

Changes in immunoglobulin A secretion induced by sympathetic and taste stimulation in the rat submandibular gland

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We studied the influence of sympathetic nerve and taste stimulation on salivary immunoglobulin A secretion in the rat submandibular gland. Although the flow of the saliva evoked by β -adrenoceptor stimulation was very small in volume, secretion evoked by both acidity and bitterness stimulation was greater than from sympathetic stimulation. A marked secretion of peroxidase was found evoked by isoprenaline. Similar levels of SIgA secretion were evoked by each stimulus. Amylase secretion was enhanced by isoprenaline, citric acid and quinine stimulation. In particular, citric acid was significantly different compared with isoprenaline. A similar level of SIgA secretion was evoked by each of the stimuli. SIgA secretion evoked by both isoprenaline and quinine was significantly inhibited by propranolol. Propranolol had only a slight inhibitory effect on citric acid stimulation. The peroxidase level in response to isoprenaline was remarkably reduced by propranolol administration. Although propranolol was less effective on peroxidase secretion evoked by citric acid, quinine-induced peroxidase stimulation was inhibited by propranolol. However, the amylase activity evoked by acidity was weaker than that of sympathetic stimulated saliva. This difference was attributable to the influence of the nervous system on IgA secretion through input to the submandibular gland via not only the sympathetic nerves, but also the parasympathetic nerves. These results indicate that the salivary secretion of SIgA is regulated by nerve impulses and that acidity imparts a greater effect on SIgA secretion than does solitary sympathetic stimulation alone. (*J Osaka Dent Univ* 2015 ; **49 : 171–178)**

Key words : SIgA ; Sympathetic stimulation ; Acidity ; Submandibular gland

INTRODUCTION

Secretion of saliva is dependent on stimuli from autonomic nerves that are the effector arms of reflexes activated predominantly by taste and chewing.¹ Nerve mediated stimulus evokes saliva secretion of water and proteins by different mechanisms. Secretion of submandibular saliva is controlled by the autonomic nervous system. The parasympathetic nervous system is the main controller of this secretion via impulses in the chorda tympani nerve that innervates it and releases acetylcholine and substance P. Both can evoke copious salivary secretion by activating muscarinic and tachykinin-1 receptors, respectively. In contrast, the sympathetic nervous system also con-

trols salivary secretion by acting on α - and β -adrenergic receptors. Sympathetic nerve stimulation induced a relatively low flow of saliva that was rich in protein and was accompanied by extensive degranulation from both acinar and granular duct cells.²

The predominant secretory immunoglobulin on mucosal surfaces is immunoglobulin A (IgA). In the mouth, it is a component of the saliva secreted by the major and minor salivary glands.³ Secretory immunoglobulin (SIgA) is present in salivary secretions and, along with other glycoproteins such as mucin, lactoferrin and peroxidase, is responsible for helping maintain the integrity of mucosal surfaces against infectious agents.⁴ SIgA is the main component of the adaptive immune system. Polymeric, J-chain-con-

taining, IgA, which is secreted by plasma cells, binds to the polymeric immunoglobulin receptor (pIgR) present on the basolateral membrane of epithelial cells and is transcytosed to the apical membrane.⁵ The pIgR-IgA complex is then cleaved with the release of SIgA into the epithelial cell secretion. In rat submandibular glands, SIgA is increased by stimulus from sympathetic nerves.⁶

The primary function of salivary alpha amylase is to break down high molecular weight carbohydrates to lower molecular weight sugars (i.e., glucose).⁷ In addition, alpha amylase also seems to play a role in maintaining mucosal immunity.^{7,8} Studies have suggested that the amylase inhibits streptococcal bacterial adherence, which inhibits further propagation or colonization of bacteria and may help regulate normal bacterial flora in the mouth.⁹

Salivary amylase activity (SAA) has been shown to increase rapidly during acute stress, and it has been suggested that it may even be used as a marker of sympathetic nervous system activity, although this concept is still debated.⁹ We utilized SAA as a parameter of the sympathetic nervous excitement level.

Several studies on rat salivary glands have shown that secretion of SIgA is increased by stimulus from sympathetic nerves.⁶ Because increased SIgA secretion into the oral cavity aids in prevention of oral disease, we examined the effect of both sympathetic nervous stimulus and taste stimulus on secretion of SIgA in comparison with other proteins in the rat submandibular gland.

MATERIALS AND METHODS

Experimental animals

Approval was obtained from the Animals Research Committee of Osaka Dental University (No.13-03008), and the experiments were performed according to the Ethical Guidelines of the International Association for the Study of Pain.¹⁰ This study was performed using the Laboratory Animal Facilities of Osaka Dental University. Twenty adult male Wistar rats weighing 280–300 g were used. The experimental animals were divided into three groups, rats stimulated by isoprenaline, citric acid and quinine. The animals were housed in the animal care center under

controlled light and environmental conditions of 12 h /12 h dark/light cycles and 20°C. Dry food and water were available *ad libitum*. The experiment began with one day of familiarization with the box.

Collection of saliva

After anesthetization by intraperitoneal injection of sodium pentobarbital (65 mg/kg), a saliva sample from the submandibular gland was collected through a polyethylene cannula (Intramedic PE-10; Becton Dickinson, Franklin Lakes, NJ, USA) inserted into the oral opening of the submandibular gland duct.

Sympathetic stimulation was performed using 2, 4 and 8 mg/kg, isoprenaline hydrochloride (IPR; Wako Pure Chemical Industries, Osaka, Japan). Five minutes after stimulation, the secreted saliva was collected for 5 min (Fig. 1).

Taste stimulation was performed using 1, 3 and 5 mM solutions of citric acid (CIT) and 5, 30 and 50 μ M solution of quinine hydrochloride (QUI), both from Wako Pure Chemical Industries. The rat oral cavities were wiped, and then 0.5 mL of each solution was injected into the mouth through the intraoral fistula. After secretory stimulation, the secreted saliva was collected for 5 min (Fig. 2). A further series of experiments examined the influence of autonomic antagonists on secretion. One minute before secretory

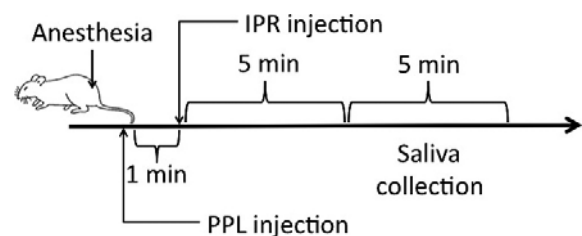


Fig. 1 Study design of saliva collection by IPR stimulation.

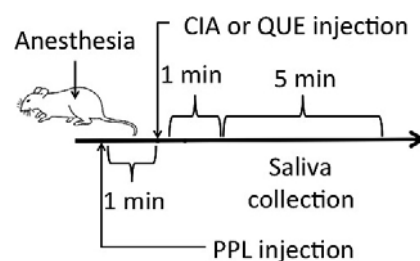


Fig. 2 Study design of saliva collection by taste stimulation.

stimulation either by IPR, CIT or QUI, 8 mg/kg of the adrenergic β -blocker propranolol hydrochloride (PPL; Wako Pure Chemical Industries) was administered intraperitoneally.

Measurement of the salivary flow rate

The saliva was collected into a micropipette connected to the cannula to measure the volume. At the end of the experiment, the gland was dissected out and weighed. The amount of secreted saliva was expressed in microliters of saliva per minute per 100 mg wet weight of the gland ($\mu\text{L}/\text{min}/100 \text{ mg gland}$).

Assay of salivary components

All samples were centrifuged before analysis. Peroxidase was assayed using the fluorogenic substrate dichlorofluorescein (Molecular Probes Europe, Leiden, Germany) which was converted to dichlorofluorescein (DCF) in the presence of hydrogen peroxidase and peroxidase.¹¹ A standard curve of DCF was prepared and peroxidase activity was expressed in μM of DCF min^{-1} (DCF unit). The concentration of SIgA in the different saliva samples was quantified by enzyme-linked immunosorbent assay (ELISA) using a rabbit anti-rat IgA (Serotec, Oxford, UK) capture antibody and a horseradish peroxidase-labeled rabbit anti-rat IgA detecting antibody. Binding was detected with a biotinylated anti-rat IgG (Sigma, IL, USA) and then with avidin-biotin complex (Dako, Ely, UK).

α -Amylase assay was measured using a hand-held salivary amylase monitor manufactured by NIPRO (Osaka, Japan). This analyzer enables automatic measurement of salivary amylase activity using a dry-chemical system, within 1 min from collection to completion of the measurement.¹² The tip of the testing strip was set under the tongue for 30 sec to collect saliva. Then, the strip was immediately inserted into the analyzer, which displays the result automatically.

RESULTS

Fluid secretion

Secretory responses were determined 5 min after different stimulation. Saliva secretion increased in a concentration-dependent manner. Fluid secretion in response to IPR stimulation increased from $9 \pm 1 \mu\text{L}/$

$\text{min}/100 \text{ mg gland}$ at 2 mg/kg, to $31 \pm 3 \mu\text{L}/\text{min}/100 \text{ mg gland}$ at 8 mg/kg. Fluid secretion in response to CIT acid stimulation increased from $289 \pm 13 \mu\text{L}/\text{min}/100 \text{ mg gland}$ at 1 mM, to $402 \pm 25 \mu\text{L}/\text{min}/100 \text{ mg gland}$ at 5 mM. In response to QUI stimulation, it increased from $132 \pm 21 \mu\text{L}/\text{min}/100 \text{ mg gland}$ at 5 μM , to $311 \pm 28 \mu\text{L}/\text{min}/100 \text{ mg gland}$ at 50 μM (Fig. 3).

Salivary flow during IPR stimulation was at a low level compared with both citric acid and quinine stimulation. We also investigated the inhibitory effects of PPL on the secretory responses induced by IPR. Although PPL markedly inhibited the fluid secretion

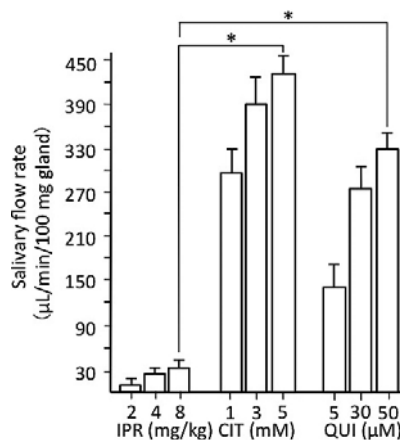


Fig. 3 Salivary flow rate induced by different reflex stimulations. The salivary secretion evoked by both CIT and QUI was significantly greater than that induced by IPR stimulation (* $p < 0.05$, t-test).

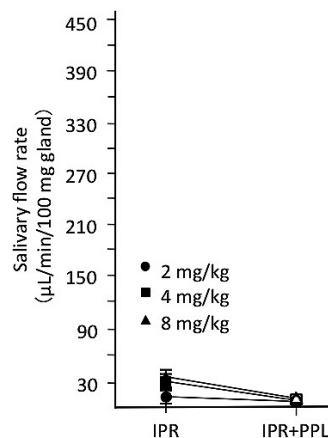


Fig. 4 Effect of PPL on the salivary flow rate evoked by IPR stimulation.

PPL inhibited the salivary secretion evoked by IPR stimulation.

evoked by IPR (Fig. 4), it was not very much influenced by CIT stimulation (Fig. 5). Fluid secretion evoked by QUI stimulation was slightly inhibited by PRP (Fig. 6).

Peroxidase secretion

Peroxidase secretion showed similar patterns to fluid secretion.

IPR evoked a marked secretion of peroxidase of between 2.2 ± 0.7 U/L to 4.3 ± 0.9 U/L. CIT-induced peroxidase activity was approximately 33%, and QUI-induced peroxidase activity was approximately

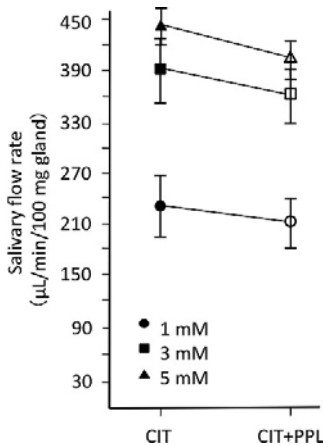


Fig. 5 Effect of PPL on the salivary flow rate evoked by CIT stimulation.

PPL did not significantly inhibit the fluid secretion evoked by CIT stimulation.

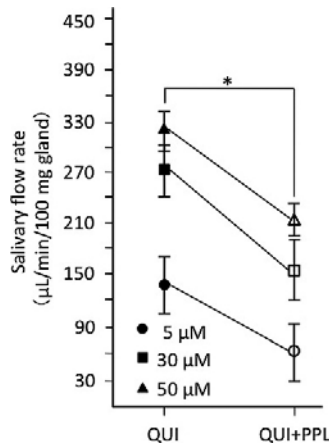


Fig. 6 Effect of PPL on the salivary flow rate evoked by QUI stimulation.

Addition of PPL significantly decreased the salivary flow rate by 50% compared with QUI alone.

25%, compared with the IPR-induced peroxidase level (Fig. 7). The inhibitory effect of PPL on the secretory responses induced by IPR was also investigated. Peroxidase level in response to IPR was markedly reduced by propranolol administration (Fig. 8). PPL was less effective on peroxidase secretion evoked by CIT (Fig. 9), while QUI-induced peroxidase activity was inhibited by PPL (Fig. 10).

SlgA secretion

The concentration of SlgA evoked by each stimulation was similar. SlgA secretion was markedly induced with stimulation by IPR ($114 \pm 11 \sim 153 \pm 15$ µg/mL), CIT ($123 \pm 13 \sim 165 \pm 23$ µg/mL) and QUI ($111 \pm 13 \sim 149 \pm 12$ µg/mL) (Fig. 11). We also investigated the

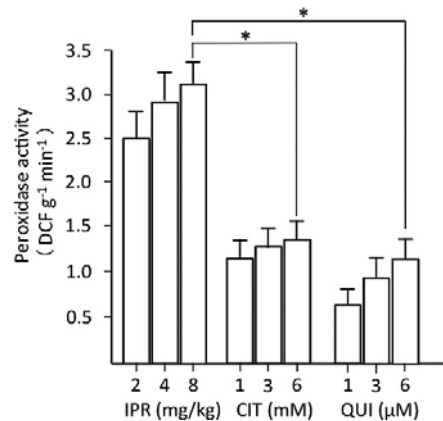


Fig. 7 Salivary peroxidase output by different reflex stimuli. Peroxidase output evoked by both CIT and QUI was significantly lower than by IPR.

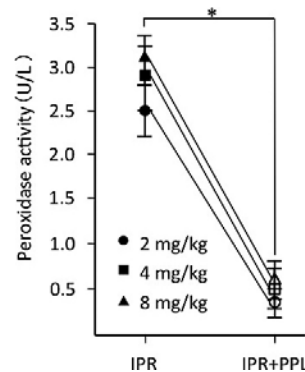


Fig. 8 Effect of PPL on the peroxidase output evoked by IPR stimulation.

PPL significantly inhibited the peroxidase secretion evoked by IPR stimulation alone.

inhibitory effect of PPL on the secretory responses induced by IPR. SIgA secretion in response to both IPR stimulation (Fig. 12) and QUI stimulation (Fig. 14) was significantly inhibited by PPL. The inhibitory effect of PPL was slight with CIT stimulation (Fig. 13).

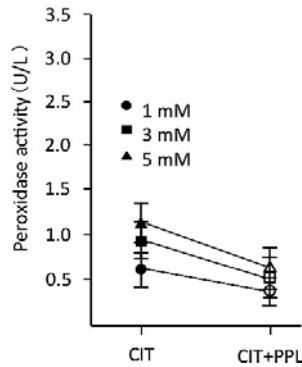


Fig. 9 Effect of PPL on the peroxidase output evoked by CIT stimulation. PPL slightly inhibited the peroxidase secretion evoked by CIT stimulation alone.

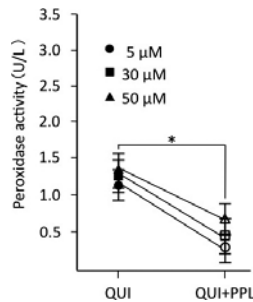


Fig. 10 Effect of PPL on the peroxidase output evoked by QUI stimulation. Peroxidase secretion evoked by addition of PPL to QUI significantly decreased by 30% compared with QUI alone.

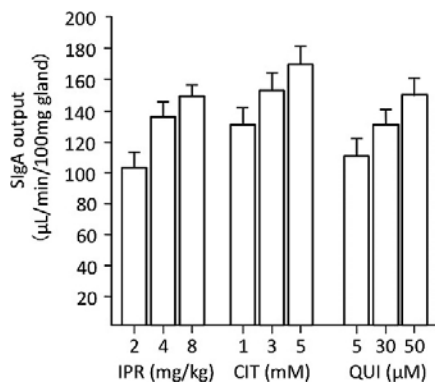


Fig. 11 Salivary SIgA output by different reflex stimuli. Output levels of SIgA evoked by IPR, CIT and QUI stimulation were similar.

Amylase activity

SAA was enhanced by IPR stimulation ($120 \pm 11 \sim 142 \pm 15$ U/L), CIT stimulation ($78 \pm 12 \sim 115 \pm 20$ U/L) and QUI stimulation ($98 \pm 14 \sim 123 \pm 26$ U/L). In

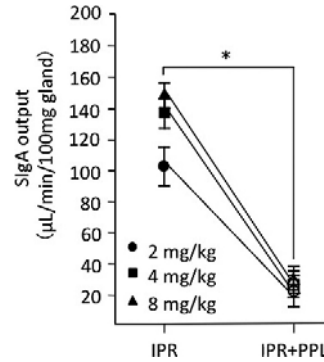


Fig. 12 Effect of PPL on the SIgA output evoked by IPR stimulation.

PPL significantly inhibited the SIgA secretion evoked by IPR stimulation.

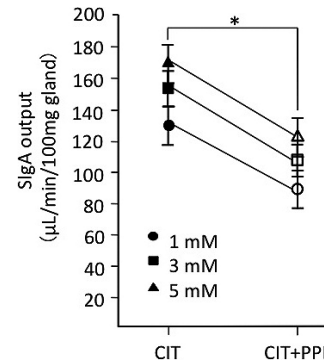


Fig. 13 Effect of PPL on the SIgA output evoked by CIT stimulation.

PPL slightly inhibited the SIgA secretion evoked by CIT stimulation.

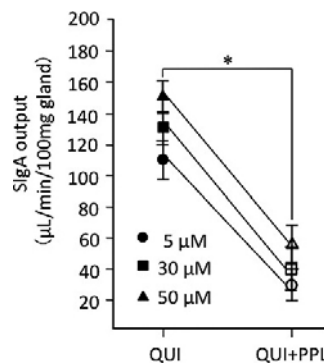


Fig. 14 Effect of PPL on the SIgA output evoked by QUI stimulation. SIgA secretion evoked by PPL addition to QUI significantly decreased by 20% compared with QUI alone.

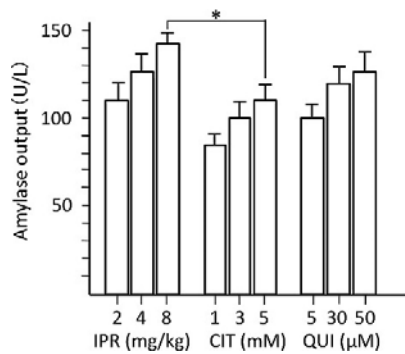


Fig. 15 Salivary SAA output by different reflex stimuli. Output levels of SAA evoked by IPR, CIT and QUI stimulation were similar.

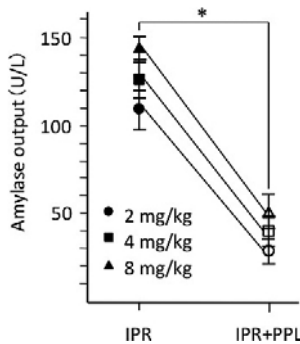


Fig. 16 Effect of PPL on the SAA output evoked by IPR stimulation. PPL significantly inhibited SAA secretion evoked by IPR stimulation.

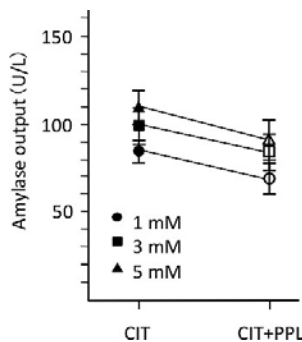


Fig. 17 Effect of PPL on the SAA output evoked by CIT stimulation. PPL slightly inhibited the SlgA secretion evoked by CIT stimulation.

particular, the SAA level evoked by CIT was significantly different compared with IPR stimulation (Fig.15). The inhibitory effects of PPL on the secretory responses were also investigated. IPR-induced en-

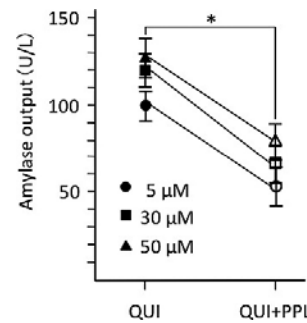


Fig. 18 Effect of PPL on the SAA output evoked by QUI stimulation. SAA secretion evoked by PPL addition to QUI significantly decreased by 35% compared with QUI alone.

hancement of SAA was significantly inhibited by PRP (Fig. 16). PPL moderately inhibited the SAA response to QUI stimulation (Fig. 18), and slightly inhibited the response to CIT stimulation (Fig. 17). IPR activates amylase secretion through β -receptors.

DISCUSSION

Saliva plays essential physiological roles in normal upper gastrointestinal tract function and oral health.¹³ It is secreted by acinar cells in the three major salivary glands, and in the minor glands.^{14,15} Nerves mainly control salivary glands, including afferent nerves induced by taste stimuli, such as acidity, bitterness, and salt,¹⁶ through gustatory taste receptors in the mucosa of the oral cavity.¹⁷ Secretion of saliva is dependent upon stimulus from autonomic nerves that are the effector arms of reflexes activated predominantly by taste. Ogawa *et al.* reported that salivary flow induced by basic taste stimuli was greatest for citric acid, followed by quinine, salt and sucrose.¹⁸ We used CIT and QUI as taste stimuli.

The salivary flow rate evoked by IPR stimulation was only 10% of that by CIT and QUI stimulation. The protein composition in the saliva induced by taste stimulation has different profiles. The secretion of peroxidase is up regulated to a much greater extent by sympathetic stimulation. Peroxidase secretion induced by QUI stimulation was greater than that induced by CIT stimulation. Although peroxidase output evoked by both IRP and QUI were markedly inhibited by PPL, CIT-induced peroxidase output was moderately inhibited by PPL.

The major antibody in saliva is IgA, which is actively transported by parenchymal cells within the salivary glands. The autonomic nerves supplying the glands *in vivo* regulate the rate of IgA secretion into saliva.¹⁹ SIgA secretion by submandibular glands is increased by stimuli from autonomic nerves. The greatest secretion of SIgA was evoked by IPR and this was inhibited by PPL. SIgA secretion induced by both CIT and QUI stimulation had a similar high level. Because it is unclear which specific autonomic receptors transduce such stimuli, we compared the secretion of fluid, SIgA and peroxidase with secretory responses during both sympathetic nerve agonist and taste stimulation. In addition, the increase in SIgA secretion caused by CIT stimulation was not adequately blocked by PPL. SIgA output evoked by QUI was inhibited by PPL.

We examined the secretion of SAA. Determining the secretion of SAA is a noninvasive method of measuring the degree of excitement of the sympathetic nervous system. We compared stimulation by the sympathetic nervous system, acidity and bitterness. Marked increases in SAA occurred in response to IPR, CIT and QUI stimulation. Although SAA evoked by both IPR and QUI stimulation was significantly inhibited by PPL, SAA output evoked by CIT was only slightly inhibited by PPL. SAA output induced by the CIT stimulation had different profiles for these conditions. These results suggest that a different balance of efferent nervous stimuli from sympathetic and parasympathetic nerves regulates the protein secreted from the submandibular gland.

This augmented SAA secretion, seen when the two nerves were activated at the same time, gave an SAA output that far exceeded the sum obtained from individual nerve stimulation. The sympathetic amylase secretion resulting from parasympathetic activity seemed to be dependent entirely on β -adrenoceptors. A high concentration of sympathetic stimulation resulted in a reduction in salivary flow, which was opposite to what happened when SAA concentration, was increased.

The present study was an attempt to show that sympathetic activity modified the SAA concentration of submandibular gland saliva when CIT stimulation was superimposed on a reflexly induced secretomo-

tor response.²⁰ We attempted to determine how sympathetic stimulation induced secretion of SIgA as well as other salivary proteins in the submandibular gland. IPR stimulation enhances the concentration of SIgA. Similar results were evoked by CIT and QUI stimulation. The concentration of SIgA was lowest with IPR, although it was similar with CIT. These results indicate that not only sympathetic nerves, but also parasympathetic nerves, up regulate SIgA secretion. We examined how both sympathetic and taste stimulation increased IgA secretion in the saliva. Because SIgA secretion evoked by CIT is not inhibited by PPL, SIgA secretion is promoted by sympathetic stimulation as well as others, such as parasympathetic stimulation. Carpenter *at al.* indicated that preganglionic parasympathectomy reduced both the stimulated and unstimulated rates of salivary IgA secretion, despite similar glandular amounts of IgA.²¹

We analyzed the secretion of antibacterial factors by the submandibular gland induced by both sympathetic nerve and taste stimulation. Our results indicated that both sympathetic and parasympathetic nerve stimulation increases SIgA secretion from the submandibular gland. Acidity seems to be beneficial in the prevention of oral disease because it enhances both the production of saliva and an increased secretion of SIgA in the saliva.

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