ing the vocal types: 1. Duration and frequency band of the filter, which is mostly addressed (called maximum filter); 2. average information per frequency band; 3. average information per time unit; 4. information of the maximum filter in relation to the average information per frequency band; 5. variation of the frequency units; 6. maximum and minimum values of the sums, if summation is extended over the time and frequency range respectively.

The calculated values for these parameters vary for the individual vocal types. By fixing an interval for each parameter, a vocalization type is defined. An individual vocalization (call) is then assigned to a special vocalization type, assuming that all of its parameter values fall into the corresponding intervals.

In order to specify the bounderies of the intervals, a random set of calls was chosen, designated by an observer as being the same type (say type A). This set was then computerized. The maximum and minimum value gained for each parameter over the whole set of calls was registered. The intervals obtained in this way define the vocal type A (44 cackle-, 94 peep- and 96 trill calls were chosen).

In the 2nd phase (a process of recognition), a large number of vocalizations was tested to establish the category to which they belong. Thereby the following results were obtained: 1. From 885 calls, which a laboratory observer[7] had classified as trill calls, 775 (87.5%) were determined actually to be of this type; 43 calls were not classified and 67 fell into other categories; 2. along the same line from 1020 peep calls, 856 (83.9%) were classified as peep; 108 calls were not classified and 56 fell into other categories; 3. from 740 cackles, 598 (80.8%) were recognized as belonging to this category; 136 calls were not determined and only 6 calls were assigned to other types.

Let us close with the following remark: Several observers can assign one and the same vocalization to different categories. Hence there is no 'correct' or 'true' classification of vocal types. The method presented here is nevertheless objective in the sense that each repetition leads to the same results, if the criteria for the classification have been determined.

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Impaired amylase release from the parotid gland of rats treated with reserpine[1]

J.R. Martinez[2] and O.H. Petersen

Department of Physiology, University of Dundee Medical School, Dundee DD1 4HN (Scotland), 9 January 1979

Summary. Using an automated system for the analysis of amylase, the release of this enzyme was compared in superfused parotid gland segments from control and reserpine treated rats. Stimulant-evoked amylase release was delayed and of smaller magnitude in the glands of the treated animals and a reduction of the transmembrane K^+ gradient caused a smaller and short lasting reduction in Ach-evoked release of amylase in the glands from these animals.

The chronic administration of reserpine to rats induces morphological and secretory changes in the salivary glands which resemble those seen in cystic fibrosis, a human hereditary disease which affects the exocrine glands and their secretions[3,4]. In addition to reduced salivary volumes and changes in the composition of saliva secreted in vivo, the in vitro release of K^+ has been found to be significantly increased in submandibular and parotid gland slices from rats treated in a chronic fashion with reserpine[5,6]. Parotid slices of reserpine treated rats also show a significantly decreased release of amylase upon stimulation with epinephrine or with isoprenaline[6]. An automated system for the continuous analysis of amylase release from pancreatic and parotid tissues superfused in vitro has been developed, which provides useful information about the kinetics of enzyme release[7,8]. This method was used in this investigation to compare the release of amylase from superfused parotid slices of normal and reserpine treated rats. The release of amylase induced by cholinergic and adrenergic secretagogues and the effects of ionic substitutions in the perfusion solution on the acetylcholine-induced enzyme release were assessed in the 2 types of parotid gland preparation.

Methods. Female Sprague-Dawley rats weighing between 200 and 290 g were used. The animals had free access to a standard pelleted diet and to water and were divided into 2 groups housed in the same animal quarters: an untreated control and a group of animals that received 7 daily doses of reserpine (0.5 mg/kg b.wt) prior to the day of the experiment, as previously described[3,4]. The animals were

Response to secretagogues*

Secretagogue	Control Amylase release (mU/mg min)	Time to reach maximum (min)	Reserpine Amylase release (mU/mg min)	Time to reach maximum (min)
None (washout)	148.8 ± 13.6	–	215.5 ± 14.6	–
Acetylcholine (10^{-5} M)	85.0 ± 4.7	2.4 ± 0.35	45.5 ± 3.9	5.8 ± 0.61
Phenylephrine (10^{-5} M)	18.0 ± 4.5	5.3 ± 2.20	5.2 ± 1.52	7.2 ± 1.9
Adrenaline (10^{-6} M)	108.0 ± 12.1	12.0 ± 0.90	79.6 ± 7.2	15.8 ± 2.5
Isoprenaline (10^{-6} M)	113.4 ± 11.2	21.0 ± 2.18	88.13 ± 12.25	27.46 ± 1.31

*Mean values ± SEM (n = 8).

killed by a blow on the head and the parotid glands were removed, placed in a Krebs-Henseleit solution and cut with a razor blade into pieces of approximately 5 mm^3. Between 150 and 200 mg of the cut segments were placed in a small translucent plastic chamber (1 ml capacity) through which oxygenated Krebs-Henseleit solution was pumped at a rate of 1 ml/min. The effluent from the chamber was fed into the input system for the amylase assay. This assay was performed continuously and automatically, as previously described[7]. Amylopectin anthranilate (Calbiochem) was used as substrate in a colloidal solution. Technicon pumps were used to pump fluid through the system and amylase output was continuously recorded as fluorescence intensity in a Technicon pen recorder.

The oxygenated Krebs-Henseleit solution used in these experiments had the following composition (mM): NaCl, 103; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13; NaH_2PO_4, 1.15; $NaHCO_3$, 25; D-glucose, 2.8; Na fumarate, 2.7; Na pyruvate, 4.9; Na glutamate, 4.9. The solution was gassed with 95% O_2 and 5% CO_2. In some experiments, the K^+ concentration was increased about 10-fold to 50 mM and the Na^+ concentration was correspondingly reduced to maintain constant osmolarity. In others, the K^+ concentration was reduced to 0.5 mM. Some experiments were performed in Krebs-Henseleit solution containing no Ca^{++} and 0.1 mM EGTA. Appropriate specific blockers (phentolamine, propranolol, atropine) were added to the perfusion solution in these experiments to prevent the effect of endogenous neurotransmitters possibly released under the altered ionic conditions. The following secretagogues were used: 1. acetylcholine (10^{-8}–10^{-5} M); adrenaline (10^{-7}–5×10^{-6} M); phenylephrine (10^{-6} M); and isoprenaline (10^{-6} M).

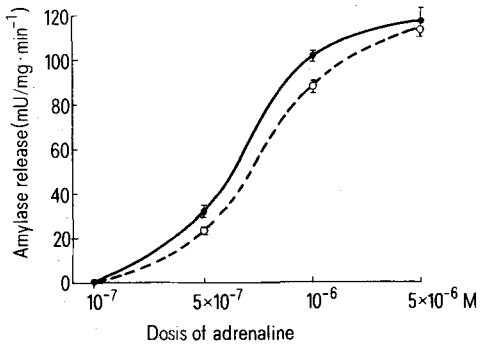

Fig. 1. Dose-response curves for adrenaline-evoked amylase release. The solid line represents results obtained from control glands while the dotted line represents results obtained on glands from reserpirized rats. Mean values ± SE (n = 5) are shown.

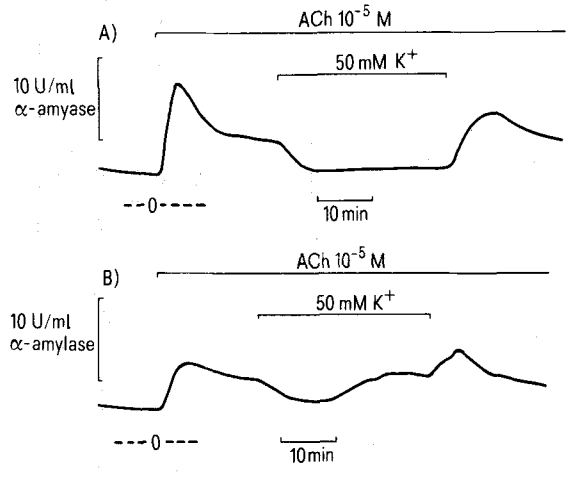

Fig. 2. The effect of changing the transmembrane K^+ gradient on Ach-evoked amylase secretion in *A* parotid tissue from normal rats and *B* parotid tissue from reserpinized rats. The figure shows concentration of amylase in tissue bath effluent as a function of time. The dotted line labelled o indicates baseline fluorescence (without tissue present). At bar labelled 50 mM K^+ the control Krebs solution ($[K^+]=4.7$ mM) was replaced by an isosmolar solution with [K] = 50 mM and with a correspondingly lowered [Na]. Phentolamine (10^{-5} M) and propranolol (5×10^{-6} M) present throughout.

Results. 1. Response to secretagogues. The table illustrates the extent of amylase released by the parotid segments of control and reserpine treated rats and the time required to reach the maximum response in the 2 groups of glands upon addition of the various secretagogues. The basal (unstimulated) secretion of amylase was significantly increased in the glands of the treated rats. The response to ACh was significantly reduced and retarded in the gland segments of the reserpine treated animals. The amylase released upon stimulation with phenylephrine and adrenaline was also significantly reduced in the glands of the treated animals, but the times required to reach a maximum response, although reduced, were not significantly different from those of control glands. The amount of amylase released in the presence of isoprenaline was not significantly different in the 2 types of gland, nor was the time required to attain the maximum release of amylase.

The dose response curve to adrenaline is illustrated in figure 1. The adrenaline response occurs within a narrow range of doses (from 10^{-7} to 5×10^{-6} M). In agreement with the response to individual doses of adrenaline described above, the dose-response curve of the parotid glands of reserpine treated animals is shifted to the right, indicating a smaller amylase secretory response for a given dose of adrenaline than in control glands.

2. Effect of ionic substitutions. The effect of a high (50 mM) K^+ concentration in the perfusate on the response to Ach is illustrated in figure 2. The upper part of this figure shows that increasing the external K^+ concentration 10-fold causes a sustained decrease in amylase release from control glands. The lower part of the figure shows that, in contrast, the reduction in amylase release induced by acetylcholine is not sustained in the parotid gland segments of reserpine treated rats, but rapidly returns to the original level obtained in normal K^+ Krebs-Henseleit solution.

Reduction in the perfusate K^+ concentration to 0.5 mM resulted in a sustained level of amylase release after acetylcholine stimulation or, in a few cases, in a slightly increased amylase release in the parotid gland segments of control rats. This increase was not as discernible in the glands of reserpine treated animals, which also showed, in a few instances, a delayed return to basal levels when the acetylcholine-containing perfusate was substituted for regular Krebs' solution after a period of perfusion with the low K^+ solution.

Perfusion with a Ca^{++} free solution containing both acetylcholine and 0.1 mM EGTA resulted in a gradual decrease in amylase release which was indistinguishable in the parotid gland segments of control and reserpine treated rats.

Discussion. The release of amylase was found to be delayed and significantly reduced in parotid gland segments of rats treated in a chronic fashion with reserpine. The comparison of the responses to the different secretagogues used suggests some possible reasons for the inhibition of enzyme release in the glands of the treated animals. In control glands, a small but significant release of amylase occurs after stimulation of cholinergic and α-adrenergic receptors, which is only partially the result of a washout phenomenon secon-

dary to fluid secretion[8,9]. During perfusion with a high K^+ solution, there was a sustained decrease in amylase release in the experiments on control glands[8] (figure 2). The parotid segments from the reserpine treated rats, however, showed an inability to maintain a reduced level of amylase release when perfused with a high K^+ solution. Decreasing the transmembrane K^+ gradient reduces ACh-evoked K^+ release in normal salivary glands[10]. The much diminished and shortlasting inhibition by high K^+ of ACh-evoked amylase release in the glands from reserpinized animals is probably related to the enhanced stimulant-evoked K^+ release in these conditions[5].

The results showing that the reserpine-induced impairment of stimulant-evoked enzyme secretion is much more marked during excitation of cholinergic or α-adrenergic receptors than during activation of β-adrenergic receptors (table) again points to an anomalous K^+ metabolism in the glands from reserpinized animals, since cholinergic or α-adrenergic stimulants, in contrast to β-adrenergic stimulants, cause a marked K^+ release[8,11].

Glands from reserpinized animals contain more Ca than controls[5,6] and since stimulant-evoked K release seems to be initiated by an increase in internal Ca^{2+} concentration[11], the results presented here may at least in part be explained by an abnormal cellular Ca metabolism.

Since the salivary glands of reserpinized animals have defects similar to those seen in cystic fibrosis[4,5] the present results would indicate that an abnormal K transport is an important element in the cellular disorder underlying this disease.

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2 Permanent address: Department of Child Health, University of Missouri School of Medicine, Columbia (Missouri 65212, USA).

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A possible preferential inhibition of chemotaxis of polymorphonuclear neutrophils by a chemical modification

T. Yamashita, K. Takamori and Y. Tanaka

Laboratory of Physiological Chemistry, School of Medicine, Juntendo University, Hongo, Bunkyo-ku, Tokyo 113 (Japan), 27 December 1978

Summary. The modification of neutrophils with amino group blocking reagents of different chemical specificities showed that dansyl chloride caused inhibition of chemotaxis without suppression of random movement. Dansylated neutrophils, like control cells, ingested bacteria. Neither the stimulated cyanide-insensitive respiration, nor lactate production during phagocytosis, was affected significantly by dansylation as compared with the inhibition of directed movement.

The movement of polymorphonuclear neutrophils (PMNs) towards a site of infection or injury is directed by a chemical gradient produced at the site. Leukocyte chemotaxis has been extensively investigated in vitro using the Boyden technique[1,2]. However, the mechanisms involved are still almost entirely unknown; how the attractant is detected and how the sensed information is translated into action. It would be very useful as a means of answering these questions to inhibit separately locomotion and chemotactic response by a chemical modification. Therefore, we tried to stain separately the directional and random movements, using amino group blocking reagents of different chemical specificities, and obtained the result that dansyl chloride inhibited chemotaxis more specifically than random movement, phagocytic ability and lactate formation in PMNs.

Materials and methods. Fluorescamine was from Roche; dansyl chloride from Sigma; sodium 2,4,6-trinitrobenzene sulfonate (TNBS) from Tokyo Chemical Industry Co. PMNs were obtained from glycogen-induced peritoneal exudates of guinea-pig[3,4]. A chemical modification of PMNs was carried out by incubating 2×10^7 cells/ml with reagents at 0 °C for a defined time. When acetone was used as a solvent for blocking reagents, its final concentration was 1% in a modification mixture. The reaction was stopped by the addition of a 2-fold molar excess of glycine. The reaction mixture was diluted 4 times with PBS for the motility assay. PMN migration was assayed as described previously[3,4] in a modified Boyden chamber in the presence (chemotaxis) and absence (random movement) of the chemoattractant (the supernatant from a sonicated suspension of *Escherichia coli* grown for 20 h at 37 °C in a polypeptone medium): 2 ml of a bacterial attractant in PBS, or PBS alone, were placed in the bottom of a

Table 1. Effects of amino group blocking reagents, glycine and acetone on chemotaxis of neutrophils

Compounds		Neutrophil migration (percent of control)	Viability (%)
Fluorescamine	100 μM	2	90
Dansyl chloride	100 μM	4	90
TNBS	1 mM	102	91
Glycine	10 mM	100	93
Acetone	4%	102	96
	8%	95	93
	12%	85	94

A chemical modification of PMNs with reagents and a treatment of PMNs with glycine or acetone were carried out at 0 °C for 60 min. After 4-fold dilution with PBS, chemotaxis was assayed. Migration values indicate the mean of 2 experiments in which the control PMN migrated 70 and 86 μm in 40 min at 37 °C, respectively.