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Effects of electrical stimulation of autonomic nervous system on degranulation of von Ebner's gland acini

Suat Gurkan[1] and Robert M. Bradley[1,2]

[1]*Department of Biologic and Materials Sciences, School of Dentistry, and* [2]*Department of Physiology, School of Medicine, University of Michigan, Ann Arbor, MI 48109 (U.S.A)*

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In a series of studies to understand interactions between taste sensation and salivary gland function, we are pursuing experiments to determine the autonomic nervous system control of von Ebner's lingual salivary glands. Electrical stimulation of the glossopharyngeal nerve, which contains the parasympathetic nerve supply to von Ebner's glands, caused a reduction in secretory granules of the glands in the rat. This depletion of granules could be blocked by prior administration of the parasympathetic antagonist, atropine. In contrast, electrical stimulation of the sympathetic nerve supply was ineffective in causing granule depletion in von Ebner's gland, but produced almost total degranulation in the parotid gland of the same animals. It is concluded that parasympathetic nerves exert the principal control over von Ebner's gland, acinar degranulation in the rat; this is compared with autonomic control of other salivary glands that have a dual peripheral control by parasympathetic and sympathetic innervation.

INTRODUCTION

There is a considerable body of literature indicating that saliva is important in taste bud function[4]. While the paired, major salivary glands supply the bulk of the saliva in the oral cavity, the lingual salivary glands (von Ebner's glands) drain into the clefts of the circumvallate and foliate papillae and thereby provide the microenvironment for the majority of the oral taste buds[21,22]. Taste stimulants have to cross the saliva in the clefts of the circumvallate and foliate papillae to reach the microvilli of the taste buds and, therefore, von Ebner's gland secretion presumably has an important role in the initial events in taste transduction.

Despite their probable importance in taste transduction, there have been relatively few studies of the physiological properties of von Ebner's glands and surprisingly little is known concerning the neural control of these glands. In a recent investigation of the effects of systemically administered, parasympathetic and sympathetic agonists and antagonists on degranulation of von Ebner's gland acini, we reported that both parasympathetic and sympathetic agonists produce a 50% reduction in secretory granule content[15]. Simultaneous administration of both agonists resulted in total degranulation. We hypothesized that the glands contain two sets of granules with different compositions, each under the control of either the parasympathetic or sympathetic nervous system.

However, a problem with using systemically administered drugs to study autonomic control of von Ebner's glands is that the site of drug action is unknown. Stimulation of the nerve supply to the gland produces a more localized site of stimulation. Therefore, in this paper we examine the extent of degranulation of von Ebner's gland acini resulting from electrical stimulation of the glossopharyngeal nerve, or parasympathetic supply, and the sympathetic trunk.

Correspondence: R.M. Bradley, Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, U.S.A.

MATERIALS AND METHODS

Adult (160–200 g), male, Sprague–Dawley rats were fasted overnight to cause accumulation of secretory granules in von Ebner's gland acini and all experiments were started the following morning. There were 3 groups of rats with 5 rats in each experimental group. In one group the glossopharyngeal nerve, carrying the parasympathetic supply to the glands, was stimulated electrically. In the second group atropine sulfate, a parasympathetic antagonist, was injected i.p. at a dose of 1.5 mg/kg and 10 min later the glossopharyngeal nerve was stimulated. In the third group the sympathetic trunk was electrically stimulated. Since von Ebner's glands are paired in the tongue, the gland contralateral to the stimulated side was used as a control.

Animals were anesthetized by an i.p. injection of sodium pentobarbital (50 mg/kg b.w.) and the trachea cannulated. Additional anesthetic was administered as necessary. The rat was placed supine and either the left glossopharyngeal nerve or the left sympathetic trunk was exposed in the neck, dissected free from connective tissue, and elevated onto a pair of stainless-steel stimulating electrodes. The glossopharyngeal nerve was stimulated for 1 h with 8 V, 1 ms pulses at 50 Hz and the sympathetic trunk was stimulated for 1 h with 4 V, 1 ms pulses at 20 Hz.

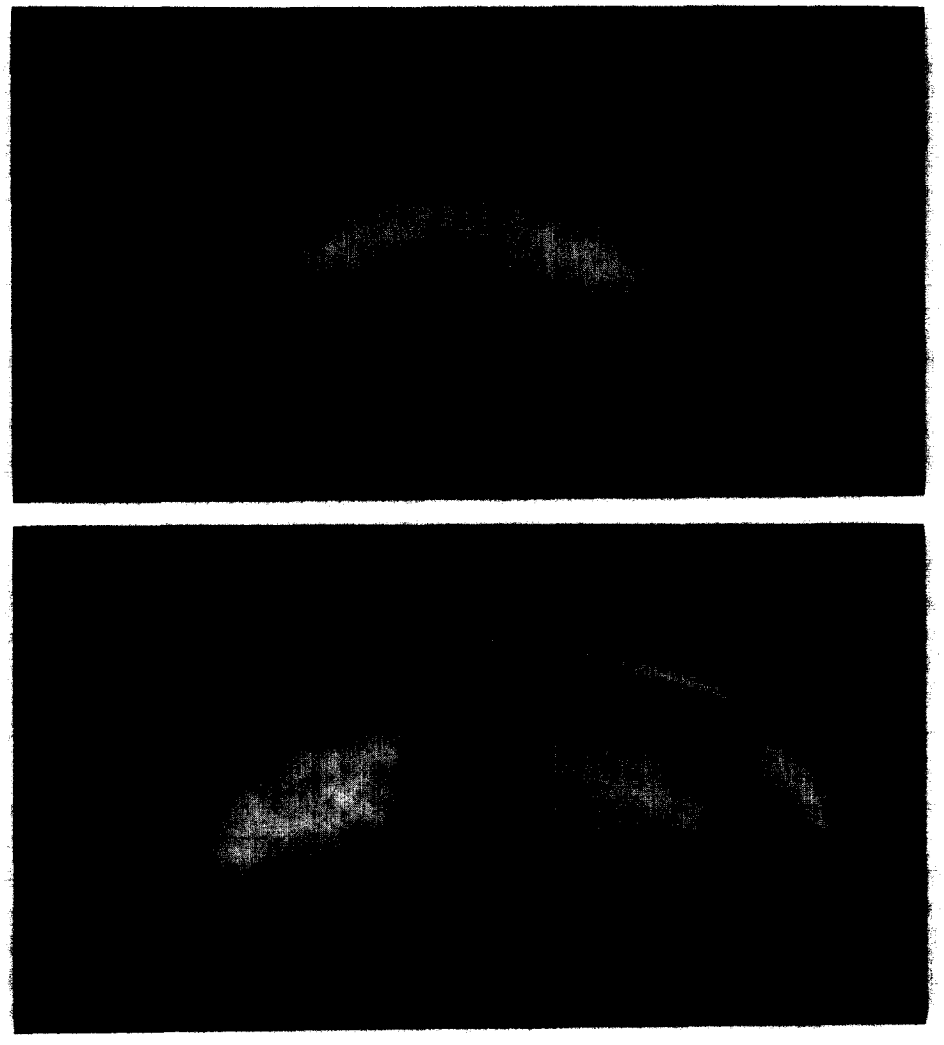

Fig. 1. Unfixed coronal sections through the tongue at the level of the foliate papillae. A: unstimulated tongue showing the bilateral von Ebner's glands. B: tongue in which the right glossopharyngeal nerve was stimulated. The appearance of the right stimulated gland appears pale in contrast with the left unstimulated gland which appears dense.

These stimulation parameters were derived from studies using electrical stimulation of the innervation of other salivary glands[7,24] and the dose of atropine sulphate was that used in our earlier study[15].

Immediately after the termination of electrical stimulation the tongue was rapidly removed and equivalent portions of von Ebner's glands from both ipsilateral (stimulated) and contralateral (unstimulated) sides of the tongue were dissected. The gland tissue was fixed overnight in 4% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.35) at 4 °C, postfixed with 1% osmium tetroxide for 1 h at room temperature, and embedded in Epon. Tissues were sectioned at 1 μm and stained with 1% Toluidine blue in 1% aqueous sodium borate for 45 s.

For each rat, cross-sections of 20 acini (10 on the ipsilateral and 10 on the contralateral side of the tongue) were selected for black-and-white photography at 1000× magnification. Measurements of acinar and secretory granule area were made from 4 × 5 in. photomicrographs using computer planimetry and the percentage of the total area occupied by the granules was calculated. For each rat a mean percentage for the 10 acini from each side of the tongue was calculated. Differences in granule areas between stimulated and unstimulated gland acini were tested using a two tailed Student's *t*-test. *P*-values less than 0.05 were considered to be significant.

RESULTS

Glossopharyngeal nerve stimulation

Electrical stimulation of the glossopharyngeal nerve has a marked effect on the gross appearance of von Ebner's glands (Fig. 1). Coronal sections through the area of the tongue containing von Ebner's glands are observed to have two distinct

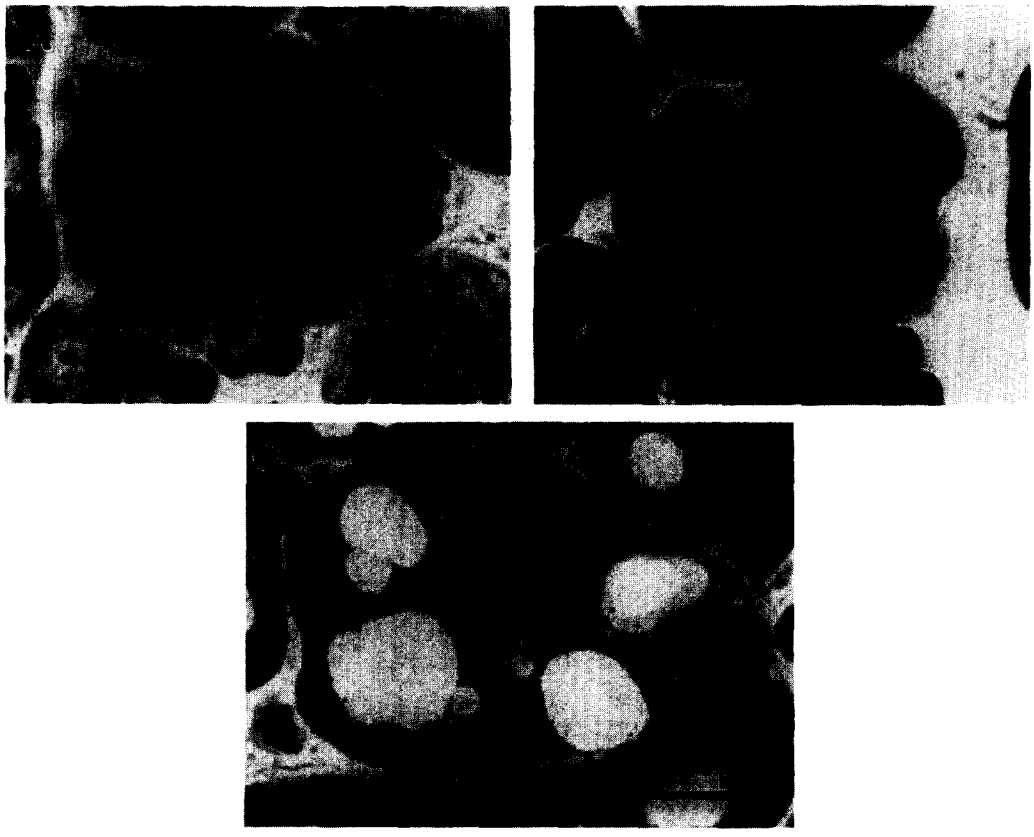

Fig. 2. Light micrographs of von Ebner's gland acini showing the effect of electrical stimulation of the glossopharyngeal nerve on reduction of secretory granule content. A: acini from unstimulated side of tongue. B: acini from stimulated side of the tongue showing marked reduction in granule content. C: acini from the stimulated side of the tongue showing extensive degranulation and vacuole production. Bar = 10 μm.

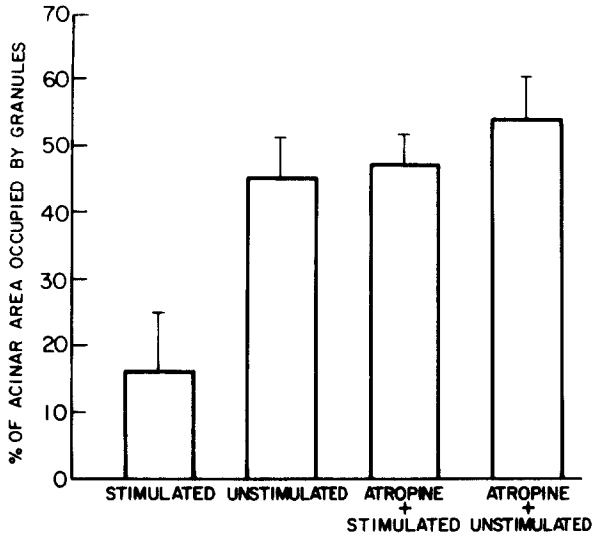

Fig. 3. Means and S.D. for area of granules of von Ebner's gland acini following electrical stimulation of one glossopharyngeal nerve, compared with the unstimulated side and the effect of atropine on blocking granule depletion initiated by stimulation of the glossopharyngeal nerve.

halves, symmetrical with respect to the midline (Fig. 1A). The half ipsilateral to the stimulated glossopharyngeal nerve appears very pale in contrast to the contralateral side which appears dense (Fig. 1B).

The difference in appearance between the contralateral and ipsilateral von Ebner's glands is due to the depletion of secretory granules (Fig. 2A, B). Following stimulation the granules are lost from the basal part of the acinar cells and are concentrated around the lumen (Fig. 2B). The granule area in the acini ipsilateral to the stimulation is only 16% of the total acinar area, compared to 47% on the contralateral unstimulated side. In some acini on the stimulated side there is total depletion of granules (Fig. 2C). The reduction in secretory granule content of acini on the stimulated side is highly significant, compared with control contralateral acini ($P < 0.0001$). The degranulation produced by nerve stimulation is presumably due to activation of the parasympathetic synapse at the gland, because administration of the

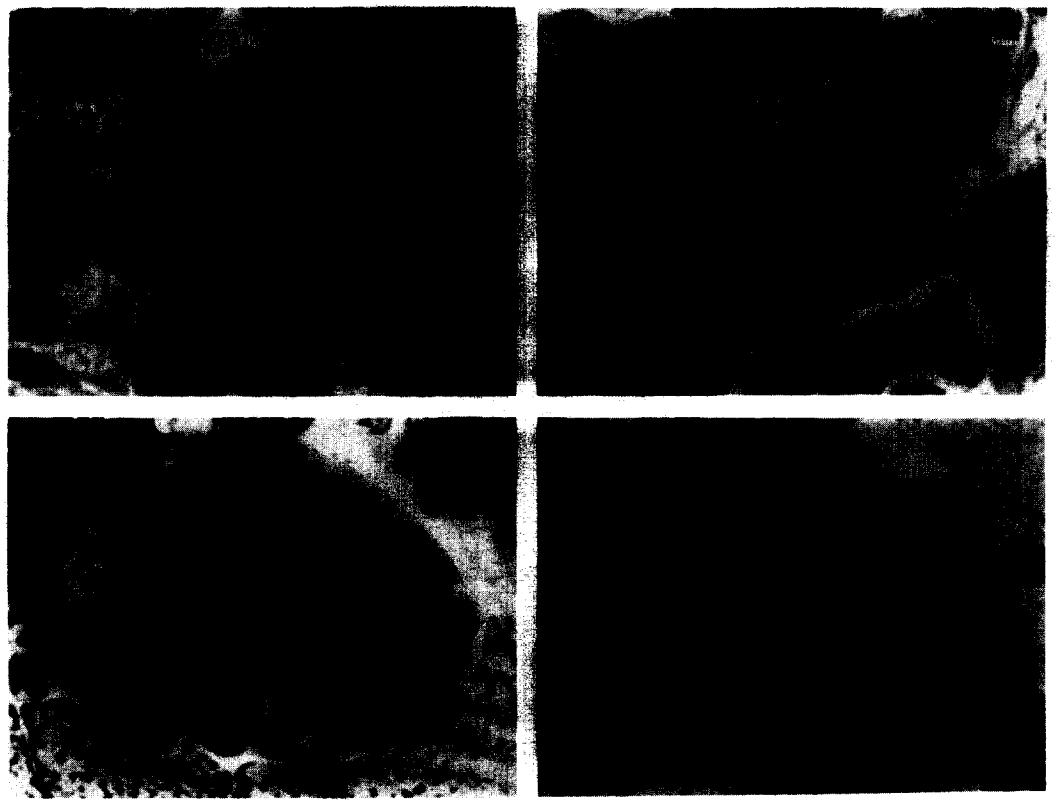

Fig. 4. Light micrographs of acini from the parotid and von Ebner's gland acini showing the effect of electrical stimulation of the sympathetic trunk nerve on reduction of secretory granule content. A: acini from unstimulated and B: stimulated parotid gland showing marked reduction in granule content on stimulated side. C: acini from the unstimulated; and D, stimulated side of the tongue showing insignificant degranulation in von Ebner's glands. Bar = 10 μm.

parasympathetic antagonist atropine prior to glossopharyngeal nerve stimulation resulted in no significant depletion (47% of total acinar area occupied by granules) when compared to control gland acini (45%) ($P > 0.05$) (Fig. 3).

Sympathetic trunk stimulation

No significant change ($P > 0.05$) in granule area in von Ebner's glands occurred after stimulation of the superior cervical trunk; the granule areas were 51 and 53% in ipsilateral (stimulated) and contralateral (unstimulated) acini, respectively, similar to tissue from unstimulated controls (Fig. 4C, D). To evaluate the effectiveness of the cervical trunk stimulation, the parotid glands were examined in the same rats. The ipsilateral parotid glands had substantial granule depletion as compared to contralateral gland (Fig. 4A, B).

DISCUSSION

Electrical stimulation of the glossopharyngeal nerve (parasympathetic supply), but not the sympathetic trunk resulted in depletion of secretory granules from von Ebner's gland acini. Similar results have been reported for electrical stimulation of the parasympathetic nerve supply of the cat parotid gland, which resulted in degranulation, whereas sympathetic stimulation produced no evident degranulation[7]. However, the results differ from those reported in studies of the rat parotid gland which, because it has been so extensively studied, has attained the status of a model system for salivary gland secretory mechanisms[10]. Parasympathetic nerve stimulation induces a copious flow of saliva with a low amylase content and little or no degranulation in rat parotid; sympathetic stimulation causes a small flow of saliva, rich in amylase, and is associated with extensive degranulation[12].

Numerous similar examples can be cited where electrical stimulation can induce very different secretory changes in acinar cells of different salivary glands and even in different cell types of the same gland depending on the specific gland or species[11,25]. It is not unprecedented, therefore, that electrical stimulation of the autonomic nerve supply to von Ebner's and the parotid glands produces different results in the rat.

Secretory granules in salivary glands contain salivary enzymes and it can therefore be concluded from the results of the present study that enzyme secretion in von Ebner's glands is under the control of the parasympathetic nervous system. However, in a previous study we demonstrated that i.p. administered parasympathetic *and* sympathetic agonists result in degranulation of von Ebner's gland acini[15]. Furthermore, in a recent study Field and Hand[9] measured secretion of amylase and lipase in rat von Ebner's glands using both in vivo and in vitro techniques and reported that parasympathetic and sympathetic agonists could produce enzyme secretion. A cholinergic agonist (carbachol), applied in vivo or in vitro, caused secretion of amylase and lipase within 1 h. Intraperitoneally administered isoproterenol, a sympathetic agonist, although eliciting little enzyme secretion after 1 h, produced significantly more enzyme reduction in the acini than carbachol after 4 h, supporting the possibility of a dual control of gland secretion.

Unfortunately Field and Hand[9] did not examine the glands morphologically after in vivo drug administration so that it is not possible to determine the degree of degranulation produced by the isoproterenol; however, it has been shown in the rat parotid gland that enzyme secretion can occur in the absence of degranulation[1]. Moreover, the results obtained using in vivo and in vitro approaches[9] were significantly different, illustrating the problems that can occur when conclusions are made using very different techniques. In fact, Garrett[10,11] has stated that in vitro and systemic drug studies, although providing insight into mechanisms of secretion, may not 'wholly reflect physiological processes'.

Even nerve stimulation is not ideal as it usually involves an unnatural stimulation pattern and will stimulate both afferent and efferent fibers in the nerve trunk. However, when investigators have used more natural stimulation, such as chewing, to stimulate salivary secretion the results have been most similar to electrical stimulation studies[13,14]. Thus electrical stimulation is a reasonable method to study the peripheral autonomic control of salivary glands.

To explain the discrepancy in our results obtained with electrical stimulation of the sympathetic trunk and those from systemic administration of a sympathetic agonist we have examined the extent of the sympathetic innervation of von Ebner's glands. First,

we know that the electrical stimulation was effective because secretory granules in parotid glands in the same animals were depleted (Fig. 4A, B). It is possible, therefore, that von Ebner's glands posses β-receptors that would respond to administration of a drug but have no sympathetic innervation. To examine the sympathetic nerve supply to von Ebner's glands we repeated the studies of Marfurt[19,20] in which horseradish peroxidase (HRP) was injected into the superior cervical ganglion to trace the sympathetic innervation to the head. Although we were able to show excellent labeling of the iris, there was no apparent labeling of fibers in the vicinity of von Ebner's glands, indicating at least with this technique the absence of a sympathetic innervation. Moreover, using a histochemical staining technique to demonstrate biogenic amines[6] we were able to show a rich sympathetic innervation in both the iris and parotid gland but only a very weak innervation in von Ebner's glands. Furthermore, there is another indication of independence between sympathetic innervation and β-receptors, because β-receptors will develop on acinar cells in the absence of sympathetic innervation[5]. The number and type of β-receptors are also influenced by chronic administration of isoproterenol, a β-adrenergic agonist[17]. Thus the use of a β-adrenergic drug could have an influence on the number of β-receptors and, therefore, the β-receptor response that would differ from electrical stimulation of the sympathetic trunk.

For the parasympathetic system, there is anatomical evidence to support its role in the control of von Ebner's glands. Injection of HRP directly under the circumvallate papilla of the rat tongue results in retrograde filling of a circumscribed collection of cells in the inferior salivary nucleus closely apposed to the second-order gustatory afferents in the solitary tract nucleus[3]. Electrical stimulation of this group of cells results in secretion of saliva from the clefts of the circumvallate papilla[16]. Thus, there is evidence that a group of parasympathetic secretomotor cells in the brainstem control secretion of von Ebner's glands. Moreover, the relationship of this cell group to the gustatory afferent cells in the solitary nucleus is suggestive of interaction between gustatory afferent information and efferent secretory control of von Ebner's glands.

It has long been known that gustatory stimuli can influence salivary secretion[8]. In human parotid glands sour taste stimuli produce a high flow of saliva whereas sweet stimuli result in a saliva rich in amylase[18]. At present little is known concerning the effect of taste stimuli on secretion of von Ebner's glands. Despite this lack of information there is considerable data on the response characteristics of the taste buds innervated by the glossopharyngeal nerve. In general the taste buds of the rat circumvallate papilla respond best to certain salts, acids and a few amino acids[2,23,26]. Use of similar substances to examine gustosalivary reflexes in other glands has shown that acids produce high flow rates of saliva low in enzymes and this is presumably the case for von Ebner's glands. Von Ebner's glands do contain amylase, but release of this enzyme has always been related to sympathetic stimulation of the salivary glands or to the presence of sweet gustatory stimuli.

The relationship of von Ebner's glands to the taste buds of the circumvallate and foliate papillae and the association between gustatory afferents and parasympathetic efferents in the brainstem, together with the importance of parasympathetic stimulation in the control of gland secretion, strongly suggests that these glands have a pivotal role in lingual gustatory function.

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