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Tonic GABAergic Inhibition of Taste-responsive Neurons in the Nucleus of the Solitary Tract

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Abstract

The effects of γ-aminobutyric acid (GABA) and the $GABA_A$ receptor antagonist bicuculline methiodide (BICM) on the activity of taste-responsive neurons in the nucleus of the solitary tract (NST) were examined electrophysiologically in urethane-anesthetized hamsters. Single neurons in the NST were recorded extracellularly and drugs (21 nl) were microinjected into the vicinity of the cell via a multibarrel pipette. The response of each cell was recorded to lingual stimulation with 0.032 M NaCl, 0.032 M sucrose, 0.0032 M citric acid and 0.032 M quinine hydrochloride (QHCl). Forty-six neurons were tested for the effects of GABA; the activity of 29 cells (63%) was inhibited by 5 mM GABA. Whether activity was elicited in these cells by repetitive anodal current stimulation (25 μA, 0.5 s, 0.1 Hz) of the tongue (n = 13 cells) or the cells were spontaneously active (n = 13 cells), GABA produced a dose-dependent (1, 2 and 5 mM) decrement in activity. Forty-seven NST neurons were tested for the effects of BICM on their responses to chemical stimulation of the tongue; the responses of 28 cells (60%) were enhanced by 10 mM BICM. The gustatory responses of 26 of these cells were tested with three concentrations (0.2, 2 and 10 mM) of BICM, which produced a dose-dependent increase in both spontaneous activity and taste-evoked responses. Nine of these neurons were sucrose-best, seven were NaCl-best, eight were acid-best and two responded best to QHCl. The responses to all four tastants were enhanced, with no difference among neuron types. For 18 cells that were tested with two or more gustatory stimuli, BICM increased their breadth of responsiveness to their two most effective stimuli. These data show that ~60% of the taste-responsive neurons in the rostral NST are inhibited by GABA and/or subject to a tonic inhibitory influence, which is mediated by $GABA_A$ receptors. The modulation of these cells by GABA provides a mechanism by which the breadth of tuning of the cell can be sharpened. Modulation of gustatory activity following a number of physiological changes could be mediated by such a GABAergic circuit.

Introduction

The rostral portion of the nucleus of the solitary tract (NST) receives topographically organized input from gustatory afferent fibers of the facial, glossopharyngeal and vagal nerves, and projects rostrally to the parabrachial nuclear complex (Allen, 1923; Åström, 1953; Norgren and Leonard, 1973; Beckstead and Norgren, 1979; Hamilton and Norgren, 1984; Hanamori and Smith, 1989). Taste-responsive neurons of the NST receive converging input from separate subpopulations of taste buds (Travers *et al.*, 1986; Sweazey and Smith, 1987) and these neurons are more broadly tuned to gustatory stimuli of different qualities than fibers of the chorda tympani (CT) nerve (Smith and Travers, 1979; Travers and Smith, 1979). Both *in vitro* and *in vivo* studies of cells in the rostral NST are beginning to reveal the role of a number of excitatory and inhibitory neurotransmitters, such as glutamate, substance P and γ-aminobutyric acid (GABA) in the processing of taste information (King *et al.*, 1993; Liu *et al.*, 1993; Wang and Bradley, 1993, 1995; Bradley *et al.*, 1996; Davis and Smith, 1997; Li and Smith, 1997; Smith *et al.*, 1998).

Responses of gustatory cells in the NST are subject to several modulatory influences, including changes in the animal's physiological state, such as gastric distension (Glenn and Erickson, 1976), changes in levels of blood glucose (Giza and Scott, 1983) or insulin (Giza and Scott, 1987), and conditioned taste aversion learning (Chang and Scott, 1984). Jugular administration of morphine decreases the NaCl responses of cells in the rat parabrachial nucleus (Hermann and Novin, 1980), but has no effect on trigeminal responses to tactile stimulation. The excitatory and inhibitory influences of descending pathways on the responsiveness of brainstem taste neurons have been demonstrated also in electrophysiological studies on decerebrate rats (Hayama *et al.*, 1985; DiLorenzo, 1988; Mark *et al.*, 1988). These and other data suggest that taste information is not simply passed on to higher centers by

brainstem taste nuclei but is subject to significant modification at these early stages of synaptic processing.

There is some evidence to suggest that GABA may be an inhibitory neurotransmitter acting on taste-responsive neurons of the NST. There are GABA-containing neurons in the gustatory zone of the NST of both rats (Lasiter and Kachele, 1988) and hamsters (Davis, 1993). Recent *in vitro* experiments in rat (Wang and Bradley, 1993) and hamster (Liu *et al.*, 1993) brain slices have shown that the responses of cells in the gustatory region of the NST can be inhibited by GABA and excited by the $GABA_A$ receptor antagonist, bicuculline methiodide (BICM), suggesting a tonic GABA-ergic inhibitory network within this region of the NST. In a previous *in vivo* experiment, we have shown that GABA can decrease the taste responses of hamster NST cells (Smith *et al.*, 1994), although the role of tonic inhibition has not been examined or the response characteristics of GABA-sensitive cells in the gustatory portion of the NST established. The present *in vivo* recording experiments were designed to test the hypothesis that GABAergic mechanisms tonically modulate the responsiveness of taste-sensitive neurons in the NST and to determine the gustatory characteristics of such neurons.

Materials and methods

Animals and surgery

Young adult male hamsters, *Mesocricetus auratus* (120–185 g), were deeply anesthetized with urethane (1.7 g/kg, i.p.) and additional anesthetic was given as needed during the course of each experiment. The animal was tracheotomized and mounted in a stereotaxic instrument using a non-traumatic head holder (Erickson, 1966). The snout was angled downward 27° from the horizontal to straighten the brainstem and minimize brain movement associated with breathing (Smith *et al.*, 1979). Body temperature was monitored and maintained at 37°C with a heating pad. A sagittal skin incision was made though the midline overlying the posterior skull and a portion of the occipital bone just dorsal to the foramen magnum was removed to reveal the cerebellum. After removing the dura, the posterior portion of the cerebellum was aspirated to expose the floor of the fourth ventricle for 3–4 mm anterior to the obex.

Single-unit recording and taste stimulation

Multibarrel glass micropipette assemblies were used for recording and microinjection of pharmacological agents. The pipettes for extracellular recording (tip diameter = 1–2 μm, resistance = 7–10 MΩ) were filled with a 2% (w/v) solution of Chicago Blue dye (Sigma Chemical Co., St Louis, MO) in 0.5 M sodium acetate. A three-barrel glass pipette (total tip diameter ≈ 35 μm) was glued to the recording electrode for microinjection of drugs; the tip of the recording electrode extended 100–120 μm beyond the microinjection pipette. The mean coordinates for the 93 taste-responsive cells recorded in these experiments were 2.10 ± 0.21 (SD) mm anterior and 1.20 ± 0.23 mm lateral to obex; they were between 0.5 and 1.0 mm ventral to the surface of the brainstem. Histological reconstruction of 10 electrode penetrations showed the electrode tip to be within the NST, medial to the solitary tract; recording sites were not systematically reconstructed. Extracellular potentials were differentially amplified (Bak MDA-4I), discriminated with a dual time-amplitude window discriminator (Bak DDIS-1), displayed on oscilloscopes and monitored with an audio monitor. The amplified action potentials were recorded along with voice cues on VCR tape. An IBM Pentium computer, configured with a CED 1401*plus* interface board and Spike2 software (Cambridge Electronic Design Ltd, Cambridge, UK), controlled chemical stimulus delivery and online data acquisition and analysis.

Taste responses were initially identified by a change in neural activity associated with the application of anodal current pulses (50 μA, 0.5 s, 0.33 Hz) to the anterior tongue and confirmed by responses to chemical stimulation of the tongue. In the experiment that examined the effect of GABA on the responses of NST neurons to anodal tongue stimulation, an anodal current pulse (25 μA, 0.5 s) was delivered to the anterior tongue every 10 s throughout the course of the experiment (Smith and Bealer, 1975). For experiments with taste solutions, the anterior tongue was stimulated with 0.032 M sucrose, 0.032 M sodium chloride, 0.032 M quinine hydrochloride (QHCl) and 0.0032 M citric acid. These concentrations evoke approximately equal multiunit taste responses in the hamster NST when applied to the anterior tongue (Duncan and Smith, 1992). The tastants were delivered by a gravity flow system composed of a two-way solenoid-operated valve connected via tubing to a distilled water rinse reservoir and a stimulus reservoir. The stimulation sequence, during which the computer acquired data, was a continuous flow initiated by the delivery of 5 s of distilled water followed by 10 s of stimulus, followed by 5 s of distilled water. The flow rate was 2 ml/s. Following each chemical stimulation, the tongue was rinsed with distilled water (>50 ml) and individual stimulations were separated by at least 2 min to avoid adaptation effects (Smith *et al.*, 1978).

Pharmacology and microinjections

GABA and BICM were dissolved in buffered physiological saline and microinjected into the NST through the multibarrel pipette assembly. Pressure pulses (30 p.s.i., 10 ms) from a Picospritzer were used to trigger the drug injection; drug volume was estimated by comparing the areas of methylene blue injections from 11 pipette assemblies to the areas of a series of known volumes (10–40 nl) injected onto filter paper. The 30 p.s.i., 10 ms pressure pulse produced a mean volume of 20.7 ± 2.3 (SD) nl per barrel from these pipettes. GABA was delivered at pipette concentrations of 1, 2 or 5 mM (pH 7.4 ± 0.1), resulting in

the delivery of ~21–105 pmol of GABA in 21 nl. The $GABA_A$ receptor antagonist, bicuculline methiodide (BICM), was delivered at pipette concentrations of 0.2, 2 or 10 mM (pH 7.4 ± 0.1), resulting in 4–210 pmol of BICM in 21 nl. Because the 0.2 mM concentration never produced an effect on these cells, it essentially served as a vehicle control injection for these experiments. GABA and BICM were obtained from Sigma.

Data analysis

The responses of each cell to chemical stimulation of the tongue were accumulated over three consecutive time periods: (i) 5 s of prestimulus water rinse, (ii) 10 s of stimulus flow and (iii) 5 s of poststimulus water rinse. The net response was calculated as the number of action potentials during the first 5 s of chemical stimulation minus the number of action potentials during the 5 s prestimulus water rinse (Vogt and Smith, 1993). Responses are reported as means ± SEM. In order to compare the effects of BICM, the means of all net responses to chemical stimulation in the control condition were compared to the means to the same stimuli in the same cells under the drug conditions. The control condition was tastant stimulation without microinjection; none of the cells that were tested with 21 nl saline injections showed any change in their firing rates and there was never a change induced by 0.2 mM BICM. A cell was defined as BICM-sensitive if the net response to the cell's best taste stimulus increased after 10 mM BICM by at least 50%; this conservative criterion ensured that any changes due to response variability were not attributed to drug effects. For statistical comparison of the effects of GABA on the responses of cells to anodal current stimulation or during spontaneous activity, the mean firing rate in each cell over a 2 min period prior to drug administration was compared to the mean firing rate during a comparable period following the drug. A cell was defined as GABA-sensitive if the firing rate during 5 mM GABA decreased by at least 50%. Differences among responses under different drug concentrations were statistically analyzed using one-way ANOVA or paired *t*-tests (SPSS for Windows, v. 6.0, SPSS, Inc., Chicago IL).

In order to measure the breadth of a cell's responsiveness to gustatory stimulation, we calculated each cell's breadth of tuning using the entropy measure (H) developed by Smith and Travers (1979). This measure is given by: $H = -K \Sigma p_i \log p_i$, where H = breadth of responsiveness, K is a scaling constant, and p_i is the proportional response to each of n stimuli. The neural response profile for each cell was converted to a proportional profile, with the response to each stimulus expressed as a proportion (p_i) of the total response to all four. For four stimuli, K = 1.661, which results in H varying from 0.0 (when a cell responds to only one stimulus) to 1.0 (when a cell responds equally to all four). To compare the response breadth to two stimuli, which is done for cells' responses before and after BICM, using K = 3.322 results in H values between 0.0 and 1.0. In either form, this equation provides a quantitative measure of the breadth of responsiveness of a gustatory neuron that varies with such variables as stimulus concentration (see Smith and Travers, 1979, for additional discussion of this measure).

Results

The effects of GABA on the activity of taste-responsive NST cells

To examine the hypothesis that GABA mediates inhibitory synaptic transmission onto taste-responsive NST cells, we tested the influence of GABA on the activity of NST cells that were first characterized for their taste sensitivity. Forty-six NST neurons were tested for their responsiveness to lingual application of sucrose, NaCl, citric acid and QHCl in order to determine their gustatory response profiles, and then subjected to administration of GABA to investigate its inhibitory effect on either the spontaneous activity of the cell or its response to repetitive anodal current stimulation of the tongue. Since a previous study had shown that GABA microinjection resulted in decreased taste-evoked responses (Smith *et al.*, 1994), this experiment was designed to investigate: (i) the dose-dependency of the effect and (ii) the proportion of cells that were sensitive to GABA and their gustatory responsiveness. Microinjection of 5 mM GABA reduced the activity of 63% of these NST neurons by >50% (n = 29). Twenty-six of these GABA-sensitive cells were then successfully tested with all three concentrations of GABA (1, 2 and 5 mM). The activity of one such cell is shown in Figure 1, where it may be seen that GABA decreased its ongoing spontaneous discharge rate in a dose-dependent fashion. At 5 mM, GABA produced a profound inhibition of the activity of this cell, which lasted for ~5 min.

Of the 26 neurons that were tested with all three doses of GABA, 13 had relatively high rates of spontaneous activity. The effects of GABA on this activity are shown in Figure 2(A), which depicts the mean firing rate under control conditions (0) and after 1, 2 and 5 mM GABA administration. Increasing concentrations of GABA produced a concentration-dependent decrease in the firing rate of these cells [one-way ANOVA, $F(3,48)$ = 6.037, P = 0.0014]. Administration of vehicle alone had no effect on any of five cells that were tested; the mean firing rate of these cells was 1.41 imp/s prior to vehicle and 1.28 imp/s after (Wilcoxon signed-ranks test, Z = 0.73, P = 0.47). Vehicle administration was not tested on all of the cells because at this volume (21 nl) we have never observed an effect of physiological saline alone on the activity of an NST neuron, and all cells that were tested with three concentrations of GABA responded in a monotonic dose-dependent fashion (as in Figure 1).

Electrical stimulation of the tongue through an anode at

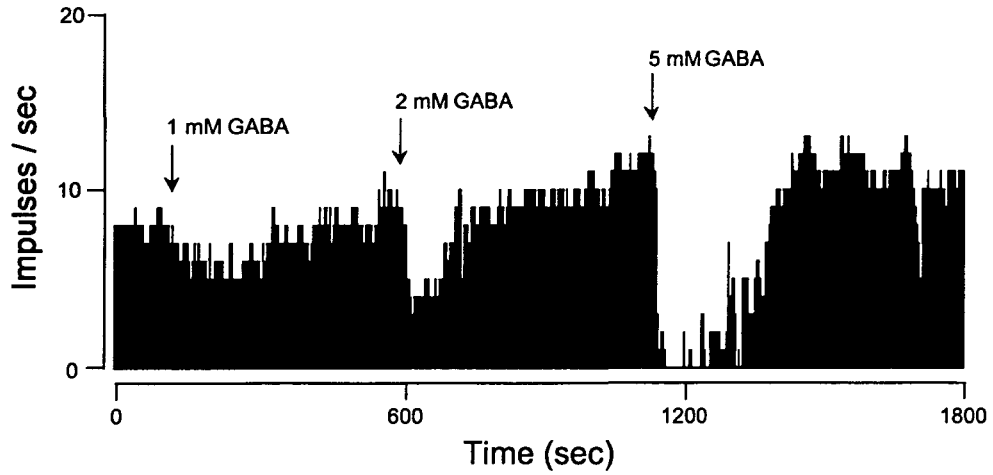

Figure 1 Impulse histogram of the spontaneous activity of a taste-responsive neuron in the rostral NST before and after microinjection of 1, 2 and 5 mM GABA. Drug application began at the arrows and consisted of a single pressure pulse with a volume of ~21 nl (producing ~21–105 pmol of GABA) microinjected 120 μm dorsal to the tip of the recording electrode.

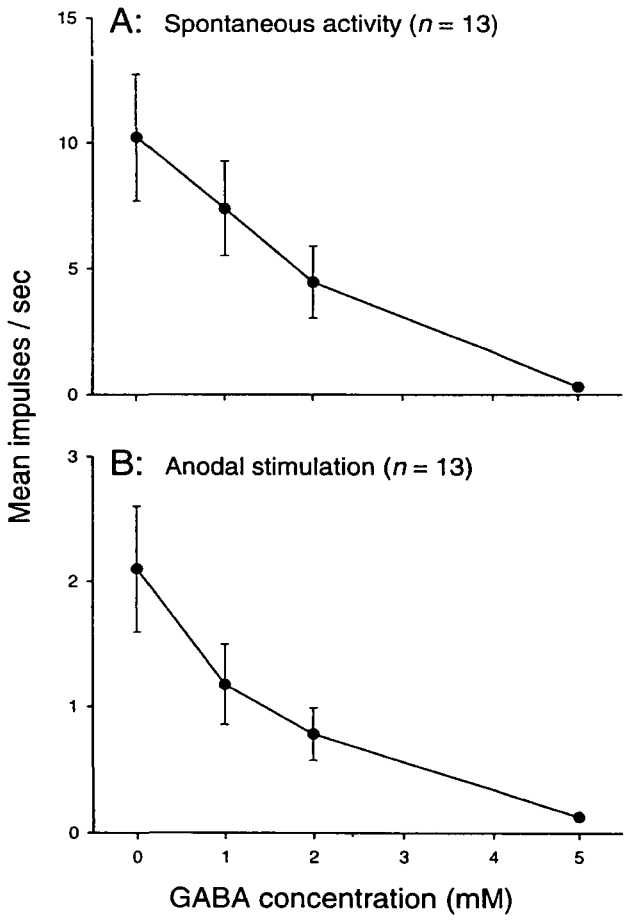

Figure 2 Mean (±SEM) firing rate in 26 taste-responsive NST neurons before (0) and after application of GABA at three pipette concentrations (1, 2 and 5 mM). **(A)** Activity in 13 cells with relatively high spontaneous activity. **(B)** Activity in 13 cells with low (<1 imp/s) spontaneous activity, which are being driven by repetitive anodal current (25 μA, 500 ms, 0.1 Hz) stimulation of the anterior tongue throughout the drug injection protocol.

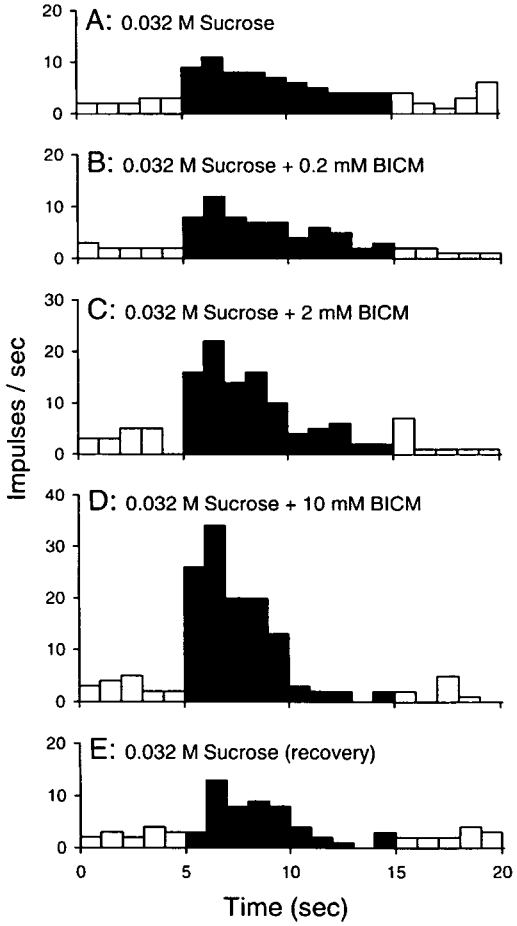

Figure 3 Peristimulus–time histograms of the responses of a single NST neuron to 0.032 M sucrose before **(A)** and immediately after microinjection of a single 21 nl pulse of 0.2 mM BICM **(B)**, 2 mM BICM **(C)** and 10 mM BICM **(D)** into the vicinity of the cell and again 10 min after the last BICM injection **(E)**. A distilled water rinse was applied to the anterior tongue during the 0–5 s interval and again at the 15 s point (open bars); the stimulus flowed from 5 to 15 s (filled bars).

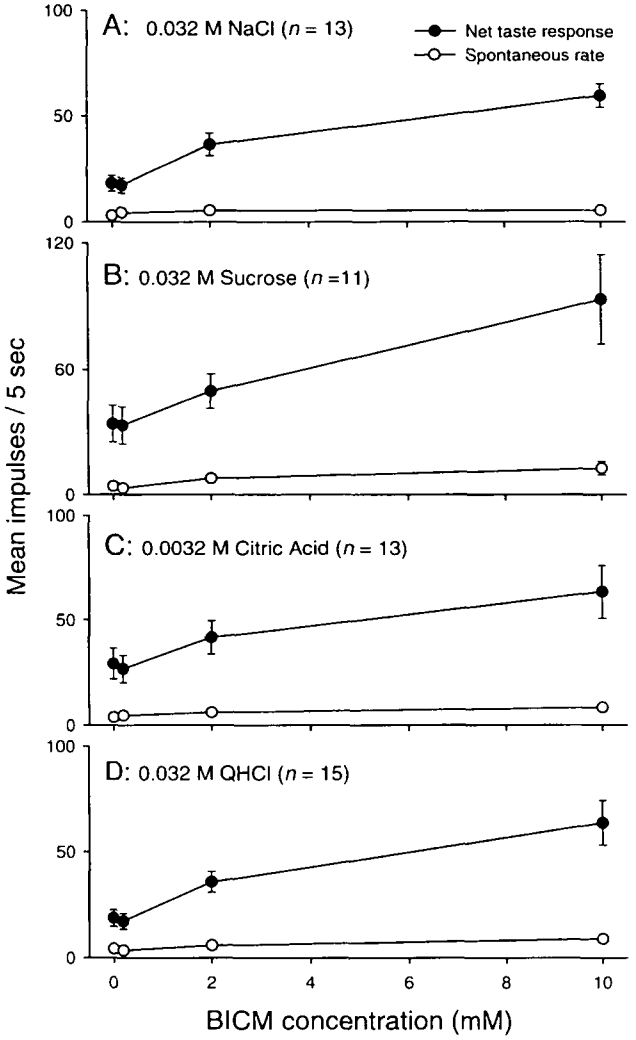

Figure 4 Mean (±SEM) firing rate in taste-responsive NST neurons before (0) and after application of BICM at three pipette concentrations (0.2, 2 and 10 mM). **(A)** Responses of 13 cells to 0.032 M NaCl (filled circles) and their spontaneous activity (open circles). The responses after 0.2 mM BICM did not differ from control (0). **(B)** Responses of 11 cells to 0.032 M sucrose and their spontaneous activity. **(C)** Responses of 13 cells to 0.0032 M citric acid and their spontaneous activity. **(D)** Responses of 15 cells to 0.032 M QHCl sucrose and their spontaneous activity.

1–50 μA produces responses in gustatory fibers of the CT nerve by the activation of electrolyte-sensitive taste receptor mechanisms (Smith and Bealer, 1975; Herness, 1987). Therefore, we used anodal stimulation of the tongue to repetitively drive gustatory input to the NST in another 13 of these taste-responsive cells in order to investigate the effects of GABA microinjection on this peripherally evoked response. These 13 cells had relatively low rates of spontaneous discharge, making it more difficult to investigate these inhibitory effects on spontaneous activity alone. As in the cells that were highly spontaneously active, GABA produced a statistically significant dose-dependent decrease in the responses of these cells to repetitive anodal stimulation [Figure 2B; $F(3,48) = 6.738$, $P = 0.0007$].

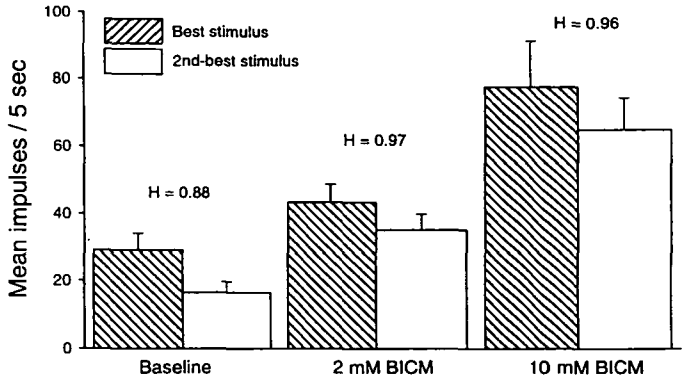

Figure 5 Mean (±SEM) responses to the best stimulus (S, N, C or Q; shaded) and the second-best stimulus (unshaded) in 18 NST cells that were tested with more than one tastant before and after BICM administration. The mean breadth of responsiveness (*H*) of these 18 cells to their two best stimuli under the three drug conditions are indicated above each pair of response values.

The effects of BICM on the gustatory responses of NST cells

In the second experiment, we tested the influence of the $GABA_A$ receptor antagonist BICM on the responses of 47 NST cells to chemical stimulation of the anterior tongue. The net responses of 28 of these neurons (60%) to their best taste stimulus (NaCl, sucrose, citric acid or QHCl) were enhanced >50% by 10 mM BICM administration. For 26 of these cells, we were able to complete a concentration series of BICM and show that their gustatory responses were enhanced in a dose-dependent manner. The responses evoked by 0.032 M sucrose in an NST cell before (Figure 3A) and after BICM are depicted in Figure 3. The response to sucrose was enhanced by BICM in this cell; 2 mM (Figure 3C) produced a 100% increase in the net 5 s response to sucrose and 10 mM (Figure 3D) produced a 213% increase. The response to sucrose returned to pre-drug levels (i.e. to within 10% of its original value) within 10 min after BICM administration (Figure 3E). Microinjection of 0.2 mM BICM (Figure 3B) had no effect on the cell's response.

Of the 26 cells that were tested with the BICM concentration series, 13 were tested with NaCl before and after all three concentrations of BICM, 11 with sucrose, 13 with citric acid and 15 with QHCl. The response to NaCl in 13 cells following BICM application (0, 0.2, 2 and 10 mM) is shown in Figure 4(A) (filled circles). The response after 0.2 mM BICM was the same as in the control (0) condition for NaCl and for all other stimuli; this lack of effect serves as a control for the pressure injection, indicating that pressure alone is not producing the change in firing rate of these cells. For all statistical analyses of the effects of BICM concentration, therefore, we did not include the data for 0.2 mM. BICM produced a statistically significant dose-dependent increase in the response to NaCl in these cells [$F(2,36)$ =

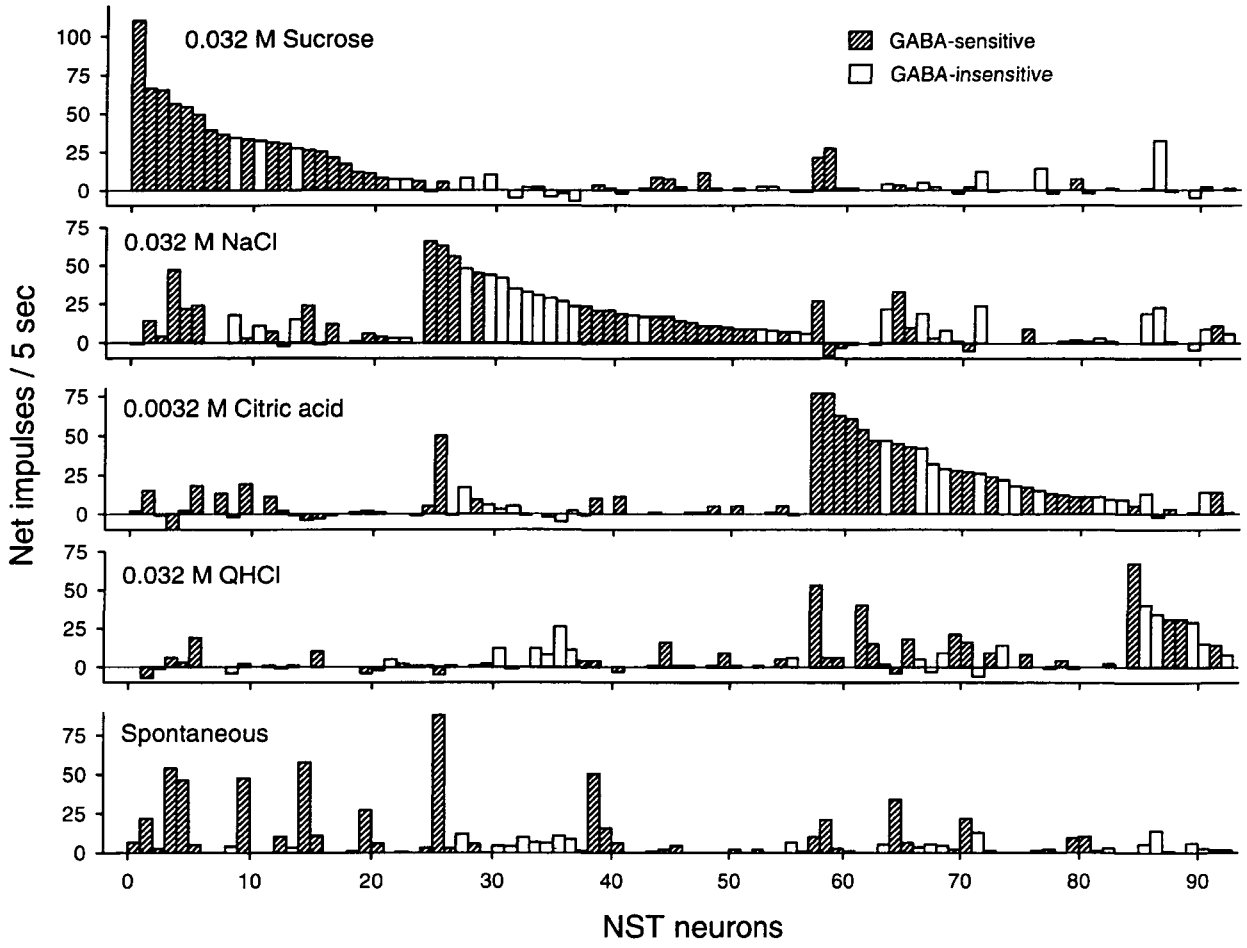

Figure 6 Responses (net imp/5 s) of 93 taste-responsive NST neurons to 0.032 M sucrose, 0.032 M NaCl, 0.0032 M citric acid, and 0.032 M QHCl (and their spontaneous discharge rates). Cells are arranged along the abscissa into four groups according to their best stimulus and within each group according to the response to that stimulus. At the extreme left are 24 sucrose-best neurons, followed by 33 NaCl-best cells, 27 acid-best neurons, and finally nine QHCl-best cells. Shaded bars indicate those cells that showed at least a 50% change in their response rate following NST microinjection of 5 mM GABA or 10 mM BICM (GABA-sensitive). Unshaded bars indicate cells that were unresponsive to GABA or BICM administration (GABA-insensitive).

17.80, $P < 0.0001$]. The effects of BICM on the spontaneous activity of these same 13 cells are shown by the open circles (Figure 4A), which depict the number of impulses occurring in the 5 s period before NaCl stimulation. Across the 26 neurons that showed dose-dependent effects of BICM on their responses to taste stimulation, the mean spontaneous activity increased significantly with increasing BICM concentration [$F(2,75) = 6.82$, $P = 0.0019$). Although blocking $GABA_A$ receptors significantly increased the spontaneous discharge of NST gustatory cells, this increase was considerably smaller than the enhancement of the cells' responses to taste stimulation (cf. open versus filled circles in Figure 4A–D).

The mean dose–response functions for the remaining taste stimuli are shown in Figure 4(B)–(D), where again it may be seen that responses following 0.2 mM BICM did not differ from baseline (0). Following 2 and 10 mM BICM there was a statistically significant dose-dependent increase in the evoked response to sucrose [$F(2,30) = 4.80$, $P = 0.016$], citric acid [$F(2,36) = 3.28$, $P = 0.049$] and QHCl [$F(2,42) = 10.27$, $P = 0.0002$]. In these samples of cells, most of the cells tested for NaCl responsiveness were NaCl-best (7/13), most of those tested for sucrose were sucrose-best (9/11), and most of those tested with citric acid were acid-best (7/13). Of the 15 cells tested with QHCl, five were NaCl-best and five were acid-best; only two were QHCl-best. Nevertheless, there was no indication that the effects of BICM were greater on stimuli tested on cells best tuned to that particular stimulus. For example, the mean increase following 10 mM BICM for NaCl in NaCl-best cells was 35.7 imp/5 s compared to 48.0 in other cell types. Similarly, the increase for sucrose in sucrose-best cells was 59.2 imp/5 s compared to 60.0 imp/5 s in the other cell types. Over all stimuli and cell types, the mean increase in response to a cell's best stimulus was 47.0 ± 7.9 imp/5 s ($n = 32$) and the response to those stimuli in other cell types ($n = 21$) increased by 39.8 ± 3.7 imp/5 s (two-tailed independent t-test, $t = 0.70$, $df = 51$, $P = 0.486$). Thus, there does not appear to be any differential

enhancement of the response of a cell's best stimulus following the release of GABA inhibition by BICM.

Despite this lack of differential enhancement, however, altering the level of GABAergic inhibition on these cells appears to produce a change in their breadth of tuning. Of the 26 neurons that were tested with BICM, 18 of these were tested with two or more tastants. Cells were always tested first with their best stimulus and then if they were broadly enough tuned and there was sufficient time, they were tested again with one or two other stimuli following the three concentrations of BICM. A comparison of the mean responses of 18 cells to their best and second-best stimuli after 0, 2 or 10 mM BICM is shown in Figure 5. Under baseline conditions, the response of the second-best stimulus was 56.2% of the response to the best stimulus; after 2 and 10 mM BICM, this proportion increased to 81.3 and 83.5% respectively. The breadth of tuning of these 18 cells to their two best stimuli was calculated using the entropy equation, modified for two stimuli (Smith and Travers, 1979; see Materials and methods). This would result in a cell that is completely specific with an entropy = 0.0 and one that is equally responsive to the two stimuli with an entropy = 1.0. Because these cells were necessarily broadly tuned in order to test more than one stimulus, these 18 cells were quite broadly responsive to their best two stimuli under baseline conditions (H = 0.88). Nevertheless, BICM had a significant effect on their breadth of responsiveness [$F(2,51)$ = 5.25, P = 0.0085]; both 2 and 10 mM BICM produced greater breadth of tuning (H = 0.97 and 0.96 respectively) to the two stimuli than occurred under baseline conditions (Duncan's multiple-range test, $P < 0.05$).

Response characteristics of GABA-sensitive NST taste neurons

Of the 93 taste-responsive neurons recorded from the NST, the responses of 57 of them were increased or decreased by >50% by 10 mM BICM or 5 mM GABA respectively. In order to assess whether these 57 GABA-sensitive cells differed in any systematic way from those taste-responsive cells that were not sensitive to GABA or BICM, we compared several response characteristics between these subpopulations of cells. The net responses to each taste stimulus (and the spontaneous activity) of these 93 cells are shown in Figure 6. In this figure, the neurons are arranged along the abscissa into four best-stimulus groups, and within each group according to the magnitude of the response to the cell's best stimulus. To the extreme left are the 24 sucrose-best neurons, arranged from the cell most responsive to the one least responsive to sucrose. Cells that were GABA-sensitive (i.e. whose responses were modified by either 5 mM GABA or 10 mM BICM by >50%) are shaded and those showing no effect of GABA or BICM are unshaded. The next 33 neurons are NaCl-best, arranged in the order of their response to NaCl. The 27 acid-best cells are next, arranged according to their response to citric acid. Finally, the nine QHCl-best neurons are depicted to the far right of the figure, arranged in descending order of their QHCl response. Sixty-one percent of these cells were sensitive to GABA or BICM. As a group, these 57 GABA-sensitive cells did not differ from the 36 GABA-insensitive cells on a number of measures.

First, the mean breadth of responsiveness (H) for the 57 GABA-sensitive cells was 0.439, which was not significantly different from that of the GABA-insensitive neurons (0.479; two-tailed independent t-test, $t = -0.733$, $df = 91$, $P = 0.466$). There were also no differences in this measure when the best-stimulus classes of cells were examined separately. Thus, the two groups of neurons defined by their GABA sensitivity did not differ significantly in their breadth of tuning. Similarly, although it appears from the figure that there might have been more sucrose-best cells that were GABA-sensitive than not, there was no significant difference in the numbers of cells in each best-stimulus category as a function of their GABA sensitivity (chi-square test, $\chi^2 = 4.97$, $df = 3$, $P > 0.05$). The cells also did not differ in their evoked response rates. The GABA-sensitive neurons had a mean net response (to sucrose, NaCl, citric acid and QHCl combined) of 11.63 ± 1.25 imp/5 s and the GABA-insensitive neurons had a mean net response of 8.91 ± 1.05 (two-tailed independent t-test, t = 1.542, df = 370, P = 0.128). However, GABA-sensitive cells had a mean spontaneous rate of 10.63 ± 2.43 imp/5 s and GABA-insensitive cells had a mean of 4.04 ± 0.66 imp/5 s; this difference was statistically significant (two-tailed independent t-test, t = 2.124, df =91, P = 0.036).

Discussion

GABAergic inhibition of taste-responsive cells

The results presented here demonstrate that GABA is an inhibitory neurotransmitter which modulates taste-responsive neurons in the NST. Microinjection of GABA into the NST produced, in 63% of taste-responsive NST neurons, a dose-dependent decrease in either the spontaneous activity or the activity stimulated by anodal current applied to the anterior tongue. The inhibition produced by GABA in the NST recovered within 3–5 min after application of the drug and there was no effect of the vehicle, indicating that the reduced discharge in these cells was not due to other factors such as pressure or toxicity. These data extend our earlier findings that microinjection of GABA *in vivo* decreases the response of hamster NST neurons to chemical stimulation of the anterior tongue (Smith *et al.*, 1994) by quantifying the proportion of NST cells that are GABA-sensitive and by demonstrating their dose dependency. These findings suggests that GABA release from intrinsic GABAergic neurons or from GABA-containing fibers that project into the NST can modulate synaptic transmission through this brainstem taste nucleus.

Whole-cell recording from *in vitro* slice preparations of both the rat (Wang and Bradley, 1993, 1995) and the hamster (Liu *et al.*, 1993) brainstem has also implicated GABA in inhibitory transmission within the gustatory zone of the rostral NST. Inhibitory postsynaptic potentials produced by electrical stimulation of the solitary tract were blocked or reduced by BICM and sometimes also by the $GABA_B$ antagonist phaclofen (Wang and Bradley, 1995). Electrical stimulation of the solitary tract would undoubtedly drive taste-sensitive afferent input to the rostral NST, but axons in the solitary tract carry tactile and temperature information as well (Travers, 1993; Travers and Norgren, 1995). The sensory properties of these afferent fibers are not determinable in an *in vitro* preparation. However, the present *in vivo* experiment makes it possible to implicate GABA in inhibitory transmission involving taste-responsive NST neurons. Both the spontaneous activity and taste-evoked (i.e. anodal current-stimulated) activity in NST cells was reduced by GABA in a dose-dependent manner. Previous *in vivo* data have shown a decrease in chemically evoked taste responses in the hamster NST following GABA microinjection (Smith *et al.*, 1994).

In a slice recording experiment in the hamster (Liu *et al.*, 1993), most GABA-mediated inhibition in the rostral NST was mediated by $GABA_A$ receptors. Of 57 GABA-sensitive cells recorded *in vitro*, only nine were inhibited by the $GABA_B$ agonist baclofen, whereas GABA-mediated responses of 48 were blocked by BICM. The $GABA_B$-mediated responses were found in cells located predominantly in the rostral lateral subdivision of the NST (Liu *et al.*, 1993). Although we did not test a $GABA_B$ antagonist in the present experiments, it appears from the *in vitro* work that the great majority of GABAergic inhibition in the rostral central subdivision of the hamster NST, which is the primary target of CT and glossopharyngeal nerve inputs (Whitehead and Frank, 1983), is mediated by $GABA_A$ receptors. In the rat NST, *in vitro* studies have shown that both $GABA_A$ and $GABA_B$ receptors are activated in many the same cells in the rostral NST (Wang and Bradley, 1995), so it is conceivable that both receptor types play a role in the GABAergic modulation of gustatory afferent activity.

Different gustatory neuron types within the NST do not appear to be differentially influenced by GABAergic mechanisms. BICM enhanced NST responses to each of the four basic tastants similarly (Figure 4) and there was no differential effect in different neuron types. Many individual neurons in the NST respond to taste solutions representing more than one taste quality (Smith and Bealer, 1975; McPheeters *et al.*, 1990), as is often the case in single afferent taste fibers (Frank, 1973; Frank *et al.*, 1988; Smith and Frank, 1993). Neurons in the hamster NST are more broadly tuned to the four basic taste stimuli than are the peripheral fibers of the CT nerve (Smith and Travers, 1979; Travers and Smith, 1979). These and other data showing convergence onto NST cells from different subpopulations of taste buds (Travers *et al.*, 1986; Sweazey and Smith, 1987; Travers and Norgren, 1991) suggest that individual neurons in the NST receive afferent input from two or more different peripheral nerve fibers. The present data suggest that all of the gustatory neuron types in the NST are inhibited via $GABA_A$ receptors regardless of their profiles of taste sensitivity or patterns of convergence. Further, the cells that were sensitive to GABA or BICM did not differ from those that were not in many of their basic response properties, including evoked response rate, best-stimulus classification or breadth of tuning. GABA-sensitive neurons, however, did have significantly higher rates of spontaneous discharge than those cells that were not modulated by GABAergic mechanisms.

Although the cells reported here (Figure 6) are somewhat less responsive and more narrowly tuned than those we have previously described in the hamster NST (Travers and Smith, 1979), this is probably due to several factors. First, the cells in the present study were recorded with 7–10 MΩ micropipettes rather than tungsten microelectrodes, which may bias the sample toward smaller, less spontaneously active cells. Second, the cells were isolated while a 0.33 Hz anodal stimulus was applied to the tongue, making it easier to find and isolate small, slowly firing cells. Finally, the use of citric acid rather than HCl as an acid stimulus produces more narrowly tuned cells because organic acids are more specific stimuli for the acid-best neurons than HCl (Smith *et al.*, 1983), which was used in previous experiments (Travers and Smith, 1979; Van Buskirk and Smith, 1981).

Tonic GABAergic inhibition in the NST

The gustatory responsiveness of cells in the rostral NST was enhanced by local injection of the $GABA_A$ antagonist BICM. This finding suggests that many taste-responsive cells in the NST are normally maintained under a tonic GABAergic inhibition. Although the sources of this inhibitory influence are currently unknown, a similar release from inhibition has been demonstrated in 400 μm thick coronal brainstem slices following BICM application (Liu *et al.*, 1993; Wang and Bradley, 1993), which indicates that much of this inhibitory influence resides within the brainstem. That some of this inhibition is also under control of mechanisms outside the brainstem has been demonstrated by the production of inhibition in NST taste cells following stimulation of the gustatory cortex (Smith *et al.*, 1998). Excitation of the ipsilateral insular cortex with either DL-homocysteic acid or electrical pulse trains produces inhibition in NST neurons that can be blocked with local microinjection of BICM. There are undoubtedly many additional sources of control over the GABAergic modulation of NST neurons that can serve to modify their responsiveness to gustatory stimulation under different conditions such as changes in blood glucose or insulin (Giza and Scott, 1983, 1987), following gastric distension (Glenn

and Erickson, 1976) or after taste aversion learning (Chang and Scott, 1984). For example, stimulation of the lateral hypothalamus produces inhibition of taste-responsive cells in the rat NST (Murzi *et al.*, 1986). In the caudal NST of the cat, hypothalamic stimulation produces a BICM-sensitive inhibition of cells receiving input from the carotid sinus and vagus nerves (Jordan *et al.*, 1988).

One of the functions of a tonic GABAergic inhibition may be to modulate the breadth of tuning of these cells. There was no significant difference in the breadth of tuning of GABA-sensitive ($H = 0.439$) and GABA-insensitive ($H = 0.479$) cells under baseline conditions. However, when the responses to the two most effective taste stimuli were examined in 18 cells that were tested with both stimuli following BICM administration, there was a significant increase in the breadth of responsiveness to these two stimuli (Figure 5). Removing the tonic influence of GABAergic inhibition resulted in the cells responding more equivalently to their best two stimuli. These data suggest that GABA may serve to modulate the breadth of responsiveness of gustatory neurons, sharpening their response to particular classes of stimuli. $GABA_A$-mediated inhibition plays a role in sharpening the responses of sensory neurons in other modalities, such as the somatosensory (Kyriazi *et al.*, 1996), auditory (Fuzessery and Hall, 1996; Suga *et al.*, 1997) and visual (Lazareva *et al.*, 1995; Sato *et al.*, 1996) systems. Further experiments in which all four taste stimuli are examined following both GABA and BICM administration could clearly demonstrate the degree to which GABAergic mechanisms contribute to shaping the responsiveness of these cells. Since less than one-third of taste-responsive neurons in the NST project rostrally to the parabrachial nuclei (Ogawa and Kaisaku, 1982; Whitehead, 1990), it would be interesting also to determine whether these projection neurons are disproportionately modulated by GABA.

Gustatory and visceral processing in the NST

Taste is considered a special visceral afferent system and shares many properties with the general visceral (cardiovascular, respiratory and gastrointestinal) afferent systems (Norgren, 1985). Afferent fibers from all of these visceral systems terminate in a rostrocaudal sequence within the NST, and the processing of information within them has several elements in common. These visceral afferent systems all appear to use glutamate as an excitatory neurotransmitter between peripheral fibers and the NST (Drewe *et al.*, 1990; Glaum and Miller, 1992; Vardhan *et al.*, 1993; Andresen and Yang, 1994; Felder and Mifflin, 1994; Wang and Bradley, 1995; Bradley *et al.*, 1996; Li and Smith, 1997), all are modulated by substance P (Yamamoto and Lagercrantz, 1985; Kubo and Kihara, 1987; King *et al.*, 1993; Bradley *et al.*, 1996; Davis and Smith, 1997), and all employ a GABAergic inhibitory mechanism for modulation of afferent information (Bousquet *et al.*, 1982; Bennett *et al.*, 1987; Jordan *et al.*, 1988; Wang and Bieger, 1991; Liu *et al.*, 1993; Wang and Bradley, 1993; Smith *et al.*, 1994; Bradley *et al.*, 1996; present results). Immunocytochemical studies have shown that cells in both caudal and rostral portions of the NST contain GABA or its principal degradative enzymes (Blessing *et al.*, 1984; Maley and Newton, 1985; Lasiter and Kachele, 1988; Davis, 1993). Thus in many respects the synaptic organization of the gustatory system has several features in common with other visceral systems within the NST. These parallels between the so-called special and the general visceral afferent systems suggest that it may be advantageous to view taste as one of several visceral sensory modalities, specifically providing input important for decisions about the ingestion of substances into the internal milieu.

Conclusion

In conclusion, the present study offers direct functional evidence that GABAergic mechanisms mediate inhibitory synaptic transmission onto taste-responsive NST neurons. Microinjection of GABA into the NST produces a dose-dependent inhibition of the activity of ~63% of taste-responsive neurons. There is a tonic $GABA_A$-mediated inhibition present in 61% of taste-responsive neurons that can be decreased in a dose-dependent manner by microinjection of the $GABA_A$ receptor antagonist BICM. These mechanisms provide a substrate for the modulation of gustatory responses in this brainstem taste nucleus and for altering the breadth of the cells' responsiveness to gustatory stimulation.

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References

Allen, W.F. (1923) *Origin and distribution of the tractus solitarius in the guinea pig.* J. Comp. Neurol., 35, 171–204.

Andresen, M.C. and **Yang, M.** (1994) *Excitatory amino acid receptors and afferent synaptic transmission in the nucleus tractus solitarius.* In Barraco, I.R.A. (ed.), Nucleus of the Solitary Tract. CRC Press, Boca Raton, FL, pp. 187–192.

Åström, K.E. (1953) *On the central course of afferent fibres in the trigeminal, facial, glossopharyngeal, and vagal nerves and their nuclei in the mouse.* Acta Physiol. Scand., 29, 209–320.

Beckstead, R.M. and **Norgren, R.** (1979) *An autoradiographic examination of the central distribution of the trigeminal, facial, glossopharyngeal, and vagal nerves in the monkey.* J. Comp. Neurol., 184, 455–472.

Bennett, J.A., McWilliam, P.N. and **Shepheard, S.L.** (1987) *A*

γ-aminobutyric-acid-mediated inhibition of neurones in the nucleus tractus solitarius of the cat. J. Physiol., 392, 417–430.

Blessing, W.W., Oertel, W.H. and **Willoughby, J.O.** (1984) *Glutamic acid decarboxylase immunoreactivity is present in perikarya of neurons in nucleus tractus solitarius of rat.* Brain Res., 322, 346–350.

Bousquet, P., Feldman, J., Bloch, R. and **Schwartz, J.** (1982) *Evidence for a neuromodulatory role of GABA at the first synapse of the baroreceptor reflex pathway: effects of GABA derivatives injected into NTS.* Nauyn-Schmiedeberg's Arch. Pharmacol., 319, 168–171.

Bradley, R.M., King, M.S., Wang, L. and **Shu, X.** (1996) *Neurotransmitter and neuromodulator activity in the gustatory zone of the nucleus tractus solitarius.* Chem. Senses, 21, 377–385.

Chang, F.-C.T. and **Scott, T.R.** (1984) *Conditioned taste aversions modify neural responses in the rat nucleus tractus solitarius.* J. Neurosci., 4, 1850–1862.

Davis, B.J. (1993) *GABA-like immunoreactivity in the gustatory zone of the nucleus of the solitary tract in the hamster: light and electron microscopic studies.* Brain Res. Bull., 30, 69–77.

Davis, B.J. and **Smith, D.V.** (1997) *Substance P modulates taste responses in the nucleus of the solitary tract of the hamster.* NeuroReport, 8, 1723–1727.

DiLorenzo, P.M. (1988) *Taste responses in the parabrachial pons of decerebrate rats.* J. Neurophysiol., 59, 1871–1887.

Drewe, J.A., Miles, R. and **Kunze, D.L.** (1990) *Excitatory amino acid receptors of guinea pig medial nucleus tractus solitarius neurons.* Am. J. Physiol., 259 (Heart Circ. Physiol. 28), H1389–H1395.

Duncan, H.J. and **Smith, D.V.** (1992) *Concentration–response functions for thirty chemical stimuli in the hamster solitary nucleus.* Chem. Senses, 17, 616.

Erickson, R.P. (1966) *Non-traumatic headholders for mammals.* Physiol. Behav., 1, 97–98.

Felder, R.B. and **Mifflin, S.W.** (1994) *Baroreceptor and chemoreceptor afferent processing in the solitary tract nucleus.* In Barraco, I.R.A. (ed.), Nucleus of the Solitary Tract. CRC Press, Boca Raton, FL, pp. 169–186.

Frank, M.E. (1973) *An analysis of hamster afferent taste nerve response functions.* J. Gen. Physiol., 61, 588–618.

Frank, M.E., Bieber, S.L. and **Smith, D.V.** (1988) *The organization of taste sensibilities in hamster chorda tympani nerve fibers.* J. Gen. Physiol., 91, 861–896.

Fuzessery, Z.M. and **Hall, J.C.** (1996) *Role of GABA in shaping frequency tuning and creating FM sweep selectivity in the inferior colliculus.* J. Neurophysiol., 76, 1059–1073.

Giza, B.K. and **Scott, T.R.** (1983) *Blood glucose selectively affects taste-evoked activity in rat nucleus tractus solitarius.* Physiol. Behav., 31, 643–650.

Giza, B.K. and **Scott, T.R.** (1987) *Intravenous insulin infusions in rats decrease gustatory-evoked responses to sugars.* Am. J. Physiol., 252 (Regul. Integ. Comp. Physiol. 21), R994–R1002.

Glaum, S.R. and **Miller, R.J.** (1992) *Metabotropic glutamate receptors mediate excitatory transmission in the nucleus of the solitary tract.* J. Neurosci., 12, 2251–2258.

Glenn, J.F. and **Erickson, R.P.** (1976) *Gastric modulation of gustatory afferent activity.* Physiol. Behav., 16, 561–568.

Hamilton, R.B. and **Norgren, R.** (1984) *Central projections of gustatory nerves in the rat.* J. Comp. Neurol., 222, 560–577.

Hanamori, T. and **Smith, D.V.** (1989) *Gustatory innervation in the rabbit: central distribution of sensory and motor components of the chorda tympani, glossopharyngeal and superior laryngeal nerves.* J. Comp. Neurol., 282, 1–14.

Hayama, T., Ito, S. and **Ogawa, H.** (1985) *Responses of solitary tract nucleus neurons to taste and mechanical stimulation of the oral cavity in decerebrate rats.* Exp. Brain Res., 60, 235–242.

Hermann, G. and **Novin, D.** (1980) *Morphine inhibition of parabrachial taste units reversed by naloxone.* Brain Res. Bull., 5(Suppl. 4), 169–173.

Herness, M.S. (1987) *Effect of amiloride on bulk flow and iontophoretic taste stimuli in the hamster.* J. Comp. Physiol., A160, 281–288.

Jacquin, T., Denavit-Saubie, M. and **Champagnat, J.** (1989) *Substance P and serotonin mutually reverse their excitatory effects in the rat nucleus tractus solitarius.* Brain Res., 502, 214–222.

Jordan, D., Mifflin, S.W. and **Spyer, K.M.** (1988) *Hypothalamic inhibition of neurones in the nucleus tractus solitarius of the cat is GABA mediated.* J. Physiol., 399, 389–404.

King, M.S., Wang, L. and **Bradley, R.M.** (1993) *Substance P excites neurons in the gustatory zone of the rat nucleus tractus solitarius.* Brain Res., 619, 120–130.

Kubo, T. and **Kihara, M.** (1987) *Blood pressure modulation by substance P in the rat nucleus tractus solitarius.* Brain Res., 413, 379–383.

Kyriazi, H.T., Carvell, G.E., Brumberg, J.C. and **Simons, D.J.** (1996) *Quantitative effects of GABA and bicuculline methiodide on receptive-field properties of neurons in real and simulated whisker barrels.* J. Neurophysiol., 75, 547–560.

Lasiter, P.S. and **Kachele, D.L.** (1988) *Organization of GABA and GABA-transaminase containing neurons in the gustatory zone of the nucleus of the solitary tract.* Brain Res. Bull., 21, 623–636.

Lazareva, N.A., Shevelev, I.A., Eysel, U.T. and **Sharaev, G.A.** (1995) *Bicuculline and orientation tuning of neurons of the visual cortex.* Neurophysiol., 27, 42–49.

Li, C.-S. and **Smith, D.V.** (1997) *Glutamate receptor antagonists block gustatory afferent input to the nucleus of the solitary tract.* J. Neurophysiol., 77, 1514–1525.

Liu, H., Behbehani, M.M. and **Smith, D.V.** (1993) *The influence of GABA on cells in the gustatory region of the hamster solitary nucleus.* Chem. Senses, 18, 285–305.

Maley, B. and **Newton, B.W.** (1985) *Immunohistochemistry of gamma-aminobutyric acid in the cat nucleus tractus solitarius.* Brain Res., 330, 364–368.

Mark, G.P., Scott, T.R., Chang, F.-C.T. and **Grill, H.J.** (1988) *Taste responses in the nucleus tractus solitarius of the chronic decerebrate rat.* Brain Res., 443, 137–148.

McPheeters, M., Hettinger, T.P., Nuding, S.C., Savoy, L.D., Whitehead, M.C. and **Frank, M.E.** (1990) *Taste-responsive neurons and their locations in the solitary nucleus of the hamster.* Neuroscience, 34, 745–758.

Murzi, E., Hernandez, L. and **Baptista, T.** (1986) *Lateral hypothalamic sites eliciting eating affect medullary taste neurons in rats.* Physiol. Behav., 36, 829–834.

Norgren, R. (1985) *Taste and the autonomic nervous system.* Chem. Senses, 10, 143–161.

Norgren, R. and **Leonard, C.M.** (1973) *Ascending central gustatory pathways.* J. Comp. Neurol., 150, 217–238.

Ogawa, H. and **Kaisaku, J.** (1982) *Physiological characteristics of the*

solitario-parabrachial relay neurons with tongue afferent inputs in rats. Exp. Brain Res., 48, 362–368.

Sato, H., Katsuyama, N., Tamura, H., Hata, Y. and **Tsumoto, T.** (1996) *Mechanisms underlying orientation selectivity of neurons in the primary visual cortex of the macaque.* J. Physiol., 494, 757–771.

Smith, D.V. and **Bealer, S.L.** (1975) *Sensitivity of the rat gustatory system to the rate of stimulus onset.* Physiol. Behav., 15, 303–314.

Smith, D.V. and **Travers, J.B.** (1979) *A metric for the breadth of tuning of gustatory neurons.* Chem. Sens. Flav., 4, 215–229.

Smith, D.V. and **Frank, M.E.** (1993) *Sensory coding by peripheral taste fibers.* In Simon, S.A. and Roper, S.D. (eds), Mechanisms of Taste Transduction. CRC Press, Boca Raton FL, pp. 295–338.

Smith, D.V., Bealer, S.L. and **Van Buskirk, R.L.** (1978) *Adaptation and recovery of the rat chorda tympani response to NaCl.* Physiol. Behav., 20, 629–636.

Smith, D.V., Travers, J.B. and **Van Buskirk, R.L.** (1979) *Brainstem correlates of gustatory similarity in the hamster.* Brain Res. Bull., 4, 359–372.

Smith, D.V., Van Buskirk, R.L., Travers, J.B. and **Bieber, S.L.** (1983) *Gustatory neuron types in the hamster brainstem.* J. Neurophysiol., 50, 522–540.

Smith, D.V., Liu, H. and **Vogt, M.B.** (1994) *Neural coding of aversive and appetitive gustatory stimuli: interactions in the hamster brain stem.* Physiol. Behav., 56, 1189–1196.

Smith, D.V., Li, C.-S. and **Davis, B.J.** (1998) *Excitatory and inhibitory modulation of taste responses in the hamster brainstem.* In Murphy, C. and Greer, C. (eds), Olfaction and Taste XII. New York Academy of Sciences, New York, in press.

Suga, N., Zhang, Y.F. and **Yan, J.** (1997) *Sharpening of frequency tuning by inhibition in the thalamic auditory nucleus of the moustached bat.* J. Neurophysiol., 77, 2098–2114.

Sweazey, R.D. and **Smith, D.V.** (1987) *Convergence onto hamster medullary taste neurons.* Brain Res., 408, 173–184.

Travers, J.B. and **Smith, D.V.** (1979) *Gustatory sensitivities in neurons of the hamster nucleus tractus solitarius.* Sens. Processes, 3, 1–26.

Travers, S.P. (1993) *Orosensory processing in neural systems of the nucleus of the solitary tract.* In Simon, S.A. and Roper, S.D. (eds), Mechanisms of Taste Transduction. CRC Press, Boca Raton FL, pp. 339–394.

Travers, S.P. and **Norgren, R.** (1991) *Coding the sweet taste in the nucleus of the solitary tract: Differential roles for anterior tongue and nasoincisor duct gustatory receptors in the rat.* J. Neurophysiol., 65, 1372–1380.

Travers, S.P. and **Norgren, R.** (1995) *Organization of orosensory responses in the nucleus of the solitary tract of the rat.* J. Neurophysiol., 73, 2144–2162.

Travers, S.P., Pfaffmann, C. and **Norgren, R.** (1986) *Convergence of lingual and palatal gustatory neural activity in the nucleus of the solitary tract.* Brain Res., 365, 305–320.

Van Buskirk, R.L. and **Smith, D.V.** (1981) *Taste sensitivity of hamster parabrachial pontine neurons.* J. Neurophysiol., 45, 144–171.

Vardhan, A., Kachroo, A. and **Supru, H.N.** (1993) *Excitatory amino acid receptors in commissural nucleus of the NTS mediate carotid chemoreceptor responses.* Am. J. Physiol., 264 (Regul. Integ. Comp. Physiol. 33), R41–R50.

Vogt, M.B. and **Smith, D.V.** (1993) *Responses of single hamster parabrachial neurons to binary taste mixtures: mutual suppression between sucrose and QHCl.* J. Neurophysiol., 69, 658–668.

Wang, L. and **Bradley, R.M.** (1993) *Influence of GABA on neurons of the gustatory zone of the rat nucleus of the solitary tract.* Brain Res., 616, 144–153.

Wang, L. and **Bradley, R.M.** (1995) *In vitro study of afferent synaptic transmission in the rostral gustatory zone of the rat nucleus of the solitary tract.* Brain Res., 702, 188–198.

Wang, Y.T. and **Bieger, D.** (1991) *Role of soliltarial GABAergic mechanisms in control of swallowing.* Am. J. Physiol., 261 (Regul. Integ. Comp. Physiol. 30), R639–R646.

Whitehead, C.M. (1990) *Subdivisions and neuron types of the nucleus of the solitary tract that project to the parabrachial nucleus in the hamster.* J. Comp. Neurol., 301, 554–574.

Whitehead, M.C. and **Frank, M.E.** (1983) *Anatomy of the gustatory system in the hamster: central projections of the chorda tympani and the lingual nerve.* J. Comp. Neurol., 220, 378–395.

Yamamoto, Y. and **Lagercrantz, H.** (1985) *Some effects of substance P on central respiratory control in rabbit pups.* Acta Physiol. Scand., 124, 449–455.

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