Biophysical Properties and Responses to Neurotransmitters of Petrosal and Geniculate Ganglion Neurons Innervating the Tongue

TOMOSHIGE KOGA1 AND ROBERT M. BRADLEY1,2
1Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor 48109-1078; and 2Department of Physiology, Medical School, University of Michigan, Ann Arbor, Michigan 48109-0622

Received 3 February 2000; accepted in final form 30 May 2000


The properties of afferent sensory neurons supplying taste receptors on the tongue were examined in vitro. Neurons in the geniculate (GG) and petrosal ganglia (PG) supplying the tongue were fluorescently labeled, acutely dissociated, and then analyzed using patch-clamp recording. Measurement of the dissociated neurons revealed that PG neurons were significantly larger than GG neurons. The active and passive membrane properties of these ganglion neurons were examined and compared with each other. There were significant differences between the properties of neurons in the PG and GG ganglia. The mean membrane time constant, spike threshold, action potential half-width, and action potential decay time of GG neurons was significantly less than those of PG neurons. Neurons in the PG had action potentials that had a fast rise and fall time (sharp action potentials) as well as action potentials with a deflection or hump on the falling phase (humped action potentials), whereas action potentials of GG neurons were all sharp. There were also significant differences in the response of PG and GG neurons to the application of acetylcholine (ACh), serotonin (5HT), substance P (SP), and GABA. Whereas PG neurons responded to ACh, 5HT, SP, and GABA, GG neurons only responded to SP and GABA. In addition, the properties of GG neurons were more homogeneous than those of the PG because all the GG neurons had sharp spikes and when responses to neurotransmitters occurred, either all or most of the neurons responded. These differences between neurons of the PG and GG may relate to the type of receptor innervated. PG ganglion neurons innervate a number of receptor types on the posterior tongue and have more heterogeneous properties, while GG neurons predominantly innervate taste buds and have more homogeneous properties.

INTRODUCTION

Investigators of the peripheral gustatory system have examined the properties of afferent taste fibers by extracellular recording from dissected single fibers. This classical approach has revealed a wealth of information on the response characteristics of afferent taste fibers (for a review see Smith and Frank 1993). In contrast, the current knowledge of the biophysical and neurochemical properties of afferent taste fibers is less complete because of the practical difficulty of examining these fibers in vivo. Researchers of nociceptors facing a similar problem have made profitable use of in vitro techniques to define the biophysical and chemical sensitivity of afferent sensory neurons innervating cutaneous receptors because the properties of the ganglion cells of these fibers reflects the properties of the afferent fibers and endings (Dray 1996). The goal of the present investigation was to apply similar techniques to afferent sensory neurons supplying taste buds to characterize both the biophysical properties and responses of these neurons to neurotransmitters that have been shown using anatomical techniques to be associated with taste receptors (Nagai et al. 1996).

Afferent fibers transmitting gustatory information from the anterior tongue and soft palate travel in the chorda tympani and greater superficial petrosal nerves with cell bodies in the geniculate ganglion (GG), while taste buds on the posterior tongue are supplied by the glosopharyngeal nerve with cell bodies in the petrosal ganglion (PG). Electrophysiological studies of the chorda tympani, greater superficial petrosal, and glosopharyngeal nerves have revealed that these nerves have heterogeneous response properties (Frank 1991; Frank et al. 1988; Nejad 1986; Ogawa et al. 1968), but it is not known if these differences are reflected in the biophysical properties of the ganglion cells. In addition to having heterogeneous response properties, afferent taste neurons also express multiple putative neurotransmitters and neuromodulators. For example, the PG has been shown to immunostain for substance P (SP), tyrosine hydroxylase, vasoactive intestinal polypeptide, calcitonin gene-related peptide, galanin, glutamate, and aspartate (Czyzyk-Kreska et al. 1991; Finley et al. 1992; Helke and Hill 1988; Helke and Niederer 1990; Helke and Rabchevsky 1991; Ichikawa et al. 1991; Okada and Miura 1992).

Not only have these neurochemicals been shown to be present in the PG and GG, they have been implicated in taste function at both the peripheral and central processes of the peripheral taste system. Serotonin (5HT) is believed to modulate chemosensory responses of taste receptor cells (Delay and Roper 1988; Nagai et al. 1998) and acetylcholine (ACh), γ-aminobutyric acid (GABA), and SP have been suggested to function at the synapse between taste buds and primary afferent neurons (Jain and Roper 1991; Nagai et al. 1986; Nagy 1982; Paran and Mattern 1975). Moreover, PG neurons respond to ACh at concentrations in the physiological range (Zhong and Nurse 1997). At the first relay in the central taste pathway, GABA and SP have been shown to function as inhibitory and

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
excitatory neurotransmitters at the synapse of the primary afferent fibers in the nucleus of solitary tract (Davis and Smith 1997; Du and Bradley 1998; Grabauskas and Bradley 1996, 1998a; King et al. 1993; Wang and Bradley 1993).

To gain insight into the biophysical properties of afferent neurons supplying taste receptors, we have used acutely isolated GG and PG neurons innervating the tongue that were retrogradely labeled by Fluorogold injected into the area of the fungiform, foliate, and circumvallate papillae. We have also characterized the responses of PG and GG neurons to application of 5HT, SP, ACh, and GABA, which have been demonstrated to be involved in the afferent taste system.

METHODS

Cell labeling and isolation

The cell labeling technique is based on methods developed in an earlier study (Bradley et al. 1985). Female Sprague-Dawley rats aged 20–40 days were anesthetized with an intramuscular injection of mixture of Rompun (10 mg/kg) and ketamine hydrochloride (90 mg/kg). The lower jaw was retracted and the tongue depressed. Using a dissecting microscope, the single circumvallate papilla was visualized and 10–15 μl of 5% solution of Fluorogold (Fluorochrome, Englewood, CO) mixed in distilled water was injected just beneath the papilla from a Hamilton syringe. To study GG neurons innervating the anterior tongue, 5–10 μl of 5% solution of Fluorogold was injected bilaterally just beneath both the foliate and fungiform papillae.

After a postoperative survival time of 3–12 days, the rats were reanesthetized with sodium pentobarbital (50 mg/kg, ip) and decapitated. The skull was opened and the forebrain removed just rostral to the brainstem. Using the exit of the facial nerve from the brainstem as a landmark, the petrous portion of the temporal bone was removed to expose the GG which was then excised. The PG was exposed in the neck by following the glossopharyngeal nerve centrally and then removed. The GG or PG were placed in HEPES buffer containing (in mM) 124 NaCl, 5 KCl, 5 MgCl₂, 10 sodium succinate, 15 dextrose, 15 HEPES, and 2 CaCl₂, and gassed with O₂. The ganglia were transferred to HEPES buffer containing 0.5 mg/ml trypsin (type III) and 0.5 mg/ml collagenase (type IVA) and incubated for 1 h at 37°C. For incubation of the GG, the enzyme concentrations were reduced by one half. The ganglia were then triturated with a series of progressively smaller diameter, fine-polished Pasteur pipettes to produce a suspension of dissociated neurons which were placed in a 35-mm-diameter plastic petri dish. The cell suspension was continuously perfused at about 2 ml/min with oxygenated HEPES buffer by gravity flow and the fluid level in the recording chamber was maintained relatively constant by suction of excess fluid. After isolation, the majority of cells were spherical, devoid of processes, and became loosely attached to the substrate after 10 min. The enzymes were obtained from Sigma and prepared before each experiment.

Electrophysiological recording

Patch electrodes, pulled in two stages from 1.5-mm OD borosilicate filament glass, were filled with a solution containing (in mM) 130 potassium gluconate, 10 HEPES, 10 EGTA, 1 MgCl₂, 1 CaCl₂, and 2 ATP. The pipette solution was adjusted to a pH of 7.2 with KOH. Electrode resistance was between 5 and 8 MΩ. Recordings were obtained between 30 min and 3 h after plating. The petri dish containing the neurons was mounted on the stage of an inverted microscope equipped with epifluorescence and Hoffman modulation optics. Fluorogold-labeled neurons were identified by fluorescence with an exposure of sufficient length to identify labeled neurons. Electrodes were manipulated under visual control and membrane potential and currents were recorded using standard whole-cell patch-clamp recording procedures with an Axoclamp 2A amplifier (Axon Instruments). Current- and voltage-clamp protocols, data acquisition, and analysis were performed using pCLAMP software (Axon Instruments). Bridge balance was carefully monitored throughout the experiments and adjusted when necessary. The junction potential due to potassium gluconate (10 mM) was subtracted from the recorded membrane voltages (Standen and Stanfield 1992). Both Fluorogold and nonlabeled neurons were investigated to determine any possible effects of the label on the recordings. Criteria for a successful recording included a minimum of 10 min recording time with a stable resting membrane potential of > –40 mV and an action potential amplitude >70 mV. The statistics are expressed as mean ± SD and differences between groups measured with the Students t-test were considered significant when P ≤ 0.05.

Drug application

Neurotransmitters or neuromodulators were prepared daily for experiments from stock solution in HEPES buffer stored at −80°C and diluted to the desired final concentration just before use. A three- or seven-barrel pipette filled with a different concentration of drug was positioned ~40 μm from the neuronal cell body. SP (0.1 μM-1 mM), ACh (1 μM-1 mM), 5HT (0.01 μM-1 mM), GABA (1 μM-1 mM), the gamma aminobutyric acid-A (GABAₐ) agonist muscimol (1 μM-1 mM), or the gamma aminobutyric acid-B (GABAₐ) agonist baclofen (0.1–2 mM) was ejected from the pipette using a Picospritzer at a low pressure (2 psi). Concentration-response curves were fitted using the Hill equation. All experiments were conducted at room temperature.

RESULTS

Identification of neurons labeled with Fluorogold

The PG and GG were surgically removed 3–12 days after injection of Fluorogold into their receptive fields. Dissociated PG and GG neurons were round or ovoid in shape and some of them had short processes. The lengths of the long axis of the isolated PG and GG neurons were 32.2 ± 4.4 and 26.0 ± 2.6 μm, respectively (mean ± SD), while their short axes were 28.6 ± 4.2 and 23.0 ± 2.6 μm. The average diameter of the PG neurons was significantly larger than those of the GG (P < 0.001) (Fig. 3A). Figure 1 is a photomicrograph of PG neurons viewed under normal (A) and fluorescent illumination (B). Two of these neurons were strongly fluorescent. Once a labeled

![FIG. 1. Dissociated petrosal ganglion neurons viewed under tungsten (A) and fluorescence (B) illumination. Two of these cells were identified as Fluorogold labeled neurons.](http://jn.physiology.org/)

neuron was identified, whole-cell recordings were made under normal illumination.

**Biophysical properties of labeled dissociated cells**

Recordings were made from 130 labeled PG and 103 labeled GG neurons. Recordings were also made from nonlabeled PG ($n = 45$) and GG ($n = 26$) neurons to determine if they were different from the labeled neurons. No neurons were spontaneously active. Depolarizing and hyperpolarizing currents were injected to investigate action potential and passive membrane properties, respectively.

Since the ganglion cells were obtained from animals aged 20–40 days, it is possible that developmental changes could be still occurring to the peripheral taste system in these animals. The full complement of rat fungiform taste buds is present by 20 days (Mistretta 1972), but taste buds in the circumvallate papilla reach adult numbers at 90 days (Hosley and Oakley 1987). Thus, at the ages studied, the receptive field of the GG neurons was probably not changing, whereas significant changes were ongoing in the receptive field of PG neurons. How this impacts on the biophysical properties of the ganglion cells is not known, but no systematic differences due to animal age were measured.

**A**

**petrosal ganglion (PG) neurons**

- **a hump neuron**
- **b non-hump neuron**

**B**

**geniculate ganglion (GG) neuron**

**FIG. 2.** Recordings from Fluorogold labeled neurons dissociated from the petrosal (A) and geniculate (B) ganglia. **Aa:** an example of a hump neuron with a deflection or hump on the descending limb of the action potential. **Ab:** a nonhump neuron with a narrow action potential. (i): neuronal responses to a depolarizing current injection applied through the patch electrode. (ii): membrane responses to a series of depolarizing and hyperpolarizing current pulses. (iii): membrane responses to a −500 pA of hyperpolarizing current pulse. Regardless of having humped or narrow spikes, all neurons had a pronounced inward rectifier response to current injection of −500 pA. **DT:** time between 90% and 10% of the action potential amplitude; **HD:** duration measured at half height of action potential.

Labeled PG neurons. Resting membrane potentials ranged from −40 to −78 mV, with a mean of $-59 \pm 8$ mV. Input resistance ranged from 300 to 951 MΩ, with a mean of $542 \pm 147$ MΩ. Membrane time constants, measured after a −50 pA current was injected, averaged $34.5 \pm 10.2$ ms. All the neurons recorded exhibited varying degrees of membrane rectification evident from their "sagging" voltage responses to intracellular injections of hyperpolarizing current pulses (Fig. 2).

Mean threshold for generation of action potential was 122 pA, which was measured in each neuron by increasing 10 pA steps of depolarizing current. Action potential amplitudes were between 72 and 128 mV with mean of $101 \pm 13$ mV. As previously described in other ganglion neurons (Gallego and Eyzaguirre 1978; Jaffe and Sampson 1976), the action potentials recorded from many neurons had a deflection or "hump" on the repolarization phase of the spike evoked by intracellular depolarizing current injection (Fig. 2Aa). Fifty-one of 130 labeled neurons were classified as hump neurons. A hump neuron generally had a longer spike half-width when compared with a sharp or nonhump neuron. Thus, duration measured at half-amplitude of hump neurons (half duration: **HD**) was significantly greater than that of nonhump neurons (Table 1). Additionally, the repolarizing time of hump neurons from 90 to...
10% of spike amplitude (decrease time: DT) was longer than that of nonhump neurons (Table 1). However, there was no significant difference in the other basic membrane properties between the neurons with different types of action potential. In general, the numbers of spikes increased with increasing magnitude depolarizing current pulses. Less than half (53/130) of the cells fired only a single action potential at the onset of a depolarizing step, even when the depolarizing current was adjusted to about twice threshold. The remaining 77 neurons responded with more than two spikes in response to an above threshold depolarizing current pulse. Mean threshold current for neurons with multiple spikes (70 pA) was significantly lower than for singly discharging neurons (169 pA). Multiple spike discharge was elicited from neurons that exhibited either hump (23/77) or nonhump spikes (54/77). Thus, 45% of the hump neurons and 68% of the nonhump neurons responded with multiple spikes, but the difference was not significant.

Comparison of GG and PG neuron biophysical properties

Membrane time constant (Fig. 3B) and input resistance (Fig. 3C) of labeled PG and GG neurons were measured from the response to a −50 pA current injection. The characteristics of the action potentials were measured in the first spike at the threshold depolarizing current injection. Values measured were action potential threshold (Fig. 3D), action potential decay time (Fig. 3E), action potential half-width (Fig. 3F), and action potential area (Fig. 3G). The mean time constant of GG neurons was significantly less than those of PG neurons. However, there was no difference in input resistance measured at the steady phase of membrane potential between the two groups. The mean spike threshold of GG neurons was significantly (P < 0.05) lower than that of PG neurons.

The most remarkable difference between the biophysical properties of PG and GG neurons was the shape of the action potential. As described above, no hump neurons were detected.

### Table 1. Intrinsic membrane properties of hump and nonhump neurons in Fluorogold labeled petrosal ganglion neurons

<table>
<thead>
<tr>
<th></th>
<th>Cell Diameter (μm)</th>
<th>RMP (mV)</th>
<th>Time Constant (ms)</th>
<th>Input Resistance (MΩ)</th>
<th>Spike Threshold (pA)</th>
<th>Spike Amplitude (mV)</th>
<th>Half-Width (ms)</th>
<th>Decay Time (ms)</th>
<th>Spike Area (mV ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hump neurons</td>
<td>(n = 51)</td>
<td>32.8 ± 4.2</td>
<td>−56 ± 7</td>
<td>37.4 ± 10.5</td>
<td>520 ± 120</td>
<td>110 ± 71</td>
<td>102.3 ± 13.4</td>
<td>5.1 ± 1.6</td>
<td>5.9 ± 2.1</td>
</tr>
<tr>
<td>Nonhump neurons</td>
<td>(n = 79)</td>
<td>31.8 ± 4.6</td>
<td>−61 ± 8</td>
<td>32.7 ± 9.6</td>
<td>556 ± 161</td>
<td>100 ± 92</td>
<td>100.7 ± 12</td>
<td>2.5 ± 1*</td>
<td>2.5 ± 1*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. *Significantly different at P < 0.01.
in GG labeled neurons. All GG neurons had narrow spikes and the decay time was much shorter (Fig. 3E). The action potential amplitude of GG neurons was significantly \( (P < 0.05) \) smaller than that of PG neurons (Fig. 3G).

**Comparison between biophysical properties of labeled and nonlabeled neurons**

Differences in the biophysical properties of labeled and nonlabeled neurons were examined by recording from an additional 45 PG and 26 GG nonlabeled neurons. Forty-two percent of the nonlabeled PG neurons were classified as hump neurons, and this ratio was similar to that of the PG labeled neurons. In addition, no significant differences were found in any biophysical properties between PG labeled and nonlabeled neurons, indicating that the label did not alter the biophysical properties of the neurons. In nonlabeled GG neurons, no differences were detected in passive membrane properties. However, 3 of 26 GG nonlabeled neurons had a hump in their action potential. As a result, the decay time and action potential area of nonlabeled neurons were significantly larger than labeled GG neurons. Similarly, the mean half-width of nonlabeled neurons was larger than the labeled neurons, but the difference was not significant.

**Effects of putative neurotransmitters**

**ACETYLCHOLINE (ACH).** Sensitivity to ACh was investigated in 48 PG and 32 GG neurons. The sensitivity to ACh was examined during \(-100 \) pA, 100 ms, hyperpolarizing current injection at 1 Hz to determine changes in membrane potential and input resistance. Neurons were considered responsive if they depolarized with a decrease in input resistance (current clamp) or produced an inward current (voltage clamp at \(-60 \) mV holding potential) during application of ACh concentrations up to 0.5 mM. Thus, if neurons did not respond to 0.5 mM ACh, they were considered to be insensitive. Concentrations of ACh were selected between 1 \( \mu \)M and 1 mM, because 0.3 mM ACh is reported to evoke almost maximal current measured under voltage clamp in PG neurons (Zhong and Nurse 1997). The duration of the ACh application was 5 s.

ACh-induced depolarization was accompanied by a decrease in input resistance in 50% of the PG neurons (Fig. 4, A and C). The response was dose-dependent and returned to control levels within 30 s after termination of the application (Fig. 4, A and E). However, the level of sensitivity to ACh, i.e., membrane potential and input resistance changes, was different from cell to cell. An ACh dose-response relationship was measured under voltage-clamp condition. The mean peak current at each ACh concentration was normalized to that elicited by 100 \( \mu \)M ACh (Fig. 3, D and E). The ACh elicited response saturated at \(-0.5 \) mM. The interval between each application was at least 3 min to avoid receptor desensitization and/or ACh-induced channel block. The complete dose-relation curve was fitted by the Hill equation

\[
I/I_{\text{max}} = 1/[1 + (\text{EC}_{50}/[\text{ACh}]^n)]
\]

where \( I \) is the measured peak current, \( I_{\text{max}} \) is the maximal response, \( n \) is the Hill coefficient, and \( \text{EC}_{50} \) is the concentration of ACh required for half-maximal activation. The \( \text{EC}_{50} \) for receptor activation was approximately 40.5 \( \mu \)M and the Hill coefficient was 1.54.

In contrast to PG neurons, the majority of GG neurons were insensitive to 0.5 mM ACh application (Fig. 4C). Only 3 of 32 GG neurons indicated some slight response as shown in Fig. 4B, and this particular neuron was the most ACh sensitive of all tested GG neurons.

**SEROTONIN (5HT).** Sensitivity to 5HT was examined in 30 PG and 21 GG neurons. 5HT rapidly depolarized over 60% of the PG neurons and initiated action potentials (Fig. 5A). The amplitude of the response to a second application of 5HT decreased when compared with the first application, especially with high concentrations of 5HT. Thus, a 5-min interval between the first and second applications was required to obtain consistent responses. The membrane potential and input resistance changes in response to 5HT were different in each cell. However, 11 of 30 PG neurons were insensitive to application of relatively high concentrations of 5HT (0.5–1 mM). Figure 5D shows the 5HT dose-response relation recorded under voltage clamp. The mean peak current at each 5HT concentration was normalized to that elicited by 100 \( \mu \)M 5HT. This concentration of 5HT elicited a saturated response (Fig. 5, C
and $D$). The complete dose-relation curve was also described by the Hill equation. The $EC_{50}$ for receptor activation was approximately 4.6 $\mu$M and the Hill coefficient was 0.93, suggesting one 5HT agonist binding site per receptor. The 5HT-induced currents were activated more rapidly at higher concentrations (Fig. 5C). A typical example of the activation and short-term desensitization phases of an inward current is indicated in 100 and 1000 $\mu$M 5HT.

In contrast to PG neurons, the majority of the GG neurons (20 of 21) were insensitive to 1 mM 5HT application (Fig. 5B). However, one neuron responded to 5HT with a rapid depolarization and a high-frequency of spikes (not shown).

SUBSTANCE P (SP). The effect of SP was first examined at concentration of 100 nM–1 $\mu$M in PG neurons. A 10-s application of 1 $\mu$M SP did not produce an observable response. However, a longer application of the same concentration of SP (30–40 s) was an effective stimulus (Fig. 6A), producing either a membrane depolarization with an input resistance decrease (Fig. 6Aa and c), or a membrane hyperpolarization with an increase in input resistance (Fig. 6Ab). More than 80% of both PG and GG neurons were sensitive to SP.

The effect of a single concentration of SP (0.5 mM) was investigated in 52 PG and 40 GG neurons. Figure 6B shows a typical response to SP on GG neurons. The hyperpolarizing and depolarizing response patterns that were observed in PG neurons were also seen in GG neurons. Most PG neurons have a depolarizing response as shown in Fig. 6, Aa and c, whereas a hyperpolarizing response accompanied by an increase in input resistance was most frequently observed in GG neurons (Fig. 6Bb). Depolarizing and hyperpolarizing responses to SP have been reported in other neurons (Dray and Pinnock 1982; King et al. 1993; Plata-Salaman et al. 1989).

GAMMA AMINOBUTYRIC ACID (GABA). The sensitivity of PG and GG neurons to GABA as well as the GABA A and GABA B receptor agonists muscimol and baclofen was investigated to determine differences in distribution of each GABA receptor subtype.

Sensitivity to GABA was examined in 17 PG and 11 GG neurons. Six of the 17 PG neurons were hyperpolarized with a decrease in input resistance (Fig. 7Ab). In eight PG neurons, however, GABA produced a small depolarization associated with a decrease in input resistance (Fig. 7Aa). In contrast, most GG neurons were hyperpolarized with a marked decrease in input resistance (Fig. 7B). In summary, 82% of PG neurons and all GG neurons were sensitive to GABA accompanied with a decrease in input resistance. The ratio of response patterns is summarized in Fig. 7C.

FIG. 5. Responses to 5HT application. $A$: an example of a depolarizing response to 100 $\mu$M 5HT in a petrosal ganglion neuron. $B$: ratio of neurons classified by their responses to 0.5–1 mM 5HT. $C$: concentration-dependent 5HT induced inward current responses recorded from a petrosal ganglion neuron. Holding potential was −60 mV. $D$: concentration-response relationships for 5HT response. Peak amplitudes of currents induced at various concentrations were normalized to current evoked by 100 $\mu$M 5HT (*). Each point is the average of 4–9 cells.

FIG. 6. Responses to substance P application in petrosal ($A$) and geniculate ($B$) ganglion neurons. $Aa$: a depolarizing response accompanied by a decrease in input resistance. $Ab$: a hyperpolarizing response with an increase in input resistance. $Ac$: a depolarizing responses accompanied by a decrease in input resistance. $B$: both depolarizing ($Ba$) and hyperpolarizing responses to application of 0.5 mM SP were observed in geniculate ganglion neurons.


**Discussion**

Compared with the dorsal root, nodose, and trigeminal ganglia, the biophysical and neuropharmacological properties of the PG and GG ganglia have received little attention. Moreover, when these ganglia have been studied, investigators have not always recorded from a selected population of ganglion cells even though there is evidence from immunohistochemical and anatomical studies that these ganglia are heterogeneous and innervate very different populations of receptors (Hall et al. 1997). The present study is thus the first in which an attempt has been made to record from a defined population of ganglion cells similar to recent experiments on tooth pulp neurons in the trigeminal ganglion (Chiego et al. 1997; Cook et al. 1998).

The results indicate that there are significant differences between the biophysical and neuropharmacological properties of neurons in the PG and GG ganglia. For example, neurons in the PG have both sharp and humped action potentials and PG neurons respond to ACh, 5HT, SP, and GABA, whereas GG neurons have sharp action potentials and only respond to SP and GABA. In addition, the properties of GG neurons are more homogeneous than those of the PG because all the neurons have sharp spikes and when responses to neurotransmitters occurred, either all or most of the neurons responded.

In other sensory ganglia, a relationship has been established between axon diameter, action potential duration, and the type of receptor innervated (Djouhri et al. 1998; Harper and Lawson 1985; Koerber et al. 1988; Rose et al. 1986; Villière and McLachlan 1996). Even when a single receptor type is innervated, the ganglion cells can have different membrane properties (Belmonte and Gallego 1983). Afferent fibers in the rat and hamster glossopharyngeal and chorda tympani nerves range in diameter from 0.2 to 4.1 μm and innervate a number of different receptors (Farbman and Hellekant 1978; Jang and Davis 1987; Miller et al. 1978). By labeling a selective receptive field, the types of receptor innervated are reduced. Thus, a label injected into the posterior tongue will label PG cells that innervate taste and somatosensory receptors and a label injected into the anterior tongue will label GG cells that innervate taste buds (Krimm and Hill 1998). The more heterogeneous properties of the GG neurons probably reflects its role in innervating taste buds (Krimm and Hill 1998). The more heterogeneous response characteristics of the GG neurons reflects its main role in innervating taste buds on the anterior tongue.

However, even though GG neurons innervating the anterior tongue have homogeneous biophysical and neuropharmacological properties, they differ in other characteristics. For example, fibers that innervate taste buds on the anterior tongue are of different diameters, the receptive field properties of chorda tympani fibers differ (Nagai et al. 1988), and the response properties of chorda tympani fibers to taste stimuli applied to the tongue are not homogeneous (Frank et al. 1988). Chorda tympani fibers with small receptive fields respond best to NaCl, whereas fibers with large receptive fields respond to a larger number of salts (Nagai et al. 1988). Additional experiments in which intracellular recordings are made from the whole ganglion while stimulating the afferent input are needed to determine if GG neurons with fibers of different diameters have different membrane properties.

While little is known about the biophysical and neuropharmacological properties of the GG, there is information on the...
PG. In early pioneering work, Gallego and his co-workers performed the first intracellular analysis of PG ganglion neurons (Belmonte and Gallego 1983; Gallego 1983). These investigators were particularly interested in neurons innervating chemoreceptors and baroreceptors involved in the cardiovascular system. Intracellular recordings with sharp electrodes were made using in vitro preparation of the whole ganglion allowing electrical stimulation of the carotid sinus nerve to isolate neurons innervating baroreceptors or carotid body chemoreceptors. As in the present study, neurons with both sharp and humped action potentials were described. All the neurons innervating the carotid body had humped action potentials and even with prolonged depolarization only produced a single spike. In contrast, the neurons innervating baroreceptors were of two types based on the conduction velocity of their axons and these neurons were capable of firing multiple action potentials when depolarized. It is possible, therefore, that the ganglion cells innervating the posterior tongue with humped and sharp action potentials innervate different populations of receptors on the posterior tongue. In other ganglia, the humped neurons have small diameter, slow conducting axons, and innervate nociceptors (Koerber et al. 1988), so it is possible that the PG neurons with humped spikes also innervate posterior tongue nociceptors, and the neurons with sharp action potentials innervate taste receptors. This possibility is supported by the results from the labeled anterior tongue neurons in the GG which supply taste receptors and all have sharp spikes.

More recently, Nurse and co-workers have examined the sensitivity of cultured PG cells to application of ACh and 5HT (Zhong et al. 1999; Zong and Nurse 1997). Application of ACh resulted in a rapid depolarization accompanied by an increase in membrane conductance in 68% of the neurons which is similar to the results obtained in the present study. In addition, the EC$_{50}$ for receptor activation in the present study was 40.5 µM and the Hill coefficient was 1.54 µM, which is similar to those reported by Zhong and Nurse (1997), who suggested that more than two ACh agonist binding sites exist per receptor from the value of the Hill coefficient. 5HT depolarized 43% of the PG cells with an increase in membrane conductance, but in a small percentage of neurons (6%) the depolarization was accompanied with a decrease in membrane conductance. The EC$_{50}$ for 5HT receptor activation in the present study was 4.6 µM and the Hill coefficient 0.93 µM. Zhong et al. (1999) report similar results and suggested that more than two binding sites are required for maximal activation of the receptor.

A number of investigators using immunohistochemical methods have demonstrated SP-like immunoreactivity in PG neurons and there is one report of intense SP immunoreactive neurons in the GG (Ichiyama et al. 1997). However, there are no reports of electrophysiological studies with SP. Guinea-pig trigeminal ganglion neurons and bullfrog dorsal root ganglion neurons are depolarized by exogenous application of SP (Dray and Pinnock 1982; Ishimatsu 1994; Spiegelman and Puil 1990). However, in a recent study in ferrets, SP in nanomolar concentrations were found to hyperpolarize the membrane potential of nodose ganglion neurons by activation of an outward calcium-dependent potassium current (Jafri and Weinreich 1998).

To our knowledge, this is the first study of the sensitivity of PG and GG to GABA. Electrophysiological studies have been performed to study the sensitivity of chick dorsal root ganglion cells (Choi and Fishbach 1981; Choi et al. 1981), rat sympathetic ganglion cells (Adams and Brown 1998; Bowery and Brown 1974), and rabbit nodose ganglion cells (Higashi et al. 1982) to GABA. In all these ganglia, GABA depolarizes the cell membrane accompanied by an increase in membrane conductance, whereas in the present study GABA hyperpolarizes...
the majority of the ganglion cells in both the PG and GG with an increase in membrane conductance. However, the earlier studies were on different ganglia and used both extracellular and intracellular recording techniques and were accomplished before the use of whole-cell recording. Not all the neurons were hyperpolarized by GABA and neurons with lower resting membrane potentials were in fact hyperpolarized by GABA (Adams and Brown 1998).

**Functional significance**

SP has been shown to be present in centrally projecting fibers of the glossoptatorygaleal nerve (Helke and Hill 1988) and in the terminal fields of the glossoptatorygaleal nerve in the nucleus of the solitary tract (Cuello and Kanazawa 1978; Hokfelt et al. 1975). Moreover, King et al. (1993) have shown that SP excites neurons of the rostral gustatory portion of the nucleus of the solitary tract (rNST), suggesting that SP may be involved with afferent transmission of taste information at the first central synapse in the taste pathway. Similarly, GABA has been shown to have a major influence on neurons processing taste information in the rNST (Grabauskas and Bradley 1996, 1998b; Wang and Bradley 1993, 1995) and this influence may be partially accounted for via presynaptic inhibition at the primary afferent excitatory synapse.

At present, the neurochemical characteristics of afferent taste fibers is not known and mechanisms of synaptic transmission between the taste bud and the primary afferent fiber are also unknown. Investigators of peripheral cutaneous afferent fibers have determined their chemosensitive properties using isolated dorsal root ganglion neurons (Dray 1996). It is possible, therefore, that the receptors on the distal afferent fibers innervating taste buds are similar to those characterized in the present study on isolated PG and GG ganglion neurons. Thus, neural transmission between glossoptatorygaleal nerves and taste buds could be mediated by ACh, SP, 5HT, and GABA, whereas transmission in taste buds innervated by the facial nerve could be mediated by 5HT and GABA but not by ACh and SP. Of course, other untested neurotransmitters could also be involved. The approach of using isolated ganglion cells to examine neurotransmission in taste buds provides a practical approach to what so far has proved to be a difficult technical problem.

This work was supported by National Institute on Deafness and Other Communication Disorders Grant DC-00288 to R. M. Bradley. T. Koga was the recipient of a subsidy from the Nakayama Foundation for Human Science.

Present address of T. Koga: Dept. of Restorative Science, Kawasaki University of Medical Welfare, Kurashiki 701-0193, Japan.

**REFERENCES**


Hokfelt et al. 1975). Moreover, King et al. (1993) have shown that SP excites neurons of the rostral gustatory portion of the nucleus of the solitary tract (rNST), suggesting that SP may be involved with afferent transmission of taste information at the first central synapse in the taste pathway. Similarly, GABA has been shown to have a major influence on neurons processing taste information in the rNST (Grabauskas and Bradley 1996, 1998b; Wang and Bradley 1993, 1995) and this influence may be partially accounted for via presynaptic inhibition at the primary afferent excitatory synapse.

At present, the neurochemical characteristics of afferent taste fibers is not known and mechanisms of synaptic transmission between the taste bud and the primary afferent fiber are also unknown. Investigators of peripheral cutaneous afferent fibers have determined their chemosensitive properties using isolated dorsal root ganglion neurons (Dray 1996). It is possible, therefore, that the receptors on the distal afferent fibers innervating taste buds are similar to those characterized in the present study on isolated PG and GG ganglion neurons. Thus, neural transmission between glossoptatorygaleal nerves and taste buds could be mediated by ACh, SP, 5HT, and GABA, whereas transmission in taste buds innervated by the facial nerve could be mediated by 5HT and GABA but not by ACh and SP. Of course, other untested neurotransmitters could also be involved. The approach of using isolated ganglion cells to examine neurotransmission in taste buds provides a practical approach to what so far has proved to be a difficult technical problem.

This work was supported by National Institute on Deafness and Other Communication Disorders Grant DC-00288 to R. M. Bradley. T. Koga was the recipient of a subsidy from the Nakayama Foundation for Human Science.

Present address of T. Koga: Dept. of Restorative Science, Kawasaki University of Medical Welfare, Kurashiki 701-0193, Japan.
PROPERTIES OF SENSORY NEURONS INNERVATING THE TONGUE


NEJAD MS. The neural activities of the greater superficial petrosal nerve of the rat in response to chemical stimulation of the palate. Chem Senses 11: 283–293, 1986.


