Synaptic Responses of Neurons Controlling the Parotid and von Ebner Salivary Glands in Rats to Stimulation of the Solitary Nucleus and Tract

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Suwabe T, Fukami H, Bradley RM. Synaptic responses of neurons controlling the parotid and von Ebner salivary glands in rats to stimulation of the solitary nucleus and tract. J Neurophysiol 99: 1267–1273, 2008. First published January 16, 2008; doi:10.1152/jn.01115.2007. Salivary secretion results from reflex stimulation of autonomic neurons via afferent sensory information relayed to neurons in the rostral nucleus of the solitary tract (rNST), which synapse with autonomic neurons of the salivatory nuclei. We investigated the synaptic properties of the afferent sensory connection to neurons in the inferior salivatory nucleus (ISN) controlling the parotid and von Ebner salivary glands. Mean synaptic latency recorded from parotid gland neurons was significantly shorter than von Ebner gland neurons. Superfusion of GABA and glycine resulted in a concentration-dependent membrane hyperpolarization. Use of glutamate receptor antagonists indicated that both AMPA and N-methyl-D-aspartate (NMDA) receptors are involved in the evoked excitation. The GABAA receptor antagonist, bicuculline (BMI) or mixture of strychnine and BMI abolished the IPSPs in all neurons. IPSP latency was longer than EPSP latency, suggesting that more than one synapse is involved in the inhibitory pathway. Results show that ISN neurons receive both excitatory and inhibitory afferent input mediated by glutamate and GABA respectively. The ISN neuron response to glycine probably derives from descending connections. Difference in the synaptic characteristics of ISN neurons controlling the parotid and von Ebner glands may relate to the different function of these two glands.

INTRODUCTION

Saliva secreted by the major and minor salivary glands plays an essential role in controlling the homeostasis of the oral cavity (Bradley and Beidler 2003). Absence or reduced salivary secretion (Xerostomia) resulting from a number of causes is a serious medical condition that leads to a marked decline in oral health (Cassolato and Turnbull 2003; Garg and Malo 1997). Secretion of saliva generally results from stimulation of the autonomic innervation of the glands or in response to drugs that mimic the actions of the autonomic innervation (Schneider et al. 1972). Thus control of the oral environment is based on the continuous processing of oral sensory information that results in salivary secretion. For example, intake of food producing a fall in oral pH will initiate a high flow of bicarbonate-containing saliva (Dawes and Jenkins 1964) to dilute and buffer the oral environment. This kind of reflex secretion derives from chemical stimulation of taste buds that transmit gustatory information to the rostral nucleus of the solitary tract (rNST). Second-order rNST neurons then synapse with autonomic secretomotor neurons controlling the salivary glands.

The parasympathetic secretomotor neurons form a column of cells called the salivatory nucleus oriented along the medial border of the NST. The preganglionic outflow from the most rostral extent of the salivatory nucleus (termed the superior salivatory nucleus) travels to the submandibular and sublingual salivary glands with the facial (VII) nerve, whereas preganglionic outflow from the more caudal salivatory nucleus (inferior salivatory nucleus, ISN) travels in the glossopharyngeal (IX) nerve to innervate the parotid and von Ebner (lingual) salivary glands (Matsuo 1999a).

Despite knowledge of the basic circuit of the gustatory–salivary reflex, and a number of studies relating salivary flow rate to taste quality (Matsuo 1999b) little is known about the synaptic mechanisms involved in the reflex with the notable exception of a recent study of the superior salivatory nucleus (Mitoh et al. 2004). In an earlier study, we showed interactions between salivary secretion from the von Ebner glands and stimulation of taste buds contained in the circumvallate and foliate papillae of the tongue (Gurkan and Bradley 1988). As the first step of in vitro study on the gusto-salivary reflex circuit, we recently characterized the morphology of ISN neurons involved in the control of the von Ebner and parotid glands (Fukami and Bradley 2005). As a next step, we now investigate the synaptic properties of the afferent sensory connection to these neurons.

METHODS

Retrograde labeling

Sprague-Dawley rats (10–24 days old) were anesthetized with 6% solution of halothane mixed with air (400–600 ml/min). Anesthetic level was assessed by tail pinching before surgery. All surgical procedures were carried out under National Institutes of Health and University of Michigan Animal Care and Use Committee–approved protocols.

A fluorescent tracer, Alexa Fluor 568 dextran (Molecular Probes, Eugene, OR), was used to retrogradely label ISN neurons innervating the von Ebner salivary glands. The lingual-tonsillar branch of the glossopharyngeal nerve was exposed by a ventral approach and cut. Crystals of the fluorescent tracer were applied to the cut central end and isolated from surrounding tissue with silicone sealer (Kwik-Cast, World Precision Instruments). To label ISN neurons innervating the parotid gland, the otic ganglion was exposed on the medial aspect of the glossopharynx-containing saliva (Dawes and Jenkins 1964) to dilute and buffer the oral environment. This kind of reflex secretion derives from chemical stimulation of taste buds that transmit gustatory information to the rostral nucleus of the solitary tract (rNST). Second-order rNST neurons then synapse with autonomic secretomotor neurons controlling the salivary glands.

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the mandibular division of the trigeminal nerve, and crystals of the tracer placed on the ganglion and coated with silicone cement. The skin wound was closed with cyanoacrylate glue. Rats recovered in an isolated cage on a heating pad and, when ambulatory, were returned to their mother’s home cage.

**Preparation of brain stem slices**

After a suitable time for transport of the label (2–4 days), the rats were reanesthetized with halothane and decapitated, and the brain was rapidly removed and cooled for 6 min in an oxygenated physiological saline solution in which NaCl was replaced with isosmotic sucrose at 4°C (Aghajanian and Rasmussen 1989). The brain stem was transected at the level of thepons and just caudal to the obex and cemented to a Vibratome (Technical Products International) stage with cyanoacrylate glue. The brain stem was sectioned horizontally for ISN neurons controlling von Ebner glands or parasagittally for ISN neurons controlling the parotid glands into 200- or 300-μm-thick slices. Slices were incubated for at least 1 h in oxygenated physiological saline at room temperature before transferring to the recording chamber. The physiological saline, which contained (in mM) 124 NaCl, 5 KCl, 2.5 CaCl₂, 1.3 MgSO₄, 26 NaHCO₃, 1.25 NaH₂PO₄, and 25 dextrose, was gassed with a 95% O₂-5% CO₂ mixture to achieve a solution pH of 7.4.

**Recording**

Brain slices were transferred to a recording chamber attached to the stage of a Nikon Eclipse E600-FN microscope and anchored with a nylon mesh. During recording, the slice was superfused at 2–2.5 ml/min with oxygenated physiological saline at room temperature. All drugs were dissolved in the physiological saline and applied by perfusion over the brain slices. Concentration of drugs used in this study was based on those used in previous experiments and by other investigators (Grabauskas and Bradley 1996; Mitoh et al. 2004; Wang and Bradley 1995).

Retrogradely labeled ISN neurons were identified using fluorescence illumination. Once identified, the labeled neuron was observed using infrared-differential contrast optics (IR-DIC) via a CCD camera (IR-1000, DAGE-MTI). A ×40 water-immersion objective lens was used to identify and observe the labeled neuron. Whole cell recordings were obtained from the labeled neurons using a patch-clamp amplifier (Axoclamp-2B, Axon Instruments). Signals were recorded through a 2-kHz low-pass filter, digitized at 20 kHz (DigiData 1200, Axon Instruments), and stored on a computer hard disk. Data acquisition was performed using pCLAMP 8 (Axon Instruments). Patch pipettes were made of borosilicate glass capillaries (TW150F-4, World Precision instruments) using a two-stage puller (PP-83, Narishige) and filled with a solution that contained (in mM) 130 potassium-glucuronate, 10 HEPES, 10 EGTA, 1 MgCl₂, 1 CaCl₂, and 2 ATP, buffered to pH 7.2 with KOH. Tip resistance of filled pipettes was 6–8 MΩ. Postsynaptic potentials (PSPs) were elicited by delivery of stimuli (0.3- to 3-mA amplitude and 0.01- to 0.05-ms duration) via a bipolar stimulating electrode consisting of tightly twisted pairs of 70-μm-diam, Teflon-insulated platinum wires. The electrode was placed under direct visual control either on the ST or on the NST adjacent to the ISN. The distance between the stimulating electrode and recording site was between 0.5 and 1 mm. All drugs were applied by changing the perfusion line to the recording chamber. Because PSPs recorded from ISN neurons are a mixture of both excitatory and inhibitory components (Bradley et al. 2005), the GABA<sub>A</sub> receptor antagonist, bicuculline methiodide (BMI) was added to the superfuse to isolate excitatory PSPs (EPSPs). Glutamate receptor blockers were used to isolate inhibitory PSPs (IPSPs). Strychnine, 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), 2-amino-5-phosphonovaleric acid (APV), and BMI were purchased from Sigma (St. Louis, MO).

**Data analysis**

Electrophysiological data were analyzed using the Clampfit program (Axon Instruments). Biophysical properties (input resistance, membrane time constants, action potential amplitude, and half-width) were determined by delivering a series of 100-ms hyperpolarizing and depolarizing current pulses (~100 to 75 pA). The junction potential caused by potassium gluconate (10 mV) was subtracted from the membrane potential values.

The time course of the PSPs was characterized by measuring its rise time, decay time, and peak amplitude. Statistical analysis was conducted using the SPSS program. The numerical values are given as means ± SE, and statistical significance (P < 0.05) was assessed using Student’s paired t-test.

**Results**

**Biophysical properties of ISN neurons innervating the von Ebner and parotid glands**

Whole cell recordings were made from a total of 56 von Ebner and 35 parotid salivary gland neurons. These neurons had a stable resting membrane potential more negative than −40 mV, a spike overshoot of ≥20 mV, and input resistance >100 MΩ.

Von Ebner salivary glands neurons had resting membrane potentials between −45 and −69 mV (−56 ± 1 mV), input resistance between 315 and 901 MΩ (641 ± 20 MΩ), time constants between 19 and 105 ms (54 ± 3 ms), spike half-widths ranging from 1.04 to 4.8 ms (2.2 ± 0.1 ms), and spike amplitude between 36 and 107 mV (91 ± 1.7 mV).

Parotid gland neurons had resting membrane potentials between −45 and −75 mV (−57 ± 1.5 mV), input resistance between 134 and 672 MΩ (320 ± 28 MΩ), time constants between 6 and 61 ms (30 ± 3 ms), spike half-width ranging from 0.65 and 2.49 ms (1.3 ± 0.1 ms), and spike amplitude between 74 and 109 mV (91 ± 1.8 mV). Input resistance and spike half-width of von Ebner gland neurons were significantly higher than parotid gland neurons (P < 0.05). The time constant of the von Ebner salivary neurons was significantly longer than parotid gland neurons (P < 0.05). These differences of spike half-width and time constant between neurons innervating the von Ebner and parotid glands probably reflect differences in input resistance.

**Characteristics of PSPs recording from ISN neurons**

Stimulation of the ST elicited PSPs in both parotid and von Ebner salivary glands neurons. When the ST was stimulated, 89% of the PSPs were depolarizing and 11% were hyperpolarizing in parotid neurons and 92% were depolarizing and 8% were hyperpolarizing in von Ebner salivary neurons. Mean PSP latency recorded from parotid salivary glands neurons (5.5 ± 0.42 ms) was significant shorter than von Ebner salivary glands neurons (7.2 ± 0.36 ms; P < 0.05).

Stimulation of the ST excites both second-order rNST neurons and inhibitory interneurons (Grabauskas and Bradley 1996; Wang and Bradley 1995) mediated by glutamate. Application of glutamate receptor antagonists therefore blocks all afferent transmission. However, synaptic potentials recorded from second-order rNST neurons consist of an EPSP/IPSP complex, and to directly analyze IPSPs evoked by stimulation of ST, IPSPs were evaluated after application of BMI by...
Superfusion of GABA (0.01–2.5 mM) resulted in membrane hyperpolarization and decrease in input resistance in all tested neurons (n = 12 in neurons controlling von Ebner glands and n = 9 in neurons controlling parotid glands). The decrease in membrane resistance was concentration-dependent (EC50 = 0.15 mM in ISN neurons controlling von Ebner glands and 0.18 mM in ISN neurons controlling parotid glands; Fig. 2A). Application of glycine (0.01–2.5 mM) also resulted in a concentration-dependent decrease in membrane resistance (Fig. 2B). Membrane hyperpolarization mediated by glycine (1 mM) and GABA (1 mM) application occurred in the presence of a neural conduction blocker, TTX (1 or 2 µM), in four neurons controlling the von Ebner gland and five neurons controlling the parotid gland (Fig. 3). These results indicate that the effects of glycine and GABA result from direct action on the ISN neurons and that ISN neurons have both functional glycine and GABA receptors.

Differences were apparent in the concentration-dependent properties of inhibition of ISN neurons innervating the parotid and von Ebner glands. The half-maximal glycine concentration value was 0.43 and 0.24 mM in ISN neurons controlling von Ebner and parotid glands, respectively, although the maximal values were not significantly different. In response to 0.25 mM glycine, ISN neurons controlling parotid glands (n = 6) showed significantly larger change of input resistance than ISN neurons controlling von Ebner glands (n = 5, 45 vs. 14%). This result suggests that ISN neurons controlling parotid glands have a lower threshold response to glycine. The glycine receptor antagonist, strychnine (2 µM), blocked the effects of glycine applied at the half-maximal concentration (0.5 mM in 3 neurons controlling von Ebner glands and 0.25 mM in 3 neurons controlling parotid glands; Fig. 2C).

**EPSPs**

Under GABA receptor block, stimulation of the ST evoked an EPSP in all ISN neurons. The mean latency between the
stimulus artifact and the rise of the EPSP was 3.1 ± 0.1 ms in 24 neurons controlling von Ebner glands and 2.5 ± 0.2 ms in 13 neurons controlling the parotid glands (Fig. 4). Although the difference in mean latency between neurons controlling von Ebner and parotid glands was statistically significant, the distribution of the latencies overlapped, indicating considerable similarities in the EPSP latencies for the two glands (Fig. 4). Differences in mean latency presumably results because the distribution of latencies recorded in parotid gland neurons is skewed to the left.

To study involvement of the different types of glutamate receptors in the evoked EPSPs, we used glutamate receptor antagonists CNQX (AMPA/kainate receptor antagonist) and APV [N-methyl-d-aspartate (NMDA) receptor antagonist]: 10 μM CNQX (n = 12; Fig. 5A) or 50 μM APV (n = 6; Fig. 5B) alone suppressed the EPSP but did not completely block the EPSP, indicating that both AMPA/kainate and NMDA glutamate receptors are involved in the evoked EPSPs.

We determined the relative contribution of the AMPA/kainate and NMDA receptors to the evoked EPSPs. Because of the voltage-dependent Mg²⁺ block of NMDA receptors (Mayer et al. 1984; Nowak et al. 1984), EPSPs were recorded in both normal physiological saline and in Mg²⁺-free saline, and the EPSP characteristics were compared. EPSP rise time (76.7 ± 5.2 ms with Mg²⁺ and 107.5 ± 6.6 ms Mg²⁺-free) and half-width increased (31.2 ± 2.7 ms with Mg²⁺ and 44.6 ± 3.8 ms Mg²⁺-free) in Mg²⁺-free saline. EPSP amplitude (11.2 ± 1.1 mV with Mg²⁺ and 10.9 ± 1.3 mV Mg²⁺-free) in 19 ISN neurons (7 controlling von Ebner glands and 12 controlling parotid glands) was not significantly effected by changing Mg²⁺ concentration. In seven neurons controlling von Ebner glands (Fig. 6A) recorded in Mg²⁺-free saline, CNQX (10 μM) alone induced a 50% decrease in EPSP amplitude (7.7 ± 0.9 mV in control), indicating that the AMPA/kainate component contributed 50% of the EPSP. Addition of APV (50 μM) and CNQX completely blocked the remaining EPSP contributed by NMDA receptors. In 12 neurons controlling the parotid glands (Fig. 6B), the AMPA/kainate component was 59% of the evoked EPSP (11.1 ± 1.4 mV in control), and by arithmetic subtraction, the NMDA component was 41% of the EPSP.

**IPSPs**

Electrophysiological data on IPSPs evoked in ISN neurons was derived using glutamate receptor antagonists. Because this block effectively eliminates synaptic input resulting from ST stimulation, IPSPs were evoked by electrical stimulation of the rNST (presumably by direct stimulation of inhibitory interneurons) 0.5–1.0 mm distant from the recording site (Grabauskas and Bradley 1999).

In control saline, electrical stimulation evoked a depolarizing PSP (9 neurons controlling von Ebner and 7 neurons controlling parotid glands; Fig. 7). After glutamate receptor block, the IPSP component of the PSP remained. The amplitude of the IPSP increased with higher intensity stimulation,
indicating that the IPSP was evoked by stimulation of neurons presynaptic to the ISN neurons. Addition of 2 μM strychnine did not effect the amplitude of the IPSPs significantly (−4.8 ± 0.9 mV before and −4.6 ± 0.8 mV with strychnine in von Ebner neurons and −3.9 ± 0.4 mV before and −3.8 ± 0.3 mV with strychnine in parotid glands neurons; P > 0.05; CNQX + APV and strychnine traces in Fig. 7). After ≥5-min washout of strychnine, a GABA_A receptor antagonist, BMI (10 μM), or mixture of strychnine and BMI abolished the IPSP in all the neurons (strychnine + BMI trace in Fig. 7). These results indicate that the evoked IPSPs are mediated primarily via GABA_A receptors activating synapses between ISN and rNST neurons.

**DISCUSSION**

These experiments are part of a series studying the neurobiology of the ISN. We have already described the morphology (Kim et al. 2004) and basic biophysical properties of the ISN neurons (Fukami and Bradley 2005), as well as responses of these neurons to substance P and 5-hydroxytryptamine (Subwabe and Bradley 2007). In this study, we concentrated on evoked synaptic responses. Stimulation of the ST elicited PSPs in neurons innervating both the parotid and von Ebner salivary glands. When the ST was stimulated, the majority of the PSPs were depolarizing and the remainder were hyperpolarizing, suggesting that afferent inputs to ISN results in facilitation of neural activity in most neurons. All the evoked potentials were mixtures of EPSPs and IPSPs. Under GABA_A receptor block, the PSPs were excitatory involving both AMPA/kainate and NMDA glutamate receptors. When the excitatory component of the PSPs was blocked, the resulting IPSPs were found to be mediated primarily via GABA_A receptors. The mean latency between the stimulus and the rise of EPSPs (6.6 ± 0.5 ms) and IPSPs (11.0 ± 0.8 ms) was significantly longer, suggesting additional synapses in the inhibitory pathway. Application of GABA and glycine resulted in a concentration-dependent decrease in membrane resistance. BMI and strychnine eliminated the effects of GABA and glycine, respectively. These changes in membrane properties occurred with TTX transmission block, indicating that the effects of GABA and glycine result from direct action on the ISN neurons and showing that ISN neurons have both functional glycine and GABA receptors. However, the glycine receptor antagonist strychnine did not significantly reduce the amplitude of the evoked IPSPs, suggesting that glycine is not released when IPSPs are evoked by local stimulation of NST.

These results are similar to patch-clamp recordings of postsynaptic currents evoked by electrical stimulation of neurons and fibers surrounding neurons in the superior salivatory nucleus. This type of electrical stimulation probably activates both descending inputs from the higher centers and/or afferent inputs from sensory nuclei in the brain stem (Mitoh et al. 2004). However, although the excitatory synaptic responses of the superior salivatory nucleus neurons are similar to the results of this study, the inhibitory synaptic activity mediated by GABA and glycine differ. Application of strychnine decreased the amplitude of the evoked synaptic currents and addition of BMI entirely abolished the remaining synaptic current. Thus glycine was apparently released from the surrounding neural elements in response to the electrical stimulation.

Based on the site of stimulation used in this study, glutamate and GABA are released either from termination of afferent fibers that compose the ST or from local interneurons in rNST. Although glycine has been shown in the NST using immunohistochemical techniques (Aprison et al. 1969; Cassell et al. 1992; Saha et al. 1999; Sweazey 1996), IPSPs in this study did not have a glycineric component, suggesting the source of the glutamate component may be afferent sensory input and parasympathetic secretomotor neurons in the ISN. Afferent input from taste receptors enters the brain stem to form the ST and synapse with second-order neurons in the rostral nucleus of the solitary tract (rNST). A population of second-order neurons projects rostrally to the parabrachial nucleus (PBN). A 2nd population projects directly to synapse with ISN neurons. It is also possible that some sensory input bypasses the rNST neurons to synapse directly with the ISN neurons since very short synaptic latencies are recorded from some ISN neurons. This monosynaptic connection possibly mediates rapid onset of saliva production in response to a drop in oral pH. Other rNST neurons connect to the ISN neurons via interneurons possibly responsible for the longer latency inhibitory responses recorded from ISN neurons. A descending input from rostral brain centers also synapses with the ISN neurons. Other synaptic interactions are possible.
glycine for inhibitory input may possibly derive from descending connections (Kobayashi et al. 1997; Matsu0 1999a) or be produced locally (Danyasz and Parsons 1998). In addition to its role as an inhibitory neurotransmitter, glycine is also a co-agonist with glutamate at NMDA receptors (Baptista and Varanda 2004; Turecek and Trussell 2001). Thus glycine may serve a number of roles in ISN neuron synaptic transmission.

The preganglionic parasympathetic neurons of the ISN form the brain stem efferent component of the reflex system controlling salivary secretion. Sensory information originating in taste and mechanoreceptors receptors in the oral cavity and descending input from higher centers relay with the ISN neurons (Bradley and Kim 2006). Although the descending connections have only been described anatomically, the afferent connections have received more attention. However, there is a general assumption that salivary reflexes are somewhat simple, consisting of excitatory input that, when activated, results in salivary secretion: the greater the input the greater the flow of saliva. This was also the assumption for reflexes involving the caudal NST (TravaglI 2007), but recent evidence has shown that the neural organization of these reflexes is complex, indicating specialization of different reflex pathways tuned to specific tasks (Bailley et al. 2007). Results of the current and past studies of the neurobiology of the salivatory neurons suggest that salivary reflexes are also more complex than originally assumed.

Although excitatory synaptic input to the ISN neurons is predictable, the results of this study show that inhibition plays a significant role in ISN neuron synaptic transmission. Substance P has been shown to modulate the excitability of NST neurons (King et al. 1993), suggesting that substance P may modulate synaptic inputs derived from rNST to ISN neurons. In our previous study, substance P and 5-HT have also been shown to modulate ISN synaptic activity, probably via descending input from more rostral brain areas (Suwabe and Bradley 2007). Examination of the intrinsic firing properties of both the ISN and superior salivatory neurons showed that these neurons possess A-type potassium channels (Fukami and Bradley 2005; Matsu0 and Kang 1998). Finally in vivo experiments of taste-initiated saliva flow have shown that, despite the fact that the neural input to the NST in response to different taste qualities may be similar, the quantity and quality of the resulting saliva is different, suggesting involved brain stem connections (Frank 1973; Kawamura and Yamamoto 1978). When all these factors are considered, the processing of sensory information by the ISN neurons controlling the salivary glands is complex.

Based on available information, a number of circuits are possible that connect the sensory input to the ISN neurons (Fig. 8). The most basic of these circuits are sensory input from oral taste and mechanoreceptors that synapse with second-order brain stem neurons that synapse with the ISN neurons. Since inhibitory processing is known to occur, GABAergic interneurons are involved that either connect reciprocally with the second-order neurons or synapse directly with the ISN neurons. Evidence indicates that second-order neurons in the NST can be either rostrally projecting neurons or interneurons (King 2006). At present, it is not clear if connections between the sensory input and the rNST are exclusive to the ISN reflex pathway or shared with rostral projections (Halsell et al. 1996). Moreover, based on anatomical tracing studies and short synaptic latencies observed in some ISN neurons, afferent sensory input may bypass NST neurons altogether and synapse monosynaptically with the ISN neurons.

ISN neurons do not simply pass on the afferent information unchanged. Because ISN neurons express hyperpolarization-activated potassium current channels and have both glutamate and GABA receptors, any afferent input that changes membrane voltage in the ISN neurons may result in modulation of hyperpolarization-activated potassium current (IKA) and thus alter transmission of the afferent information. For example, GABAergic synaptic inputs may hyperpolarize and result in IKA activation. In contrast, depolarizations via glutamatergic and descending synaptic inputs would inactivate IKA and augment neuron output. This raises the intriguing possibility that sensory input derived from different taste qualities (e.g., sour and sweet) would result in different patterns of activity because of modulation of IKA that produces subtle changes in the output of the salivary reflexes.

Finally differences in the synaptic properties (latency, differences in agonist response concentration functions) and cell morphology (Bradley and Kim 2006; Fukami and Bradley 2005) of neurons controlling the von Ebner and parotid salivary glands may reflect differences in their function. The parotid glands secrete directly into the oral cavity and contribute to the pooled saliva from the other major salivary glands. The parotid gland secretion therefore provides significant contribution to the environment of the whole mouth. In contrast, the von Ebner glands are more specialized, and their secretion forms the microenvironment of the taste buds lining the clefs of the circumvallate and foliate papillae and secrete a lipase and a protein that is exclusive to these salivary glands (Bradley and Beidler 2003; Field et al. 1989; Schmale et al. 1993). Thus salivary secretion and the autonomic reflexes controlling these two salivary glands suggest very different reflex connections related to their functional role.

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