Ca^{2+}, these ions are accumulated in the cells [34]. It is possible that in this case the secretion induction is more rapid than when the Ca^{2+} has to be released from intracellular pools. On the other hand, it has been shown that catecholamines stimulate the water secretion in the presence of exogenous Ca^{2+}, which results in an inhibition of the amylase secretion [3, 36]. Therefore, it is possible that exogenous Ca^{2+} initially has some positive or no effect on the amylase secretion and later inhibits this secretory process (Fig 2).

Regulation of the secretion from the sublingual gland by the autonomic nervous system

The mucin secretion from the sublingual gland can be stimulated by acetylcholine, whereas noradrenalin and adrenalin induce only a slow secretion from this gland (Fig. 5). Histologically, sympathetic nerve endings could not be detected in the mucous acini of the sublingual gland (Plate 2). It is possible that the mucous cells contain some receptors for hormones transported by blood and that the catecholamines are able to induce a slight secretion via these receptors. In contrast to the parotid gland, addition of Ca^{2+} is necessary for the catecholamine-induced mucin secretion from the sublingual gland, and though cyclic AMP is present in this gland, the level of this compound can not be enhanced by isoproterenol in vivo (Fig. 8). Apparently, the adrenergic β-receptor is not present on the mucous secretory cells. However, the protein secretion from the sublingual gland is stimulated by both acetylcholine and the catecholamines. Neither the acetylcholine-, nor the catecholamine-induced protein secretory processes seem to need exogenous Ca^{2+}. Apparently, the protein secretion from the sublingual gland is regulated in a different way than the mucin secretion It is not known which cell type is responsible for the protein secretion. It is possible that the secreted proteins, at least in part, are derived from the serous demilune cells of the sublingual gland and that these cells, in contrast to the mucous cells, are innervated both sympathetically and parasympathetically. However, with the Falck technique, sympathetic innervation could not be observed (Plate 2) and, moreover, dibutyryl cyclic AMP can not induce protein secretion from this gland (Fig. 6). Thus, apparently, the protein secretion from the sublingual gland is also regulated in a different way than the serous secretion from the parotid gland. Histologically, Kim et al. [14] did not detect any secretion from the demilune cells of the sublingual gland of the rat after injection of the β-mimeticum isoproterenol, and the stimulator of the muscarinic receptor, pilocarpine. If the demilune cells are involved in the protein secretory process of the sublingual gland, it seems possible that this process is regulated via receptors of another type than those present on the serous and mucous acinar cells.

Pilocarpine seems to enhance the cyclic AMP level in the sublingual gland slightly (Fig. 8), but dibutyryl cyclic AMP is able to induce only a slow mucin secretion from this gland (Fig. 5). This indicates that cyclic AMP is not the second messenger for the cholinergic secretion in this gland. It is possible that the influence of pilocarpine on the cyclic AMP level is a secondary effect caused by stimulation of, for

example, hormonal receptors on the plasma membrane.

Which receptors are involved in the various secretory processes and which is the role of cyclic AMP in the salivary glands is under current investigation.

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