Differential Gurmarin Suppression of Sweet Taste Responses in Rat Solitary Nucleus Neurons

Christian H. Lemon,1 Toshiaki Imoto,2 and David V. Smith1
1Department of Anatomy and Neurobiology, University of Tennessee College of Medicine, Memphis, Tennessee 38163; and 2Division of Integrative Physiology, Department of Functional, Morphological, and Regulation Science, Faculty of Medicine, Tottori University, Yonago 683-0826, Japan

Submitted 6 March 2003; accepted in final form 9 April 2003

Lemon, Christian H., Toshiaki Imoto, and David V. Smith. Differential gurmarin suppression of sweet taste responses in rat solitary nucleus neurons. J Neurophysiol 90: 911–923, 2003; 10.1152/jn.00215.2003. We examined the effect of the sweet transduction blocker gurmarin on taste responses recorded from neurons in the rat solitary nucleus (NST) to determine how gurmarin sensitivity is distributed across neuronal type. Initially, responses evoked by washing the anterior tongue and palate with 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.01 M quinine-HCl were recorded from 35 neurons. For some cells, responses to a sucrose concentration series (0.01–1.0 M) or an array of sweet-tasting compounds were also measured. Gurmarin (10 μg/ml, 2–4 ml) was then applied to the tongue and palate. Stimuli were reapplied after 10–15 min. Neurons were segregated into groups based on similarities among their initial response profiles using hierarchical cluster analysis (HCA). Results indicated that sucrose responses recorded from neurons representative of each HCA-defined class were suppressed by gurmarin. However, a disproportionate percentage of cells in each group displayed sucrose responses that were substantially attenuated after gurmarin treatment. Postgurmarin sucrose responses recorded from neurons that composed 57% of class S, 40% of class N, and 33% of class H were suppressed by ≥50% relative to control. On average, attenuation was statistically significant only in class S and N neurons. Although the magnitude of gurmarin-induced response suppression did not differ across sucrose concentration, responses to different sweet-tasting compounds were differentially affected. Responses to NaCl, HCl, or quinine were not suppressed by gurmarin. Results suggest that information from gurmarin-sensitive and -insensitive receptor processes converges onto single NST neurons.

INTRODUCTION

Substances that selectively modify specific physiological functions have proven useful for exploring the neural representation of sensory information. For taste, lingual application of gurmarin, a protein isolated from the plant Gymnema sylvestre (Imoto et al. 1991), significantly attenuates integrated chorda tympani (CT) nerve responses to sweet-tasting substances in rats (Imoto et al. 1991; Miyasaka and Imoto 1995) and C57BL mice (Ninomiya and Imoto 1995; Ninomiya et al. 1997, 1998). Additionally, palatal gurmarin treatment suppresses integrated responses to sugars, sodium saccharin, and sweet-tasting amino acids recorded from the greater superficial petrosal (GSP) nerve in rats (Harada and Kasahara 2000). Gurmarin does not affect responses to nonsweet stimuli representative of other basic taste quality classes (e.g., NaCl, HCl and quinine) in the CT (Imoto et al. 1991; Miyasaka and Imoto 1995) and GSP (Harada and Kasahara 2000) nerves in rats and the CT nerve in mice (Ninomiya and Imoto 1995; Ninomiya et al. 1997, 1998).

Lingual gurmarin treatment does not affect integrated responses to sweeteners recorded from the glossopharyngeal nerve of C57BL mice (Ninomiya et al. 1997). Data obtained from this species suggest the existence of two different types of receptors for sweets, gurmarin-sensitive and -insensitive, that are differentially distributed across the tongue. Moreover, a recent extension of these findings shows that gurmarin application inhibits responses to sucrose in only a subset of sugar-best CT fibers in this strain of mouse (Ninomiya et al. 1999). Those fibers that are affected exhibit absolute or near absolute suppression, with responses to 0.5 M sucrose suppressed to ~10% of control on average. This differential effect is somewhat analogous to the influence of amiloride on the neural processing of salt information as lingual application of amiloride suppresses responses to salts only in NaCl-best CT fibers (Hettinger and Frank 1990; Ninomiya and Funakoshi 1988) and NaCl-best (Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996; St. John and Smith 2000) and sucrose-best (Smith et al. 1996; St. John and Smith 2000) neurons in the nucleus of the solitary tract (NST). Therefore it is possible that input arising from gurmarin-sensitive and -insensitive sweet transduction mechanisms is segregated to particular classes of taste-responsive neurons in the brain stem.

To test the hypothesis that gurmarin sensitivity is differentially distributed across gustatory neuron types in the rat NST, we recorded trains of actions potentials evoked by various taste stimuli, including sucrose and other sweeteners, from single NST neurons prior to and after lingual/palatal application of gurmarin. These experiments attempt to relate specific transduction mechanisms to the organization of gustatory neural circuitry within the CNS.

A portion of these results was presented at the 2002 meeting of the Association for Chemoreception Sciences, Sarasota, FL.

Address for reprint requests: C. H. Lemon, Dept. of Anatomy and Neurobiology, University of Tennessee College of Medicine, 855 Monroe Ave., Suite 515, Memphis, TN 38163. (E-mail: chris@utmem.edu).

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.
METHO DS

Animals and surgery

Thirty-four adult male Sprague Dawley rats, weighing 210–550 g, were used as subjects. Rats were housed individually in a vivarium, which maintained a 12-h light/dark schedule and ambient temperature of ~23°C. Food and water were available ad libitum. Subjects were deeply anesthetized with urethan (1.5 g/kg ip) and prepared for electrophysiological recording. Each rat was tracheotomized and secured in a nontraumatic head holder that deflected its snout ~27° downward: this configuration served to minimize brain stem movements associated with breathing. The occipital bone was removed and parts of the cerebellum were gently aspirated to expose the brain stem and allow access to the NST. Body temperature was maintained at ~37°C by a heating pad.

Single-unit electrophysiology

Etched tungsten microelectrodes, insulated except for the tip (impedance = 0.5–8 MΩ at 1 kHz, FHC, Bowdoinham, ME), were used to record extracellular action potentials from single NST neurons. For each preparation, the area of the brain stem where the rostral pole of the solitary tract resided was visually located using vascular landmarks present on the dorsal surface of the exposed tissue. A hydraulic micromanipulator was then used to slowly advance the microelectrode through the brain stem. The portion of the NST that contained neurons responsive to lingual stimulation was initially identified by a change in neural activity associated with the passage of anodal current (10 μA/500 ms) across the anterior tongue; cells were then verified as taste-driven by application of various gustatory stimuli (see following text). The gustatory-responsive portion of the NST was located ~1 mm ventral to the brain stem surface.

Electrophysiological activity was band-pass filtered (bandwidth = 0.3–10 kHz), differentially amplified (Grass PS11 with high-impedance probe) and subsequently routed to various monitors and analytic devices. Spikes that arose from single neurons were identified based on waveform consistency, which was continuously observed throughout each recording session using a storage oscilloscope and, following analog to digital conversion (sampling rate = 25 kHz), a template-matching algorithm (Power 1401 RISC acquisition interface coupled with Spike 2 software, CED, Cambridge, UK). Well-isolated neurons with robust responses to 0.5 M sucrose were used for experimentation. Trains of action potentials that arose during recording sessions were pulse-code modulated and stored, along with voice and trial marker cues, on VHS tape. Digital records of aggregate electrophysiological activity, including template-matched spikes, were downloaded to storage media for off-line quantitative analysis.

Taste stimuli

Generally, neurons were tested with two of three groups of taste stimuli. Tastants within each group were presented individually to, and subsequently rinsed from, the oral cavity of each preparation under normal (i.e., control) conditions and after oral application of gurmarin. Stimulus trials of interest were replicated as many times as possible. Every neuron was tested with a set of stimuli that consisted of representatives of the four basic taste qualities (herein referred to as the basic stimulus set), which were presented in random order. Some cells were then subjected to a half-logarithmic step ascending concentration series of sucrose, whereas others were tested using a randomized array of various other sweet-tasting compounds (see Table 1).

Tastants were made from reagent grade stock dissolved in deionized water. Solutions were delivered at room temperature to the anterior tongue and palate via a gravity flow system at a rate of ~2.5 ml/s. A three-way solenoid fluid valve, which was controlled by the acquisition system, regulated solution delivery. A curved, polyethylene tube that extended from the output port of this valve was directed toward the palate of each subject. Visual inspection revealed that this configuration allowed solutions to effectively bath both the palate and anterior tongue as solutions were deflected downward on encountering the palate. Moreover, a test using methylene blue dye was performed on one preparation to verify that this method of taste stimulus delivery adequately bathed both the anterior tongue and palate. Using the described flow system, dye was delivered to the oral cavity for 10 s. The distribution of stain was then assessed with a dissecting microscope, which revealed that the entire anterior tongue and soft palate were dyed. A subsequent test verified that the naso-nasal cinnus ducts (NIDs) were also stained.

During data acquisition, taste stimuli were presented to each subject using a specific protocol. The tongue and palate were first rinsed with deionized water for 10 s followed immediately by a taste stimulus for 10 s. The tongue and palate were then rinsed with ~50 ml of deionized water and >2 min was allowed to elapse between trials. The stimulus delivery system was thoroughly rinsed with deionized water between presentation trials.

Experimental protocol and gurmarin application

Once applied, gurmarin is a difficult substance to remove from gustatory epithelia. Integrated CT responses to sucrose required >4 h for complete recovery after gurmarin treatment despite repetitive distilled water rinses of the tongue (Miyasaka and Imoto 1995). Although methods do exist by which gurmarin removal may be chemically facilitated (Ninomiya et al. 1998), only slight recovery of mouse CT fiber responses to sucrose was observed at 10 min post-gurmarin treatment (Ninomiya et al. 1999). Moreover, repeated anti-gurmarin reactions, which required up to 100 min, were necessary for complete recovery of CT sucrose responses to baseline levels (Miyasaka and Imoto 1995). Therefore we chose a simple pre-/postgurmarin design, assuming that responses to nontaste stimuli would serve as controls for the viability of the neurons from which we recorded, as responses to NaCl, HCl, and quinine in the CT (Imoto et al. 1991; Miyasaka and Imoto 1995) and GSP (Harada and Kasahara 2000) nerves in rats and the CT nerve in mice (Ninomiya and Imoto 1995; Ninomiya et al. 1997, 1998) are not altered by gurmarin treatment.

Once single-unit responses evoked during control stimulus presentations were recorded, 10 μg/ml gurmarin (dissolved in deionized water) was applied to the tongue and palate and responses were re-recorded. After the gurmarin treatment, deionized water rinses of the tongue were continued until responses were restored to control levels. This procedure was repeated until complete recovery of baseline level responses were observed. When complete recovery was achieved, deionized water rinses were stopped and the rats were then prepared for single-unit electrophysiological recording.

TABLE 1. Taste stimuli and abbreviations

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Concentration, M</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimuli representative of basic taste qualities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose (sweet)</td>
<td>0.5</td>
<td>suc</td>
</tr>
<tr>
<td>Sodium chloride (salty)</td>
<td>0.1</td>
<td>NaCl</td>
</tr>
<tr>
<td>Hydrochloric acid (sour)</td>
<td>0.01</td>
<td>HCl</td>
</tr>
<tr>
<td>Quinine hydrochloride (bitter)</td>
<td>0.01</td>
<td>quinine</td>
</tr>
<tr>
<td>Concentration series</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Other sweet compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>0.5</td>
<td>fru</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.3</td>
<td>gal</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.5</td>
<td>glu</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.3</td>
<td>gly</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.5</td>
<td>mal</td>
</tr>
<tr>
<td>Sodium saccharin</td>
<td>0.01</td>
<td>sac</td>
</tr>
<tr>
<td>d-asparagine</td>
<td>0.1</td>
<td>asp</td>
</tr>
<tr>
<td>L-histidine</td>
<td>0.1</td>
<td>his</td>
</tr>
</tbody>
</table>
water; \(\sim 2.4 \, \mu M\) was applied to both the tongue and palate of each subject using a blunt-tipped syringe (2–4 ml total volume). For each preparation, the application of gurmarin to the tongue and palate was visually verified. Moreover, a test using methylene blue dye was performed on one preparation to verify that our gurmarin application procedure did indeed adequately bathe both the anterior tongue and palate, including the NIDs.

Treatment of the tongue with 10 \(\mu g/ml\) gurmarin has been shown to produce significant and near maximal attenuation of integrated CT nerve responses to 0.5 M sucrose in the rat (Miyasaka and Imoto 1995). When applied to the rat palate, 10 \(\mu g/ml\) gurmarin yielded significant and substantial suppression of integrated superficial petrosal nerve responses to sucrose and other compounds described as sweet tasting by humans (Harada and Kasahara 2000). However, the effect of gurmarin is not instantaneous after application, with 4.8 \(\mu M\) gurmarin requiring \(\sim 5\) min to produce maximal suppression of sucrose responses recorded from single mouse CT fibers (Ninomiya et al. 1999). Therefore we allowed 10–15 min to elapse after gurmarin treatment before proceeding with experimentation. After this time, each component of the stimulus set used under control conditions was presented as previously described and evoked responses recorded. After completion of this phase, an additional experiment was conducted on some neurons using a stronger concentration of gurmarin to determine if effects, or lack thereof, observed at the standard concentration were indeed limits. For these cells, we doubled the concentration of gurmarin (20 \(\mu g/ml; \sim 4.8 \, \mu M\)), applied it as previously described and retested the stimulus set 10–15 min posttreatment.

**Data analysis**

The basic metric used to quantify gustatory responses in NST neurons was net response magnitude, expressed in spikes/s. This measure was calculated as the average number of spikes that occurred each s during the first 5 s of stimulus presentation minus the average firing rate per s during the 5-s water rinse period that immediately preceded this epoch (i.e., spontaneous discharge). These data served as input to all subsequent statistical analyses. For each neuron, responses evoked by chemical stimuli were considered significant if the net response exceeded the mean \(\pm 2.54\) SDs of the spontaneous discharge rate.

To describe the breadth of tuning of each neuron, a measure of response profile entropy (Shannon and Weaver 1949; Smith and Travers 1979) was calculated using net responses to each component of the basic stimulus set. Entropy is defined as

\[
H = -K \left( \sum_{i=1}^{n} P_i \log P_i \right)
\]

where \(P_i\) represents the response to the \(i\)th stimulus expressed as a proportion of the total response to \(n\) stimuli and \(K\) is a scaling constant. For four stimuli, \(K = 1.661\), which results in \(H\) ranging from a minimum of 0 (i.e., neuron responds to only 1 stimulus) to a maximum of 1 (i.e., neuron responds equally well to all stimuli). Due to the dominance of excitatory responses in our data set (134 of 140 responses used for entropy calculations were >0) and the observance that no inhibitory responses were found to be significantly less than average spontaneous discharge, \(P_i \log P_i\) was set to zero during the calculation of \(H\) if the response evoked by the \(i\)th stimulus was \(\leq 0\).

Cells were categorized into types based on the stimulus within the basic set that evoked the maximal net response (i.e., their best stimulus) prior to gurmarin treatment. Additionally, hierarchical cluster analysis (HCA; conducted using Statistica, StatSoft, Tulsa, OK) was performed to quantitatively and objectively identify sets of neurons with pregurmarin tuning profiles (i.e., net responses to only basic set stimuli) that were most similar. Input to HCA consisted of a distance matrix representing pairwise neuronal tuning profile similarity/dissimilarity, where \(1 - \) Pearson’s product-moment correlation \((r)\) served as the distance metric. The unweighted pair-group average amalgamation schedule was used.

The effect of gurmarin on taste responses was statistically evaluated by using appropriate ANOVAs. Significant interactions were sometimes explored using planned interaction comparisons as our general hypothesis dictated a priori predictions with regard to the outcome of specific experiments (i.e., postgurmarin stimulus responses were compared with only their respective controls). For each ANOVA, degrees of freedom and \(P\) values for within-subject tests were corrected using the Greenhouse-Geisser adjustment to protect against violations of sphericity. Although these corrections were made prior to establishing \(P\) levels, only the uncorrected degrees of freedom and \(P\) values are reported. Post hoc comparisons among within-subject level means were accomplished through the use of paired samples t-test in which each observed score was evaluated using a Dunn critical value. This sort of multiple comparison procedure (MCP) is the only sort of post hoc test for repeated level means that has adequate control of \(\alpha\) for all pairwise comparisons (Toothaker 1991).

**RESULTS**

**General response characteristics**

Trains of action potentials were recorded from 35 NST neurons with robust responses to 0.5 M sucrose [mean response to 0.5 M sucrose across all neurons \(= 10.5 \pm 1.0\) (SE) net spikes/s]. Our conservative statistical criterion indicated that 34 of these cells significantly responded to 0.5 M sucrose. Two neurons in our sample were recorded simultaneously from one preparation. Eleven neurons were found to be sucrose-best, 18 were classified as NaCl-best, and 6 were typed as HCl-best. No quinine-best units were observed in our sample. Overall, these neurons were broadly responsive to the basic stimuli (\(H = 0.79 \pm 0.02\) SE). Nineteen neurons significantly responded to all components of the basic stimulus set, 13 significantly responded to only three of these stimuli, 2 significantly responded to only two tasters, and 1 cell significantly responded to only one stimulus. Figure 1 displays the across neuron pattern of response evoked by each basic stimulus and the average spontaneous discharge observed for each neuron prior to gurmarin treatment.

HCA was used to objectively classify neurons into heterogeneous types based on similarities/dissimilarities among their tuning profiles measured in response to the control presentation of each component of the basic stimulus array. The final HCA solution is represented graphically by the dendrogram in Fig. 2. HCA suggested three classes of neurons. All sucrose-best neurons were linked into class \(S\) (\(n = 14; \bar{H} = 0.77 \pm 0.03\) SE); three NaCl-best units with robust responses to sucrose were also bound to this cluster. Class N (\(n = 15; \bar{H} = 0.78 \pm 0.04\) SE) was composed almost entirely of NaCl-best neurons, the exceptions being a pair of HCl-best cells with strong NaCl responses. Class H (\(n = 6; \bar{H} = 0.89 \pm 0.02\) SE) neurons responded strongly to HCl, NaCl, and quinine.

**Effects of gurmarin on neuronal responses to sucrose**

Trains of action potentials that were evoked by presentation of each component of the basic stimulus set were recorded before and after gurmarin (10 \(\mu g/ml\)) application from all 35 neurons in our sample. Gurmarin treatment was found to attenuate sucrose-evoked spike discharge for the majority of these cells although the degree of suppression varied across
affected neurons. Many cells displayed residual responses to sucrose after gurmarin treatment; postgurmarin responses to sucrose remained significantly greater than average spontaneous discharge for 22 neurons. Complete gurmarin-induced inhibition (i.e., 0 net spikes/s) of a response to 0.5 M sucrose was observed during one trial for one neuron within our sample. Gurmarin treatment did not activate NST neurons, implying that 10 μg/ml gurmarin does not produce a gustatory quality sensation in the rat. Figure 3 shows digital oscilloscope records for two NST neurons in which the magnitude of the postgurmarin response to 0.5 M sucrose was either almost completely (Fig. 3A) or partly (Fig. 3B) suppressed relative to
within-neuron control (i.e., unadulterated sucrose response). For the neuron in Fig. 3A, the response to sucrose was almost fully inhibited by gurmarin (96% suppression). For the unit in Fig. 3B, the magnitude of the postgurmarin response to sucrose was attenuated by 68%. For both neurons, the magnitude of the response evoked by 0.1 M NaCl was not altered by gurmarin treatment.

The orotopic receptive field of the neuron depicted in Fig. 3B was at least partially composed of the fungiform papillae as this cell was driven by anodal stimulation of the anterior tongue. As methylene blue dye tests indicated that our gurmarin application procedure effectively bathed both the anterior tongue and palate, and taste stimulus delivery was limited to these same regions, the partial effect observed for this neuron could not be attributed to inadequate gurmarin application. Moreover, we acquired data from five neurons that clearly received input from the fungiform papillae but for which gurmarin treatment failed to attenuate responses to 0.5 M sucrose (criterion = ≥35% suppression relative to within-neuron control; mean suppression = 0 ± 3.42%; maximum suppression = 11%).

Assuming a criterion of ≥35% attenuation relative to within-neuron control, gurmarin application suppressed responses to 0.5 M sucrose for 22 (63%) neurons in our sample. If the criterion was raised to 50% attenuation, 16 (45%) cells were affected. For three (9%) neurons, postgurmarin sucrose responses were attenuated by 90% relative to control. These descriptors are summarized in Fig. 4, which displays across-neuron patterns of response to 0.5 M sucrose measured under control conditions (Fig. 4A) and after oral application of 10 µg/ml gurmarin (Fig. 4B). The difference between these patterns is shown in Fig. 4C. Moreover, these data indicate that sucrose responses recorded from neurons representative of each HCA-determined neuronal class were affected by gurmarin. However, a disproportionate percentage of cells in each class displayed sucrose responses that were substantially attenuated after gurmarin treatment. Eight (57%) class S, six (40%) class N, and two (33%) class H neurons exhibited postgurmarin responses to sucrose that were suppressed by ≥50% relative to control. Figure 4C further describes the magnitude of the effect as observed across neuronal type.

The null hypothesis that gurmarin application did not differentially affect basic taste response magnitudes across neuronal class was statistically evaluated using a neuron (35 cases) by neuronal group (3 levels; each HCA-defined neuronal class served as a level) by gurmarin treatment (2 levels) by stimulus (4 levels) mixed ANOVA. This hypothesis was not accepted as a significant neuronal group by gurmarin treatment by stimulus interaction was found \( F(6,96) = 3.34, P = 0.005 \). Planned interaction comparisons revealed that responses to sucrose were significantly attenuated after oral application of gurmarin in class S \( F(1,32) = 18.12, P = 0.0002 \) and N \( F(1,32) = 12.14, P = 0.001 \) neurons whereas sucrose responses in class H cells were unaffected \( (\alpha = 0.01) \). For each neuronal class,
gurmarin treatment did not affect responses to NaCl, HCl, or quinine relative to control (\(p < 0.01\)). Figure 5 graphically summarizes these data.

To determine if the observed effects, or lack thereof, of 10 \( \mu \text{g/ml} \) gurmarin approximated limits, taste responses were recorded from three neurons following oral application of 20 \( \mu \text{g/ml} \) gurmarin. Data regarding responses to sucrose that were recorded from these cells are graphed in Fig. 6. For the neuron depicted in Fig. 6A, gurmarin suppressed responses to 0.1, 0.32, 0.5, and 1.0 M sucrose, although concentration-response functions measured following oral application of 10 and 20 \( \mu \text{g/ml} \) gurmarin were not different. Moreover, the response evoked by 0.5 M sucrose after 10 \( \mu \text{g/ml} \) gurmarin treatment was nearly identical to that measured after application of 20 \( \mu \text{g/ml} \) gurmarin (difference = 1 net spike / 5 s), which indicated that 10 \( \mu \text{g/ml} \) gurmarin produced a maximal effect. As seen in Fig. 6B, 10 or 20 \( \mu \text{g/ml} \) gurmarin did not affect responses to 0.5 M sucrose measured from two other neurons.

Both of these cells received verified input from the fungiform papillae but were unaffected by gurmarin treatment. As observed with the standard concentration, oral application of 20 \( \mu \text{g/ml} \) gurmarin did not activate NST neurons.

**Sucrose concentration-response functions**

To determine if the effect of gurmarin was differential across sucrose concentrations, a half-logarithmic step ascending concentration series of sucrose, which ranged from 0.01 to 1.0 M, was presented to 19 neurons (class S: \( n = 7 \); class N: \( n = 10 \); class H: \( n = 2 \)) both before and after gurmarin application. A neuron by gurmarin treatment (2 levels) by sucrose concentration (5 levels) mixed ANOVA revealed that sucrose responses recorded from these cells were significantly influenced by gurmarin application and stimulus concentration [gurmarin by sucrose concentration interaction: \( F(4,60) = 7.74, p = 0.00004 \)]. Planned interaction comparisons indicated that post-
gurmarin responses to 0.1 M \(F(1,15) = 9.58, P = 0.007\), 0.32 M \(F(1,15) = 8.64, P = 0.01\) and 1.0 M \(F(1,15) = 10.35, P = 0.006\) sucrose were significantly attenuated relative to control. Although measured responses were negligible, responses to 0.01 and 0.032 M sucrose were not significantly suppressed after gurmarin application \((\alpha = 0.05)\). Considering only those concentrations where significant suppression was noted, the amount by which postgurmarin sucrose responses were attenuated did not significantly differ across sucrose concentration (pairwise comparisons of 0.1, 0.32, and 1.0 M sucrose pre-/postgurmarin response magnitude differences, Dunn MCP, \(\alpha = 0.05\); see Fig. 7).

**Effects of gurmarin on neuronal responses to various sweet-tasting compounds**

The effect of oral application of 10 \(\mu\)g/ml gurmarin on responses to various sweet-tasting compounds was explored in seven neurons. Given the small \(n\) and the sometimes disparate effects observed across the cells, we present data from experiments conducted on individual neurons in Fig. 8. For neuron N14, gurmarin treatment attenuated responses to sucrose, fructose, glycine, \(\alpha\)-asparagine, and \(\alpha\)-histidine by \(\approx 50\%\) relative to control. Given the presence of residual postgurmarin sweet responses, these data suggest that this neuron received input from gurmarin-sensitive and -insensitive receptor mechanisms that were rather broadly tuned. In contrast, the overall lack of a gurmarin treatment effect noted for neuron N7 implied that sweet responses in this cell were mediated by input derived entirely from gurmarin-insensitive sweet transduction processes. However, data obtained from neurons N15, S5, and H5 were most interesting. For neuron N15, gurmarin treatment did not affect the response to sucrose or fructose. However, post-gurmarin responses to glucose and maltose were suppressed by \(\approx 50\%\). Moreover, the post-gurmarin response to galactose was

---

**FIG. 4.** Across neuron patterns of response evoked by 0.5 M sucrose under control conditions (A) and after oral application of 10 \(\mu\)g/ml gurmarin (B). Neurons are segregated according to hierarchical cluster analysis (HCA) class, rank ordered within each class according to the net response magnitude evoked by sucrose under control conditions and identified along the abscissa using labels described in Fig. 1. If applicable, responses were averaged across multiple sucrose presentation trials recorded from the same neurons during each condition. An averaged response is denoted by an integer, which indicates the number of trials reflected by the mean. C: across-neuron difference between the control and postgurmarin across neuron patterns of response evoked by 0.5 M sucrose. The percentage criterion by which the postgurmarin response to 0.5 M sucrose was attenuated relative to control is indicated for each neuron.
fully inhibited. Sweet responses were also differentially affected by gurmarin in neuron H5. For this cell, responses to sucrose and fructose were resistant to gurmarin treatment. However, responses to Na-saccharin, D-asparagine, and D-histidine were suppressed by \( \frac{50}{100} \) relative to control, whereas the postgurmarin glucose response was fully inhibited. These data suggest that some NST neurons may receive convergent input from gurmarin-sensitive and -insensitive receptor mechanisms that are differentially tuned. Data obtained from neuron S5 further exemplify this point. This cell received information regarding the presence of maltose on gustatory epithelia from exclusively gurmarin-sensitive sweet transduction processes as the response to maltose was completely inhibited after gurmarin treatment. Although the postgurmarin response to sucrose was attenuated by \( \frac{50}{100} \) relative to control, the residual indicates that both gurmarin-sensitive and -insensitive receptors contributed to this response. Therefore the tuning characteristics of the gurmarin-sensitive and -insensitive receptor processes that initiated the transmission of sucrose and maltose information to this neuron differed, as the gurmarin-insensitive component was not responsive to 0.5 M maltose.

**DISCUSSION**

We recorded taste responses from 35 individual NST neurons before and after oral application of the sweet transduction blocker gurmarin to determine how gurmarin sensitivity is distributed across neuronal type. The majority of NST neurons in our sample received input from gurmarin-sensitive transduction processes. However, many of these neurons exhibited residual postgurmarin responses to sucrose. As our gurmarin application procedure effectively bathed both the anterior tongue and palate, and taste stimulus delivery was limited to these areas, the presence of residual responses implied that these neurons were driven by both gurmarin-sensitive and -insensitive receptor mechanisms. Sucrose responses recorded from neurons representative of each HCA-defined class were affected by gurmarin, suggesting that information derived from gurmarin-sensitive receptor processes is not restricted to a single NST neuronal type on arrival at the CNS. However, a differential proportion of cells within each neuronal class exhibited postgurmarin responses to 0.5 M sucrose that were attenuated by \( \frac{50}{100} \) relative to control. Based on this criterion, the largest number of affected neurons was found in class S, followed by classes N and H, respectively. On average, postgurmarin responses to 0.5 M sucrose were found to be significantly suppressed only in class S neurons. Additionally, the effect of gurmarin was sometimes differential across different sweet-tasting compounds within individual neurons, which implied that some NST cells may receive convergent input from gurmarin-sensitive and -insensitive receptor mechanisms that are differentially tuned to various sweet-tasting ligands. Responses to NaCl, HCl and quinine were not affected by gurmarin treatment.

**Information from gurmarin-sensitive receptors is not restricted to a single NST neuronal type**

The existence of gurmarin-sensitive and -insensitive receptor mechanisms was first suggested by data concerning the effects of gurmarin on whole-nerve responses to sweeteners recorded from rodents. Although integrated CT (Imoto et al. 1991; Miyasaka and Imoto 1995; Ninomiya and Imoto 1995; Ninomiya et al. 1998) and GSP (Harada and Kasahara 2000) responses to sucrose were found to be significantly suppressed after gurmarin treatment, a residual postgurmarin response to this stimulus was evident in these recordings. Moreover, the inhibitory effect of gurmarin on CT responses to 0.5 M sucrose became asymptotic, though not absolute, at \( \sim 5 \mu M \), which produced \( \sim 80\% \) suppression relative to control; no further suppression was observed even if the tongue was treated with 240 \( \mu M \) gurmarin (Miyasaka and Imoto 1995). For C57BL
mice, the effects of gurmarin have been shown to be nerve specific. Whereas integrated CT responses to various sweeteners were suppressed by gurmarin to \(~50\%) of control in this species (Ninomiya and Imoto 1995; Ninomiya et al. 1997), those recorded from the glossopharyngeal nerve were recalcitrant to gurmarin treatment (Ninomiya et al. 1997). As gurmarin is believed to act on an apical receptor binding site (Miyasaka and Imoto 1995; Yoshie et al. 1994), these data suggest that at least two receptor processes for sweeteners exist in rodents, classified on the basis of their sensitivity to gurmarin (Ninomiya et al. 1999). Moreover, single sucrose-best CT neurons can be segregated into types on the basis of their susceptibility to lingual gurmarin treatment (Ninomiya et al. 1999), suggesting selective synaptic coupling between taste receptor cells (TRCs) that express gurmarin-sensitive or -insensitive receptors and particular subsets of sucrose-best CT fibers.

Selective coupling between specific types of TRCs and peripheral gustatory neurons has been shown by the effects of lingual amiloride treatment on responses to NaCl in single CT fibers. Salt responses recorded only from those fibers responding best to NaCl were susceptible to amiloride treatment (Hettinger and Frank 1990; Ninomiya and Funakoshi 1988), suggesting that this fiber type exclusively innervates TRCs that express amiloride-sensitive Na\(^+\) transduction processes. This segregation of amiloride-sensitive salt information to a particular type of CT neuron is similar to the observation that gurmarin-sensitive sweet information is selectively distributed to a particular type of sucrose-best CT fiber (Ninomiya et al. 1999). Because amiloride-sensitive salt information is predominantly relayed to NaCl-best (Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996; St. John and Smith 2000) and sucrose-best (Smith et al. 1996; St. John and Smith 2000) NST neurons, it could be hypothesized that gurmarin-sensitive sweet information is also differentially distributed across physiologically defined NST neuronal types. Such organization may have implications for how sweet information is encoded by neural activity in the CNS, as the arrangement of amiloride-sensitive input contributes to the neural representation of salt information in the NST (Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; St. John and Smith 2000) and gustatory behavioral discrimination between salts (Spector et al. 1996).

To our knowledge, the present study is the first to explore the distribution of gurmarin sensitivity across taste-driven neurons in the CNS. Although gurmarin-sensitive information was not restricted to a particular class of neuron, a disproportionate percentage of cells from each neuronal class exhibited sucrose

**FIG. 6.** Effects of oral application of 10 and 20 \(\mu g/ml\) gurmarin on sucrose responses recorded from three neurons. A: data from neuron N13. Although different from control, responses to 0.5 M sucrose and sucrose concentration-response functions measured after treatment with the standard and doubled concentration of gurmarin were similar. B: data from neurons N10 and N15. Responses to 0.5 M sucrose measured under control conditions (trials <0) and after either 10 (trials 1 and 2) or 20 (trial 3) \(\mu g/ml\) gurmarin treatment were nearly identical.
responses that were substantially attenuated following gurmarin treatment. Over half of the class S neurons exhibited postgurmarin sucrose responses that were attenuated by \( \geq 50\% \) relative to control; the same could be said for less than half of the N or H class cells. Overall, postgurmarin responses to sucrose were significantly attenuated only in class S and N neurons. In some respects, the distribution of gurmarin sensitivity across NST neuronal types relative to those in the periphery (Ninomiya et al. 1999) is similar to that observed for amiloride-sensitive salt input as the apparent restriction of information derived from a particular receptor process to a specific peripheral neuron class is not absolute in the CNS. However, amiloride is more effective at reducing responses to \( \text{Na}^+ \) salts in those NST cells that respond maximally to these stimuli relative to the average gurmarin-induced attenuation of responding to sucrose observed in class S cells in the present study (see Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996; St. John and Smith 2000). Although an amiloride-insensitive component is apparent, salt responses in NaCl-best NST neurons are predominantly derived from amiloride-sensitive salt input (Boughter and Smith 1998; Boughter et al. 1999; Giza and Scott 1991; Scott and Giza 1990; Smith et al. 1996; St. John and Smith 2000), whereas gurmarin-sensitive and -insensitive receptor mechanisms contributed almost equally, on average, to sucrose responses in class S cells (see Fig. 5). Although they are somewhat differently organized, the neuronal circuits that underlie amiloride-sensitive salt and gurmarin-sensitive sweet input to the brain distribute information to more than one category of NST neuron.

Convergence of neural information in the gustatory NST has been directly demonstrated (Ogawa et al. 1984; Sweazeys and Smith 1987; Travers and Smith 1986; Vogt and Mistretta 1990) and implied (Boughter and Smith 1998; Doetsch and Erickson 1970; Hill et al. 1983; Smith et al. 1996; St. John and Smith 2000; Travers and Smith 1979) by a number of studies. Moreover, many NST neurons in the present study appeared to receive convergent input from gurmarin-sensitive and -insensitive receptor mechanisms. This was partially suggested by the observation that, for the majority of these cells, residual postgurmarin responses to 0.5 M sucrose were found that exceeded statistical threshold, whereas the absence of gurmarin on sucrose responses recorded from single mouse CT fibers is purportedly more absolute (Ninomiya et al. 1999); companion rat single-fiber data do not presently exist. However, the presence of these residual responses by themselves may not necessarily reflect input derived from gurmarin-insensitive receptor components (Ninomiya et al. 1999) as factors such as inadequate gurmarin concentration and/or treatment could also result in such responses. However, our experimental measures taken to address these possibilities suggest otherwise.

We encountered taste-driven NST neurons during experimentation that did not appreciably respond to sucrose and thus were not included in our study. The composition of our neuronal sample reflects this: we recorded from many neurons that were subsequently typed as class S (40\% of sample) and N (43\% of sample) that responded well to sucrose. However, only 6 (17\% of sample) sucrose-responding class H cells were found. Many cells with strong responses to HCl, NaCl, and quinine were encountered that did not respond to sucrose, suggesting that some cells may not receive sucrose-mediated input from the anterior tongue and palate, areas known to be populated with TRCs that express sweet receptor mechanisms (Gilbertson et al. 2001). Moreover, this low \( n \) may have influenced our findings regarding the effect of gurmarin on responses to sucrose across neuronal class as failure to observe a significant effect of gurmarin in class H neurons may be attributable to low statistical power. Assuming this caveat to be true and that further investigation would yield a sizable number of sucrose-responsive and gurmarin-sensitive class H NST neurons, our analogy between the differential distribution of gurmarin-sensitive sweet and amiloride-sensitive salt information across NST neuronal types could be rendered less appropriate, although a recent report showed that amiloride significantly attenuated responses to NaCl in some HCl-best NST neurons (St. John and Smith 2000).

**Effects of gurmarin varied across sweeteners within individual neurons**

Although neurophysiological data are limited, various psychophysical studies have suggested multiple receptor mechanisms for sweet compounds. Intensity matching experiments in humans indicated that concentrations of fructose, glucose, and sucrose could be found that rendered these stimuli indistinguishable (Breslin et al. 1996). However, higher concentrations of maltose could not be matched using this procedure, suggesting that maltose activates a separate receptor process. It was recently demonstrated that whole-mouth adaptation to fructose increased discriminability between fructose and glucose in humans, indicating that these two sugars possibly stimulate separate receptor sites (Tharp and Breslin 2002). Other human cross-adaptation experiments have reported similar findings of incomplete cross-adaptation among various sweet stimuli (Faury et al. 1980; Froloff et al. 1998; Schifman et al. 1981). Moreover, discrimination experiments, in which intensity was rendered an irrelevant cue (Spector et al. 1997), have shown that rats can discern sucrose from maltose, suggesting inde-
ependent receptor mechanisms for these stimuli in the rodent. Our data complement these findings by showing that some neurons received input from gurmarin-sensitive and -insensitive receptor processes that responded differentially to sweet compounds (see Fig. 8), suggesting that some receptor mechanisms are sensitive to only a subset of, and not all, sweet-tasting ligands. Moreover, our data indicate that some neurons may receive input from TRCs expressing gurmarin-sensitive receptor processes that are unresponsive to sucrose (see Fig. 8). Although our low n with regard to this type of data warrants further investigation, a complementary differential effect of gurmarin on responses to a sweetener array has been observed in integrated GSP nerve recordings in rats. Phasic responses to sucrose, fructose, lactose, and maltose were significantly inhibited, whereas responses to galactose and glucose were unaffected following palatal gurmarin treatment (Harada and Kasahara 2000). The convergence of peripheral fibers that are driven by differentially tuned gurmarin-sensitive and -insensitive receptor processes onto NST neurons could account for the observed idiosyncratic effects of gurmarin on neuronal responses to various sweeteners.

Recent advances in molecular biology suggest the existence of a single mammalian receptor for sweets, T1R2/T1R3, as this candidate responded to all sweet taste stimuli tested (Li et al. 2002). However, other reports suggest that T1R2/T1R3 is extremely selective, recognizing only a limited range of sweet compounds (Nelson et al. 2001). Moreover, T1R2 is purportedly undetectable in most fungiform taste papillae (Hoon et al. 1999), yet some fungiform TRCs clearly respond to sucrose (Gilbertson et al. 2001). Although our understanding of sweet

![Fig. 8. Effect of oral application of gurmarin on responses to various sweet compounds as observed for 7 neurons. Gurmarin treatment was sometimes selective for particular stimuli within a cell. *, postgurmarin response attenuation of ≥50%; **, ≥100% suppression relative to control. See Table 1 for stimulus abbreviations.](http://jn.physiology.org/lookup/doi/10.1152/jn.00758.2002)
taste reception is far from complete, it is generally agreed that sweet stimuli activate TRCs through at least two transduction pathways: one involves the generation of cyclic nucleotides, the other modulates levels of inositol triphosphate (Herness and Gilbertson 2001). Receptor and transduction processes are the initial steps toward gustatory perception, which is ultimately a product of neural information processing in the brain. Further understanding of the neural mechanisms underlying sweet perception will necessitate the derivation of the relationships between receptor mechanisms and the physiology of neurons in the CNS.

Implications for gustatory neural information processing

Although scant, data regarding the effects of gurmarin on gustatory behavioral tasks do exist. Gurmarin was found to suppress the avoidance of sucrose for C57BL mice trained in a conditioned taste aversion paradigm (Nakashima et al. 2001). Additionally, rats fed a diet containing G. sylvestre exhibited a transient reduction in preference for sucrose: intake decreased and subsequently recovered several days later at a time when gurmarin binding proteins, which suppress the activity of gurmarin, appeared in the saliva of these subjects (Katsukawa et al. 1999). The present study demonstrated that sucrose responses recorded from class S and N neurons were significantly attenuated after gurmarin treatment, indicating that these cells processed gurmarin-sensitive sweet input. Therefore activity generated by class S and N neurons possibly contributes to such sucrose-mediated behavioral tasks. This correlate reinforces the notion of a distributed neural code for taste in the CNS, where activity generated by a network of individual cells of different physiologically defined types underlies the neuronal representation of multiple stimulus qualities and parameters (Erickson 1968; Pfaffmann 1959; Scott and Giza 2000; Smith and St. John 1999).

The authors thank Dr. John D. Bougther Jr. for valuable comments on this manuscript.

DISCLOSURES

This work was supported in part by National Institute of Deafness and Other Communication Disorders Grants DC-005270 to C. H. Lemon and DC-00353 to D. V. Smith.

REFERENCES


J Neurophysiol • VOL 90 • AUGUST 2003 • www.jn.org


