

The effects of histamine, pyrilamine, cimetidine, and ranitidine on secretion of lingual lipase and amylase from rat von Ebner's glands

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Abstract

Minced von Ebner's glands of rat tongue were incubated *in vitro* with histamine and histamine receptor antagonists. At various time intervals, media and homogenates of the tissue were assayed for lingual lipase and amylase activity and percentage secretion calculated. Histamine elicited moderate secretion (~10%) of lingual lipase and amylase. In contrast, pyrilamine, an H₁ receptor antagonist, elicited >60% secretion. There were statistically significant differences between the percentage secretion of lingual lipase and amylase for basal secretion, as well as for histamine- and pyrilamine-evoked secretion above basal. The H₂ receptor inhibitors, cimetidine and ranitidine, stimulated secretion of only amylase, but not lingual lipase. When combined with histamine, these antagonists partially inhibited only the secretion of histamine-evoked lingual lipase, but not amylase. The differences in percentage secretion between the two enzymes indicate that exocytosis may not be the only process involved in protein secretion. The anomalous effects of the H₁ and H₂ receptor antagonists necessitate a more detailed characterization of the receptors of von Ebner's glands.

Introduction

Histamine initiates a variety of physiological responses that are mediated through receptors, H₁, H₂, and H₃, and inhibited by receptor antagonists. These receptors were classified by agonist effects and the inhibition by specific antagonists of histamine-evoked responses [1-3]. Some antagonists for the H₁ receptor are antihistamines, inhibitors of the allergic response evoked by histamine. Classi-

cally, gastric and other exocrine secretion was considered to be mediated through the H₂ receptor [1, 2, 4]. Only a few functions of the H₃ receptor have been defined. The H₃ receptor was found to be a mediator of the regulation of histamine release and synthesis [1, 2, 5].

Histamine stimulates secretion of gastric juice [6] and both acid and pepsinogen from the stomach [2, 4, 7, 8]. Histamine also stimulates the exocrine pancreas to secrete fluid [9] and digestive enzymes [10-12] and the major salivary glands (parotid and submandibular) [9, 13, 14] to secrete fluids.

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The effect of histamine on minor salivary gland secretion has not been investigated. Thus, it is of interest to investigate the role of histamine in the regulation of secretion of protein from rat von Ebner's (lingual serous) glands, a minor exocrine salivary gland. Von Ebner's glands of rat tongue are located in the posterior portion of the tongue, embedded between the muscle fibers beneath the vallate and foliate papillae [15]. A unique function of these minor salivary glands is to secrete the digestive enzyme, lingual lipase [16]. Lingual lipase is the enzyme responsible for the first step of fat digestion. Lingual lipase hydrolyzes triacylglycerol at the acid pH of the stomach producing amphipathic products that form emulsions which aid in the further digestion of fats by pancreatic lipase [17, 18]. Von Ebner's glands also secrete amylase [16]. The regulation of protein secretion from von Ebner's glands differs from other salivary glands. Protein secretion from the parotid gland is mediated mainly by the β -adrenergic receptor and fluid secretion is mediated by the cholinergic receptor [19]. Cholinergic stimulation (carbamylcholine chloride, pilocarpine) is the primary mechanism of protein secretion from von Ebner's glands. Isoproterenol (β -adrenergic) elicited minor secretion and phenylephrine (α -adrenergic) evoked no response [16]. In addition, in von Ebner's gland protein secretion is also elicited by substance P [20].

In the present study, the effects of histamine on the secretion of lingual lipase and amylase from von Ebner's glands were investigated. The role of H_1 and H_2 receptors in histamine-evoked secretion was evaluated with the H_1 receptor antagonist, pyrilamine, and the H_2 receptor antagonists, cimetidine and ranitidine.

Materials and methods

Chemicals

Histamine-free base and pyrilamine maleate salt were purchased from Sigma, St. Louis, MO. Cimetidine hydrochloride (injection) was obtained from SK&F Lab Co, Cidra, PR and ranitidine hydrochloride (injection) from Glaxo, Research Triangle Park, NC. Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco Laboratories, Grand Island, NY. The radioisotopes used in the lingual lipase assay, tri-[9,10- 3H]-oleoylglycerol and [9,10- 3H]-oleic acid, were from Dupont NEN Research Products, Boston, MA. L- α -phosphatidyl

choline, type III-E, taurodeoxycholine, trioleoylglycerol (99%), and starch were from Sigma, St. Louis, MO. All other chemicals were of reagent grade.

Tissue preparation and incubation

These experiments were carried out as previously described, with some modifications [16]. Briefly, Sprague-Dawley male rats, 200 ± 30 g, certified free of sialodacryoadenitis and rat corona viruses, were obtained from Charles River Laboratories, Raleigh, NC and housed under controlled temperature, humidity, and lighting (on at 0800 and off at 2000 h daily), and allowed unlimited access to water and a commercial pelleted diet. For each experiment, eight rats were fasted overnight, anesthetized with nembutal (50 mg/kg, i.p.), and exsanguinated. Tongues were removed and the glands dissected one at a time, being careful to exclude any mucous glands which are found posterior and lateral to von Ebner's glands. The glands were minced and pooled in 1.33 ml DMEM, previously gassed with 95% O_2 , 5% CO_2 . The glands were gassed after each addition of tissue. When all the glands were dissected, DMEM was added and the tissue randomly divided into eight 50 ml round-bottom polycarbonate tubes. Controls, to determine basal secretion, were done in duplicate with each experiment and all test samples were in duplicate. After centrifugation to $240 \times g$, the media were aspirated and discarded. One milliliter DMEM or 1 ml of a DMEM solution of a receptor antagonist was added to the tubes for preincubation. The tubes were gassed and incubated for 20 min at $37^\circ C$ in an AO water bath shaker at 90 cycles/min. The media were aspirated and discarded and 1 ml of DMEM alone (basal secretion) or DMEM solutions of the agonist, antagonist, or the solution of the agonist plus the solution of the antagonist were added to the tissue. The incubation was carried out with gassing every 15 min. Aliquots of 0.2 ml were removed (0.1 ml into two cryogenic tubes) at 30, 60, and 90 min and replaced with 0.2 ml of the appropriate solution. Experiments with pyrilamine and some experiments with histamine were also aliquoted at 15 min. At the final time interval (90 min), all the media were aspirated and stored in two cryogenic tubes/sample in liquid nitrogen. The tissue was washed with DMEM, frozen on dry ice, thawed, and then homogenized with a Polytron (Brinkmann Instruments) for 30 s in Tyrode's solution (glucose-free),

total volume = 4 ml. The homogenates were centrifuged for 15 min at $850 \times g$ and the supernatants were stored in two cryogenic tubes/sample in liquid nitrogen.

Enzyme assays

Amylase was assayed the next day by the method of Bernfeld [21]. A unit of enzyme activity is defined as a milligram equivalent of maltose formed in 3 min at 30°C. Lingual lipase was assayed the following day by the method of Field and Scow [22]. A unit of enzyme activity is defined as a micromole of fatty acid produced per minute at 37°C. Since the samples were stored in two cryogenic tubes/sample, each assay could be performed on samples that had only been thawed once.

Agonists and antagonists tested

Solutions of agonists and antagonists were prepared in DMEM. The dose response was evaluated at concentrations of histamine in DMEM of 0.01, 0.1, 1, and 10 mM and the dose response to pyrilamine was evaluated at 0.001, 0.01, 1, 5, and 10 mM. In all other experiments, the concentration of histamine, the H₂ receptor antagonists, cimetidine and ranitidine, and the H₁ receptor antagonist, pyrilamine, was 10 mM. The pH of the histamine solutions was adjusted with HCl to 7.6, which was the pH of the gassed DMEM. Cimetidine and ranitidine solutions did not need pH adjustment. Pylamine solutions were adjusted with NaOH to pH 7.6.

Calculations

The data were expressed as percentage secretion. The units of enzyme activity were calculated for media and homogenate samples at each time interval. Appropriate corrections were made for the enzyme activity in the volume of sample removed at each time interval. The total enzyme activity was the sum of the units in the media and in the tissue homogenates at the longest time interval. Percentage secretion was calculated as the units secreted divided by the total activity times 100 at each time interval. In some cases, to evaluate the secretion that resulted from the treatment alone, which was expressed as percentage secretion above basal, the percentage secretion of the controls (basal secretion) was subtracted from the total percentage secretion.

Statistics

All statistics were performed using the SAS statistical software package (SAS Institute, Cary, NC). Parametric statistics were used only after the data were checked for normality and homoscedasticity. Deviations from normality were tested with the Wilk-Shapiro test and the homoscedasticity was checked by visual examination of residual plots from the ANOVA model. In some cases, it was necessary to take log_e transformations of the data in order to meet the parametric assumptions [23]. If the data were clearly nonnormal even after transformation, then the Wilcoxon two-tailed signed-rank test on differences was used. For the parametric analysis, the split-plot ANOVA was used to test the effects of treatment where multiple samples were derived from the same tissue preparation. Individual comparisons were made only if the overall ANOVA was significant at the $p < 0.05$ level. When multiple comparisons were made in the same analysis, the reported p -value was adjusted by the Bonferroni correction factor [24]. Differences were considered statistically significant when $p < 0.05$.

Results

Basal secretion

The control values representing basal secretion of tissue incubated with DMEM only for all the experiments are shown in Table 1. The percentage secretion of amylase was greater than and statistically significantly different from the percentage secretion of lingual lipase at 15, 30, and 60 min. At 90 min the difference was not significant. Thus, under nonstimulating conditions at 15, 30, and 60 min, the proportion of amylase and lingual lipase secreted was not the same, amylase being greater.

Histamine

The secretory response of von Ebner's glands to histamine was evaluated at histamine concentrations of 0.01–10 mM. The results of the 60 min incubation are shown in Fig. 1. The secretory response was dose-dependent with histamine concentration of 10 mM giving the greatest secretory response. This concentration was used in all subsequent experiments.

Table 1

Basal percentage secretion of lingual lipase and amylase of the untreated controls. The *p* values were determined by the nonparametric two-tailed Wilcoxon signed-rank test on differences between lingual lipase and amylase

	Time (min)	<i>n</i>	Percentage secretion mean \pm SE	<i>p</i>
Lingual lipase	15	10	3.40 \pm 0.56	0.002
Amylase			7.55 \pm 0.68	
Lingual lipase	30	28	6.58 \pm 0.69	0.0001
Amylase			9.42 \pm 0.40	
Lingual lipase	60	28	10.07 \pm 0.79	0.0001
Amylase			13.15 \pm 0.48	
Lingual lipase	90	23	14.02 \pm 1.14	0.057
Amylase			15.27 \pm 0.72	

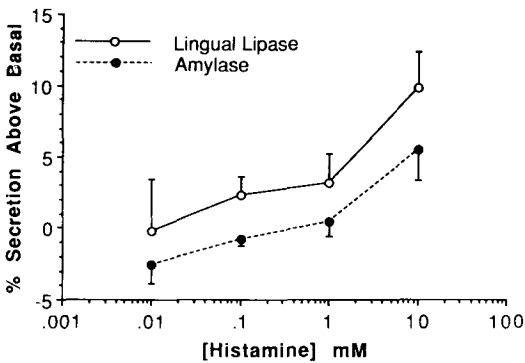


Figure 1
This is a dose-response curve showing the percentage secretion of lingual lipase and amylase above basal evoked by 0.01–10 mM histamine when incubated with minced von Ebner's tissue for 60 min. The values for basal secretion were 12.38 \pm 1.66% and 13.49 \pm 0.83% for lingual lipase and amylase, respectively (*n* = 3–11).

Fig. 2 shows the percentage secretion above basal resulting from the incubation of minced von Ebner's glands with 10 mM histamine with sampling at 15, 30, 60, and 90 min. There were significant differences between the percentage lingual lipase secreted and the percentage amylase secreted at 60 and 90 min. However, when the data of the total percentage secretion were evaluated by the Wilcoxon signed-rank test, the difference between the percentage secretion of lingual lipase and amylase was statistically significant, *p* = 0.021, only at 30 min. The percentage secretion elicited by histamine was significantly different from the basal response for both enzymes, but neither of the enzymes was significantly different from basal at 15 min using Wilcoxon signed-rank test.

H₂ receptor antagonists

To characterize further the process of secretion stimulated by histamine, antagonists to the H₂ receptor were investigated. Ten millimolar cimet-

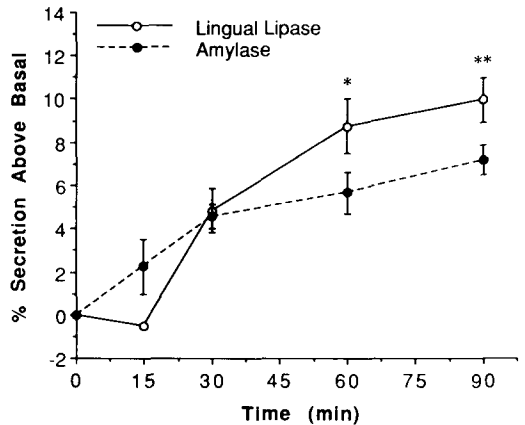


Figure 2
Time course of the percentage secretion above basal evoked by 10 mM histamine. Minced von Ebner's glands were incubated with 10 mM histamine. Media samples were taken at 15, 30, 60, and 90 min and assayed for lingual lipase and amylase. Homogenates of the tissue were assayed at 90 min. The values for basal secretion were 5.37 \pm 1.14%, 5.54 \pm 0.88%, 9.41 \pm 1.25%, 11.49 \pm 1.29% for lingual lipase, and 9.73 \pm 1.65%, 8.96 \pm 0.77%, 12.96 \pm 0.88%, 13.57 \pm 1.08% for amylase, *n* = 3, 13, 11, and 8, for 15, 30, 60, and 90 min, respectively. * and ** indicate significant differences between the percentage secretion of lingual lipase and amylase. The data were evaluated by the nonparametric Wilcoxon signed-rank test; *p* = 0.024 at 60 min and *p* = 0.0078 for 90 min. The secretion of both enzymes elicited by histamine was significantly different from basal secretion at 30, 60, and 90 min, but not at 15 min; *p* = 0.0007, 0.091, and 0.0078 for lingual lipase and *p* = 0.0002, 0.0005, 0.001 for amylase at 30, 60, and 90 min, respectively.

dine or ranitidine was preincubated with the tissue for 20 min prior to incubating the tissue with the antagonist plus 10 mM histamine. As seen in Fig. 3A, cimetidine inhibited the secretion of lingual lipase evoked by histamine at 30 and 60 min. However, as shown in Fig. 3B, the secretion of amylase

was not significantly inhibited by the presence of cimetidine at any time interval.

Table 2 reveals that there was no significant effect on the secretion of lingual lipase by cimetidine alone at any time interval. However, the release of amylase in the presence of cimetidine was significantly different from the controls at 60 and 90 min. Thus, 10 mM cimetidine evoked secretory responses in the release of amylase and lingual lipase that differed. Cimetidine also had different effects on the secretion of lingual lipase and amylase evoked by 10 mM histamine.

The results of the incubation of tissue with histamine or histamine plus ranitidine are shown in Fig. 4A and B. As with cimetidine, 10 mM ranitidine significantly inhibited secretion of lingual lipase elicited by 10 mM histamine at 30, 60, and 90 min. Ranitidine had no effect on the secretion of amylase elicited by histamine. As shown in Table 3, ranitidine alone does not stimulate secretion of lingual lipase. However, it does significantly stimulate the secretion of amylase at 30, 60, and 90 min. The secretory response to ranitidine treatment is similar to that of cimetidine treatment.

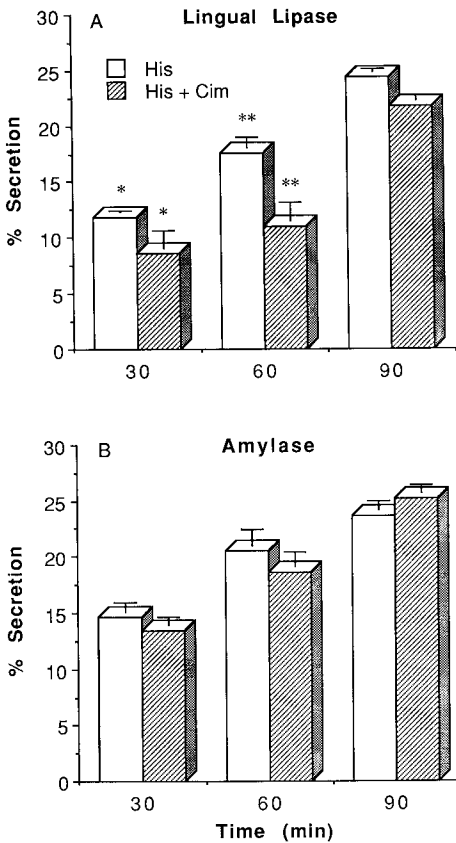


Figure 3

The effect of the H_2 receptor antagonist, cimetidine (Cim), on secretion from minced von Ebner's glands evoked by 10 M histamine (His). Media samples taken at 30, 60, and 90 min and homogenates of the tissue taken at 90 min were assayed for lingual lipase and amylase. Cim (10 mM) was incubated with the tissue for 20 min prior to the incubation of Cim + His. The data were evaluated by the split-plot ANOVA with Bonferroni's correction for each enzyme comparing percentage secretion evoked by His alone or combined with Cim at each time interval. The basal values and n are shown in Table 2. (A) Lingual lipase; * indicates statistically significant differences at 30 min between secretion evoked by His and His combined with Cim. $p=0.027$; ** indicates significant differences at 60 min between secretion evoked by His and His combined with Cim, $p=0.0003$. (B) Amylase; no statistically significant differences were found between treatment with His alone or His in combination with Cim.

H_1 receptor antagonist

The classic H_1 receptor antagonist, pyrilamine, was incubated with minced von Ebner's gland both alone and in combination with histamine. The initial studies revealed that pyrilamine induced an overwhelming secretory response that overshadowed any inhibitory effect it might have had on secretion stimulated by histamine.

All the statistical analysis for the experiments involving pyrilamine were done using the split-plot ANOVA with Bonferroni's correction on log_e transformed data with multiple and individual comparisons.

No statistically significant difference was found between the percentage secretion of tissue treated with histamine or with histamine in combination with pyrilamine for either of the enzymes (data not shown). Figure 5 is a dose-response curve showing the percentage secretion above basal evoked by 0.001–10 mM pyrilamine incubated with the tissue for 60 min. The secretory response was dose-dependent starting with 0.1 mM pyrilamine, the lowest dose that elicited a response, and increasing to a maximum at 10 mM. Figure 6 shows the percentage secretion above basal of both enzymes at various time intervals after incubating von Ebner's

Table 2

The effect of cimetidine on the secretion of lingual lipase and amylase. The control values represent basal secretion. Separate split-plot ANOVAs were performed for each enzyme. The *p*-values represent the probability of a significant treatment effect after adjusting for the three comparisons per enzyme with the Bonferroni correction factor.

	Time (min)	<i>n</i>	Lingual lipase percentage secretion mean \pm SE	<i>p</i>	Amylase percentage secretion mean \pm SE	<i>p</i>
Control	30	4	5.26 \pm 1.51		8.87 \pm 0.55	
Cimetidine			4.06 \pm 1.21	NS	9.47 \pm 0.37	NS
Control	60	4	8.64 \pm 1.64		12.51 \pm 0.60	
Cimetidine			8.17 \pm 1.27	NS	14.64 \pm 0.93	0.003
Control	90	4	11.27 \pm 1.99		15.54 \pm 0.82	
Cimetidine			12.66 \pm 1.45	NS	19.36 \pm 0.72	0.0003

Table 3

The effect of ranitidine on the secretion of lingual lipase and amylase. The control values represent basal secretion. Split-plot ANOVAs were done on each enzyme at each time interval in order to achieve normality in the residuals. The *p*-values have been corrected for the three comparisons made per enzyme with the appropriate Bonferroni factor. They represent the probability of a significant treatment effect.

	Time (min)	<i>n</i>	Lingual lipase percentage secretion mean \pm SE	<i>p</i>	Amylase percentage secretion mean \pm SE	<i>p</i>
Control	30	5	3.63 \pm 0.68		7.89 \pm 0.76	
Ranitidine			3.66 \pm 0.88	NS	12.34 \pm 1.22	0.0105
Control	60	5	6.76 \pm 1.07		11.67 \pm 0.87	
Ranitidine			4.78 \pm 0.79	NS	15.76 \pm 0.66	0.0012
Control	90	5	10.51 \pm 1.88		13.88 \pm 0.99	
Ranitidine			10.27 \pm 2.88	NS	19.58 \pm 1.16	0.0072

glands with 10 mM pyrilamine. In addition to the statistically significant differences between the percentage secretion of lingual lipase and amylase shown in Fig. 6, there were also significant differences between basal secretion and secretion evoked by pyrilamine, $p=0.0003$ at all time intervals. The percentage secretion either above basal or total of lingual lipase and amylase resulting from pyrilamine treatment was higher than the percentage secretion elicited by carbachol [16]. In these experiments with pyrilamine, statistically significant differences were found in basal secretion between the percentage secretion of lingual lipase and amylase at 15, 30, and 60 min, $p=0.0240$, 0.0039 , and 0.0276 , respectively, $n=3$ at 15 min and $n=6$ at 30, 60, and 90 min. However, when basal secretion was not subtracted from the total percentage secretion, there were no significant differences between the total percentage secretion of either of the en-

zymes produced during pyrilamine treatment. Furthermore, multiple comparisons of time by enzyme interactions revealed no indication that the slopes of the lingual lipase and amylase curves were not parallel.

Discussion

These studies show that histamine plays a role in the regulation of protein secretion from von Ebner's gland. Regulation of protein secretion from von Ebner's gland can be more readily compared to the regulation of protein secretion from the exocrine pancreas than from the parotid gland, a major salivary gland. Both the pancreas [8] and von Ebner's glands secrete protein primarily in response to cholinergic stimulation [16], whereas parotid glands secrete protein primarily in response to β -adrenergic stimulation [19]. Liebow and

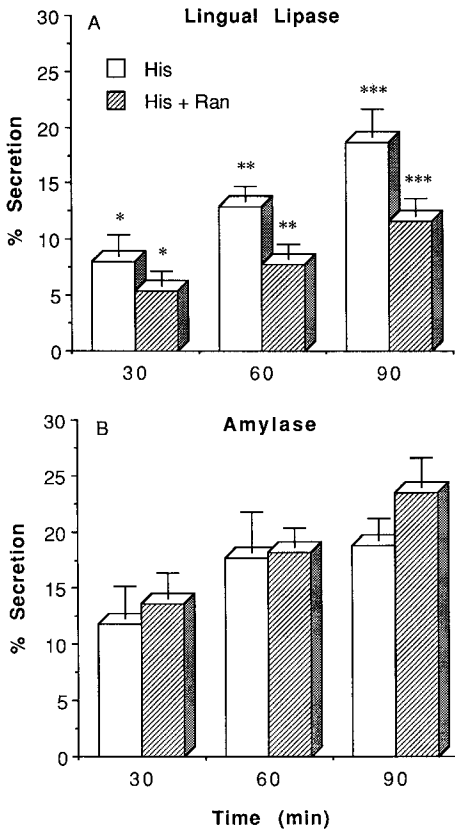


Figure 4

The effect of the H_2 receptor inhibitor, ranitidine (Ran), on secretion from minced von Ebner's glands evoked by 10 mM histamine (His). Media samples taken at 30, 60, and 90 min and homogenates of the tissue taken at 90 min were assayed for lingual lipase and amylase. Ran (10 mM) was incubated with the tissue for 20 min prior to the incubation with Ran + His. The data were evaluated by the split-plot ANOVA with Bonferroni's correction. One ANOVA was done for each enzyme comparing His with His + Ran at each time interval. The values for basal secretion for lingual lipase were $3.44 \pm 1.04\%$, $5.90 \pm 0.95\%$, and $8.31 \pm 0.28\%$ at 30, 60, and 90 min, respectively. For amylase the values for basal were $6.91 \pm 0.81\%$, $10.85 \pm 1.27\%$, and $12.69 \pm 0.97\%$ at 30, 60, and 90 min, respectively; $n=3$. (A) Lingual lipase; * indicates statistically significant differences at 30 min between secretion evoked by His and His combined with Ran, $p=0.015$; ** indicates significant differences at 60 min between secretion evoked by His and His combined with Ran, $p=0.0009$; *** indicates significant differences at 90 min between secretion evoked by His and His combined with Ran, $p=0.0003$. (B) Amylase; no statistically significant differences were found between treatment with His alone or His in combination with Ran.

Franklin [10] found that 1 mM histamine stimulates pancreatic enzyme secretion from *in vitro* isolated rabbit pancreas preparations and Pariente

et al. [25] found that intravenous infusions of histamine also increased enzyme secretion from the rabbit pancreas. In addition, amylase secretion was elicited by 0.01 mM histamine from isolated guinea-pig pancreas [11], any by 1 mM histamine in isolated segments of guinea-pig pancreas [12]. In contrast, histamine evoked fluid secretion from the parotid [13] and submandibular glands [9, 13] of cats and dogs.

Evidence for the physiological role of histamine as a mediator in parasympathetic induction of salivary secretion and in secretion from the stomach comes from the work of Lorenz and coworkers. Histamine was found in stomach tissue [6, 26], the pancreas [26], parotid and submandibular glands [27], in submandibular and parotid saliva [14] and the tongue [26]. Especially interesting are the findings by Lorenz et al. [26] that histamine is present in the area of the tongue, between the root and the body, where von Ebner's gland is located and that it can be released from this area by compound 48/80. In addition, enzymes involved in histamine metabolism were found in many species in the parotid and submandibular glands and the gastric mucosa. Histidine decarboxylase was found in the parotid [28] and the submandibular [27, 28] glands. Histamine methyl transferase was detected in the parotid and submandibular glands [28, 29] and in the gastric mucosa [29]. Diamine oxidase was demonstrated in the parotid and submandibular glands [28, 30] and in the gastric mucosa [30].

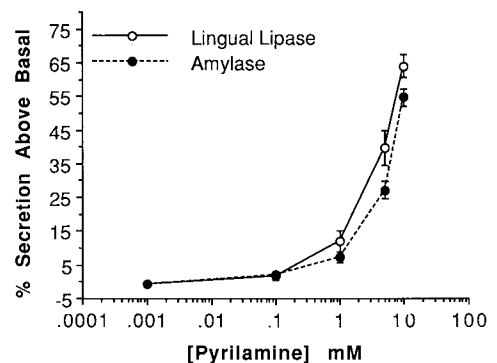


Figure 5

This is a dose-response curve showing the percentage secretion above basal of lingual lipase and amylase evoked by 0.001–10 mM pyrilamine incubated with minced von Ebner's tissue for 60 min. The values for basal secretion were $9.94 \pm 0.91\%$ and $13.38 \pm 0.37\%$ percentage secretion for lingual lipase and amylase, respectively; $n=4-6$, $n=1$ for data at 0.001 mM.

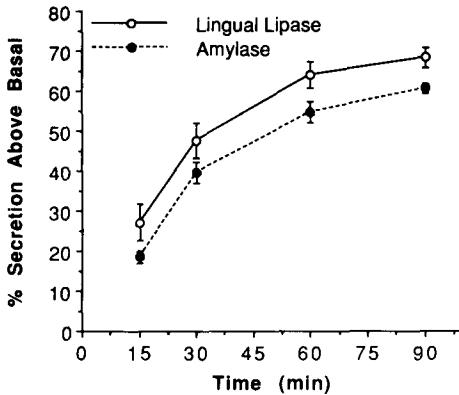


Figure 6

Time course of the percentage secretion above basal of lingual lipase and amylase when minced von Ebner's glands were incubated with 10 mM pyrilamine. Media sampling was done at 15, 30, 60, and 90 min. Homogenates of the tissue taken at 90 min and all media samples were assayed for lingual lipase and amylase. Statistical evaluation of all pyrilamine data was by the split-plot ANOVA using Bonferroni's correction on \log_e transformed data with multiple and individual comparisons. The percentage secretion of lingual lipase was significantly different from the percentage secretion of amylase at 30, 60, and 90 min, $p=0.0021$, 0.0042 , 0.0264 , respectively, $n=6$. The 15 min data were not included in the overall ANOVA due to smaller sample size, $n=3$. The values for basal secretion for lingual lipase were $3.42 \pm 0.45\%$, $5.76 \pm 0.59\%$, $10.79 \pm 0.97\%$, $14.69 \pm 0.67\%$, at 15, 30, 60, and 90 min, respectively. For amylase the values for basal were $6.60 \pm 0.81\%$, $9.15 \pm 0.42\%$, $13.13 \pm 0.42\%$, $15.89 \pm 0.36\%$ at 15, 30, 60, and 90 min, respectively.

Mast cells, a source of released histamine, were found in the submandibular gland, but they are not the sole source of histamine released from the gland [27]. Mast cells have also been found in the tongue [26] and in von Ebner's gland [Redman, R.S., unpublished observations]. In von Ebner's gland they are widely scattered, some are adjacent to the acinus, and many are near the ducts, in the stroma, blood vessels, and muscle bundles. The density of these mast cells is similar to those seen in the tongue [26]. These findings of histamine content in and release from the stomach, parotid and submandibular glands, and the tongue and the detection of enzymes of histamine metabolism in the stomach, parotid and submandibular glands provide further evidence that histamine plays a role in the regulation of secretion.

Studies to characterize the histamine receptor in von Ebner's gland revealed unusual findings. Treatment of minced von Ebner's glands with the H_2

receptor antagonists, cimetidine and ranitidine, evoked differential results in the stimulation of secretion of lingual lipase and amylase and in the inhibition of the secretion of each enzyme elicited by histamine. Both antagonists stimulated secretion of amylase but not lingual lipase and they both had some inhibitory effect on the histamine-stimulated secretion of lingual lipase, but they had no effect on the histamine-stimulated secretion of amylase. The results gave no clear indication that histamine binds to an H_2 receptor or even that this receptor is present in von Ebner's gland. The results are similar to those found in the rabbit pancreas by Pariente et al. [25, 31]. Injection of cimetidine into an infusion of anesthetized rabbits increased both pancreatic juice flow and enzyme output [31], and in addition, infusion of histamine plus cimetidine increased the flow rate and protein output above infusion with histamine alone [25].

H_1 and H_2 receptors act through different signal transduction pathways. The H_2 receptor stimulates the adenylate cyclase system [2, 5, 32] and the H_1 receptor acts primarily through the phosphatidyl inositol system [1, 2, 33]. Increased cytosolic Ca^{2+} was found in the cytosol of the exocrine pancreas after stimulation with histamine [12]. Thus, the H_1 receptor antagonist, pyrilamine, was tested as an inhibitor of histamine evoked secretion of lingual lipase and amylase. A most unexpected finding was that pyrilamine was a very potent stimulator of lingual lipase and amylase secretion. It was more effective in stimulating secretion of lingual lipase and amylase than carbamylcholine chloride. Greater than 60% lingual lipase and amylase were secreted above basal in 90 min of incubation with 10 mM pyrilamine, whereas *in vitro* incubations with carbamylcholine chloride resulted in secretion of $55.9 \pm 2.4\%$ lingual lipase and $28.0 \pm 2.5\%$ amylase in 90 min [16]. Only *in vivo* i.p. injections of pilocarpine caused greater secretion. Von Ebner's glands were maximally depleted of both enzymes in 1 h, with $25.9 \pm 3.4\%$ lingual lipase and $31.8 \pm 6.4\%$ amylase remaining in the glands [16]. Pyrilamine, which is also known as mepyramine (Merck Index, 9th edition, 7767, 1976), is the classical antagonist of the H_1 receptor and has been used to characterize H_1 receptors for many years [2]. However, in von Ebner's gland, pyrilamine functions as a secretagogue for protein secretion and could possibly have a receptor that differs from the H_1 receptor. Leurs et al. [33] found that in rat liver plasma membranes, [3H] mepyramine labels non- H_1 re-

ceptors. These authors suggest the presence of an ethylenediamine recognition site and warn against using mepyramine binding as the sole indicator of the presence of the H₁ receptor. Lorenz et al. [9] also found that in dog submandibular glands, unlike other antihistamines which inhibit salivation stimulated by histamine, mepyramine, stimulates fluid secretion and the release of histamine. In addition to the anomalous histamine receptor results, the present studies have confirmed previous findings of differences in the percentage secretion of lingual lipase and amylase when stimulated to secrete by carbachol, isoproterenol, forskolin [16], and substance P [20]. In the present studies, statistically significant differences were found in basal secretion of lingual lipase and amylase at 15, 30, and 60 min and also in percentage secretion above basal when the tissue was treated with histamine at 60 and 90 min or pyrilamine at 15, 30, and 60 min. However, when the total percentage secretion was analyzed, there was no significant difference in the percentage secretion during pyrilamine treatment at all time intervals and only statistically different at 30 min with histamine. When the basal secretion values at each time interval are subtracted from the results of treated samples, the assumption is made that basal secretion continues during the secretagogue stimulation. This could probably occur if basal secretion is by a pathway that is different from stimulated secretion. Explanation of the phenomena of differential secretion of two enzymes is very difficult. If the proteins are packaged in the same secretory granules and exocytosis is the only means of secretion from secretory granules, the percentage secretion of both enzymes should be the same and both enzymes should be similarly effected by agonists and antagonists. However, there are many instances of "non-parallel" protein secretion from the pancreas [34]. Among the possible reasons for these results are the presence of more than one secretory pathway, a variety of secretory granules containing different enzyme compositions, or interactions of the enzymes with membrane proteins of the secretory granules that may effect the release of the enzymes [35]. It is also possible that constitutive secretion [36] may play a role in these phenomena. The results presented in this paper indicate that in order to learn more about the regulation of secretion from von Ebner's gland, there must be further characterization of the receptors of von Ebner's gland.

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