

RESEARCH PAPER

Substance P as a putative efferent transmitter mediates GABAergic inhibition in mouse taste buds

Correspondence Anthony Y Huang, Department of Anatomy, Southern Illinois University School of Medicine, 1135 Lincoln Dr., Carbondale, IL 62901, USA. E-mail: ahuang@siu.edu

Received 13 July 2017; **Revised** 21 November 2017; **Accepted** 23 December 2017

Anthony Y Huang^{1,2}  and Sandy Y Wu¹

¹Department of Anatomy, Southern Illinois University School of Medicine, Carbondale, IL, USA, and ²Center for Integrated Research in Cognitive and Neural Science, Southern Illinois University School of Medicine, Carbondale, IL, USA

BACKGROUND AND PURPOSE

Capsaicin-mediated modulation of taste nerve responses is thought to be produced indirectly by the actions of neuropeptides, for example, CGRP and substance P (SP), on taste cells implying they play a role in taste sensitivity. During the processing of gustatory information in taste buds, CGRP shapes peripheral taste signals *via* serotonergic signalling. The underlying assumption has been that SP exerts its effects on taste transmitter secretion in taste buds of mice.

EXPERIMENTAL APPROACH

To test this assumption, we investigated the net effect of SP on taste-evoked ATP secretion from mouse taste buds, using functional calcium imaging with CHO cells expressing high-affinity transmitter receptors as cellular biosensors.

KEY RESULTS

Our results showed that SP elicited PLC activation-dependent intracellular Ca²⁺ transients in taste cells *via* neurokinin 1 receptors, most likely on glutamate–aspartate transporter-expressing Type I cells. Furthermore, SP caused Type I cells to secrete GABA.

CONCLUSION AND IMPLICATIONS

Combined with the recent findings that GABA depresses taste-evoked ATP secretion, the current results indicate that SP elicited secretion of GABA, which provided negative feedback onto Type II (receptor) cells to reduce taste-evoked ATP secretion. These findings are consistent with a role for SP as an inhibitory transmitter that shapes the peripheral taste signals, *via* GABAergic signalling, during the processing of gustatory information in taste buds. Notably, the results suggest that SP is intimately associated with GABA in mammalian taste signal processing and demonstrate an unanticipated route for sensory information flow within the taste bud.

Abbreviations

GLAST, glutamate–aspartate transporter; IP₃, inositol 1,4,5-trisphosphate; NK1, neurokinin 1 receptor; TRPV1, transient receptor potential vanilloid 1

Introduction

Taste signal transmission consists of cell–cell circuits *via* transmitters secreted by separate morphotypes of taste cells; these transmitters include 5-HT (Kaya *et al.*, 2004; Huang *et al.*, 2005; Jaber *et al.*, 2014), **ATP** (Finger *et al.*, 2005; Huang *et al.*, 2007; 2009; Murata *et al.*, 2010), noradrenaline (NA) (Dvoryanchikov *et al.*, 2007; Huang *et al.*, 2008a) and **GABA** (Cao *et al.*, 2009; Dvoryanchikov *et al.*, 2011; Huang *et al.*, 2012). Responding directly to sour (acid) taste stimuli (Huang *et al.*, 2006, 2008b; Chang *et al.*, 2010) and high concentrations of KCl (Huang *et al.*, 2007; Oka *et al.*, 2013), Type III (presynaptic) cells release 5-HT, NA and GABA (Huang *et al.*, 2007; 2008b; 2011a). Upon sweet or bitter taste stimulation, Type II (receptor) cells secrete ATP *via* membrane channels (Huang *et al.*, 2007; Taruno *et al.*, 2013). ATP acts on two targets within taste buds: (i) gustatory afferent fibres that propagate taste signals to the brain (Finger *et al.*, 2005; Huang *et al.*, 2011b; Vandenbeuch *et al.*, 2015); and (ii) Type III (presynaptic) cells, which release 5-HT and NA when stimulated (Huang *et al.*, 2008b; 2009).

Early electrophysiological data revealed that capsaicin, an agonist of transient receptor potential vanilloid 1 (**TRPV1**) channels, modulates taste nerve responses to other taste compounds (Wang *et al.*, 1995; Osada *et al.*, 1997; Roper, 2014). This modulation was interpreted as being produced indirectly, *via* stimulation of capsaicin-sensitive nerve terminals in the lingual epithelium that release stored neuropeptides [e.g. CGRP and **substance P (SP)**] onto taste cells by an axon reflex (Holzer, 1988; Maggi and Meli, 1988; Wang *et al.*, 1995; Simon *et al.*, 2003; Sato *et al.*, 2012). Indeed, immunohistochemistry revealed that neuropeptides and TRPV1 coexist in the sensory neurons innervating the circumvallate papillae (Ishida *et al.*, 2002). Despite these findings, our understanding of peptidergic modulation is limited.

SP, an 11 amino acid peptide member of the tachykinin family (Karagiannides *et al.*, 2008), transmits nociceptive sensations. SP is produced in chemosensory neurons that contribute to chemesthesis, the general chemical sensitivity of mucus membranes in oronasal cavities perceived as pungency, irritation or heat (Green, 2012; Roper, 2014). In addition to evoking nociceptive responses to chemical irritants as part of a common chemical sense, the extensive innervation of those areas where taste buds are expected to be present may contribute to specific taste-related chemical sensitivities (Nagy *et al.*, 1982; Yamasaki *et al.*, 1984; Montavon *et al.*, 1996; Ishida *et al.*, 2002; Sato *et al.*, 2012). Several studies of polymodal nociceptors in taste buds, using electrophysiology (Simons *et al.*, 2003; Talavera *et al.*, 2005), Ca^{2+} imaging (Grant, 2012; Huang and Wu, 2015) and psychophysical testing (Simons *et al.*, 2002; Kapaun and Dando, 2016), support the notion that oral nociceptive inputs on gustatory transmission may underlie the ability to modulate the perceived intensity of some taste qualities.

Here, we used Ca^{2+} imaging with cellular biosensors to test whether SP acts on taste cells to alter taste bud output, that is, ATP secretion. Our findings show that SP can shape the peripheral taste signals before their transmission across gustatory fibres.

Methods

Ethical approval

All procedures conducted followed the guidelines of the National Institute of Health (NIH Office of Animal Care and Use) and were approved by the Southern Illinois University Animal Care and Use Committee (Animal Welfare Assurance Number, A-3078-01). Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath and Lilley, 2015).

Experimental animals

We used adult male C57BL/6J (<https://www.jax.org/strain/000664>) ($n = 51$) and GAD67-GFP (<https://www.jax.org/strain/006334>) mice ($n = 5$) obtained from Jackson Laboratory. Animals (6–8 weeks old; average weight 23–26 g) were fed *ad libitum* and housed in a temperature-controlled room ($22 \pm 1^\circ\text{C}$) under artificial illumination (lights on from 05:00 to 17:00 h) and 55% relative humidity. Mice were killed by exposure to CO_2 followed by cervical dislocation. This procedure minimizes distress (NIH guideline, https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_euthanasia_adult.pdf). Tongues were then removed for further dissection.

Isolated taste buds and/or taste cells

Dispersed taste buds and taste cells were isolated as described previously (Huang and Wu, 2015; 2016). Briefly, we injected an enzyme cocktail containing $1 \text{ mg}\cdot\text{mL}^{-1}$ collagenase A (Roche, Indianapolis, IN, USA), $2.5 \text{ mg}\cdot\text{mL}^{-1}$ dispase II (Roche), $2 \text{ mg}\cdot\text{mL}^{-1}$ elastase (Worthington, Lakewood, NJ, USA) and $1 \text{ mg}\cdot\text{mL}^{-1}$ trypsin inhibitor (Sigma, St. Louis, MO, USA) beneath the epithelium surrounding circumvallate papillae and removed the lingual epithelium. Isolated taste buds were collected in glass micropipettes and transferred to a recording chamber (Warner Instrument, Hamden, CT, USA) with a glass coverslip base. To isolate single taste cells, individual taste buds were triturated in the recording chamber using a glass micropipette.

Ca^{2+} imaging

Taste cells located in the shallow recording chamber were loaded with $5 \mu\text{M}$ Fura 2-AM (Invitrogen, Life Technologies, USA) following their isolation. During the experiments, taste buds and taste cells were continuously perfused with Tyrode's buffer, composed of the following (in mM): 140 NaCl, 5 KCl, 2 CaCl_2 , 1 MgCl_2 , 10 HEPES, 10 glucose, 10 Na-pyruvate, 5 NaHCO_3 , pH 7.2, 310–320 Osm. For Ca^{2+} -free Tyrode's solution, MgCl_2 was substituted for CaCl_2 . The conventional Ca^{2+} imaging recording was carried out using Indec Workbench v.6 software (INDEC Biosystem, Mountain View, CA, USA). Fura 2-loaded cells were excited at 340 and 380 nm, and emission images were collected at $\geq 510 \text{ nm}$. The ratio of F340/F380 was converted to approximate $[\text{Ca}^{2+}]_i$ as described by Grynkiewicz *et al.* (1985). The fluorescence ratios of free and Ca^{2+} -bound Fura 2 at 340 nm and the fluorescence ratios of free and Ca^{2+} -bound Fura 2 at 380 nm were determined using a Fura 2 Calcium Imaging Calibration Kit (Invitrogen, Life Technologies) (e.g. Huang *et al.*, 2011a). The average baseline (resting) Ca^{2+} of taste cells in these experiments was $160 \pm 42 \text{ nM}$ ($n = 39$ cells), which corresponds well with

values reported previously (Huang *et al.*, 2011a; Huang and Wu, 2015; 2016). Our criteria for accepting Ca^{2+} responses for analysis are described fully in the previous publications (Huang *et al.*, 2012; Huang and Wu, 2015). In brief, responses were quantified as peak minus baseline $[\text{Ca}^{2+}]$ (i.e. $\Delta[\text{Ca}^{2+}]$). We accepted Ca^{2+} responses only if they could be elicited repetitively in the same cell by the same stimulus, and evoked responses were at least 2× baseline $[\text{Ca}^{2+}]$ fluctuation.

Isolated taste buds and taste cells were stimulated by bath-perfusion of ATP, KCl (50 mM, substituted equimolar for NaCl), taste mix and SP (up to 100 nM). Stimuli were bath-applied for 30 s followed by a return to the buffer perfusion for at least 3–5 min between trials. This perfusion paradigm allows the stimuli to mix thoroughly within the recording chamber and to reach a final concentration in the bath followed by a complete washout. Moreover, this procedure produced reliable and consistent stimulus-evoked responses from isolated taste cells, as described in Huang *et al.* (2009, 2011a) and Huang and Wu (2015, 2016). All experiments were conducted at room temperature.

Stimuli and solutions

All stimuli and pharmacological agents were made up in Tyrode's buffer. Isolated taste buds and taste cells were stimulated by bath-perfusion of KCl (50 mM, substituted equimolar for NaCl), taste mix (10 μM **cycloheximide**, 1 mM sucralose, 0.1 mM SC45647, 1 mM denatonium) and SP (10 nM). Stimuli were bath-applied for 30 s followed by a return to buffer perfusion for at least 3–5 min between trials. This perfusion paradigm allows the stimuli to mix thoroughly within the recording chamber followed by a sufficient washout. Moreover, this procedure produced reliable and consistent stimulus-evoked responses from isolated taste cells.

Biosensor cells

GABA biosensors consisted of CHO cells stably expressing heteromeric **GABA_B receptors** (**GABA_{B1}** and **GABA_{B2}** receptors) and the G-protein α subunit, $G_{\alpha\text{qo5}}$ (Huang *et al.*, 2011a). We also used CHO cells expressing **P2X2/P2X3** receptors (hereafter called ATP biosensors) (Huang *et al.*, 2007; Huang 2011b). An aliquot of 5 μM Fura 2-loaded biosensor cells (GABA or ATP) was transferred to the recording chamber containing isolated taste buds. Immediately after GABA or ATP biosensors had settled to the bottom of the chamber, they were probed with a single application of GABA (100 nM) or ATP (0.3 to 1 μM). Highly sensitive biosensors were drawn onto a fire-polished glass micropipette to test transmitter release from taste buds (Figure 5A).

Controls to establish the sensitivity and selectivity of GABA (Huang *et al.*, 2011a) and ATP biosensors (Huang *et al.*, 2007) were as described previously. Biosensor cells alone did not show the Ca^{2+} mobilization in response to bath-applied KCl (50 mM) or taste stimuli used in the study as described previously (Huang *et al.*, 2007; 2011a). Lastly, we verified that the Ca^{2+} responses of GABA biosensors were not affected by the pharmacological agents used in this study, including SP and **RP67580**, a **neurokinin 1 (NK₁) receptor** antagonist, apart from **CGP55845**, a GABA_B receptor antagonist, which was used to corroborate responses in biosensor cells generated by GABA receptors.

Importantly, we verified that GABA biosensor cells retained full receptor agonist sensitivity (and thus Ca^{2+} responses) in the presence of RP67580 used in this study (see below Figure 5B).

Double immunostaining for NK₁ receptors, glutamate–aspartate transporter (GLAST) and GABA

Isolated taste buds were fixed in 4% paraformaldehyde in PBS for 10–20 min at 4°C and immunostained following the procedures as described in Huang and Wu (2015, 2016). **GLAST** is exclusively expressed in Type I cells in vallate taste buds (Lawton *et al.*, 2000; Vandenbeuch *et al.*, 2013; Yoshida *et al.*, 2015). To localize two different antigens, NK₁ receptor/GLAST and/or GABA/GLAST, in isolated taste buds, a mixture of two primary antibodies generated in two different species against different antigens was used. We used rabbit anti-NK₁ (1:200; catalogue #ATR-001; Alomone Labs, Jerusalem, Israel) for NK₁ receptors (Muñoz *et al.*, 2011), guinea pig anti-GLAST (1:200; catalogue #AB-2571717; Frontier Institute, Hokkaido, Japan) for Type I cells (Yoshida *et al.*, 2015) and rabbit anti-GABA (1:1500; catalogue #A2050; Sigma) for taste cell transmitter (Dvoryanchikov *et al.*, 2011; Huang *et al.*, 2011a). The immunoreactivity of NK₁ receptors and GABA was determined by incubation with biotin-conjugated donkey anti-rabbit IgG (1:200; catalogue #711-065-152; Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) followed by a tyramide reaction using a tyramide signal amplification (TSA) kit with Alexa Fluor 568 (catalogue #T-20934; Molecular Probes, Life technologies, Eugene, OR, USA). GLAST primary antibody binding was revealed using a donkey anti-guinea pig secondary antibody conjugated with Alexa Fluor 488 (1:200; catalogue #706-545-148; Jackson ImmunoResearch Laboratories Inc.).

Briefly, fixed taste buds were washed with PBS and were blocked with 10% normal donkey serum for 1 h at room temperature. Primary antibodies were applied to taste buds for overnight at room temperature. Thereafter, for tyramide-based detection, after incubation in the primary antiserum, the taste buds were washed three times for 10 min each in PBS followed by incubation in a biotin-conjugated donkey anti-rabbit secondary antibody (1:200) for 1 h at room temperature. The solutions from a Vectastain ABC kit (Vector Labs) were applied to the sections for 1 h at room temperature. After washes in PBS, a tyramide reaction using a TSA kit with Alexa Fluor 568 (Molecular Probes, Life technologies) was performed for 15 min. Double-immunostaining was performed after the tyramide reaction. Donkey anti-guinea pig secondary antibody (1:200) was applied to the taste buds for 0.5 h at room temperature. Taste buds were washed in PBS and coverslipped with ProLong Diamond Antifade Fluoromount (catalogue #P36961; Invitrogen, Life technologies). Negative controls were processed with the omission of primary antibodies. Alternatively, synthesizing NK₁ receptor control antigen (0.1 $\mu\text{g}\cdot\text{mg}^{-1}$; Alomone Labs) and GLAST antigen proteins (1 $\mu\text{g}\cdot\text{mL}^{-1}$; Frontier Institute) were used in a blocking assay. In these control studies, neither NK₁ receptor, GLAST nor GABA immunoreactivity was present. Images were obtained

with an Olympus Optical laser-scanning confocal microscope using Fluoview software.

Alternatively, taste buds, isolated from circumvallate papillae of GAD67-GFP mice, were fixed for 10 min in 4% paraformaldehyde in 0.01 M PBS (pH 7.2–7.4) and immunostained following the procedures described in Dvoryanchikov *et al.* (2011) and Huang *et al.* (2011a). Taste buds were then rinsed three times in PBS and incubated with 10% normal goat serum for 1 h at room temperature. We used rabbit anti-GABA (1:1500; catalogue #A2050; Sigma) for 60–90 min at room temperature. Immunoreactivity of GABA was determined by incubation with Alexa Fluor 568-conjugated goat anti-rabbit IgG (1:200; catalogue #A-11011; Molecular Probes, Life Technologies) and then washed again three times in PBS. Negative controls were processed in parallel in every experiment with primary antibody omitted. No nonspecific fluorescence was detected. Images were obtained with an Olympus Optical laser-scanning confocal microscope using Fluoview software.

Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis *et al.*, 2015). The experimental procedures or treatment and data analyses were carried out with randomization and blinding. In these studies, we performed a minimum of five independent experiments, where individual data points were based on at least technical duplicates each. Following acquisition, Ca^{2+} imaging recordings were viewed and analysed using Indec Workbench v.6 software (INDEC Biosystem). Converting ΔCa^{2+} to approximate $[\text{Ca}^{2+}]_i$ was routinely calculated as described by Grynkiewicz *et al.* (1985). For statistical analyses, we used non-parametric methods, including normalization of data, to reduce variability of baseline between independent experiments. The one-way ANOVA and non-parametric analyses were followed by Tukey's *post hoc* tests when F reached significance and Student's paired *t*-tests were applied to determine whether changes in responses following a given treatment were

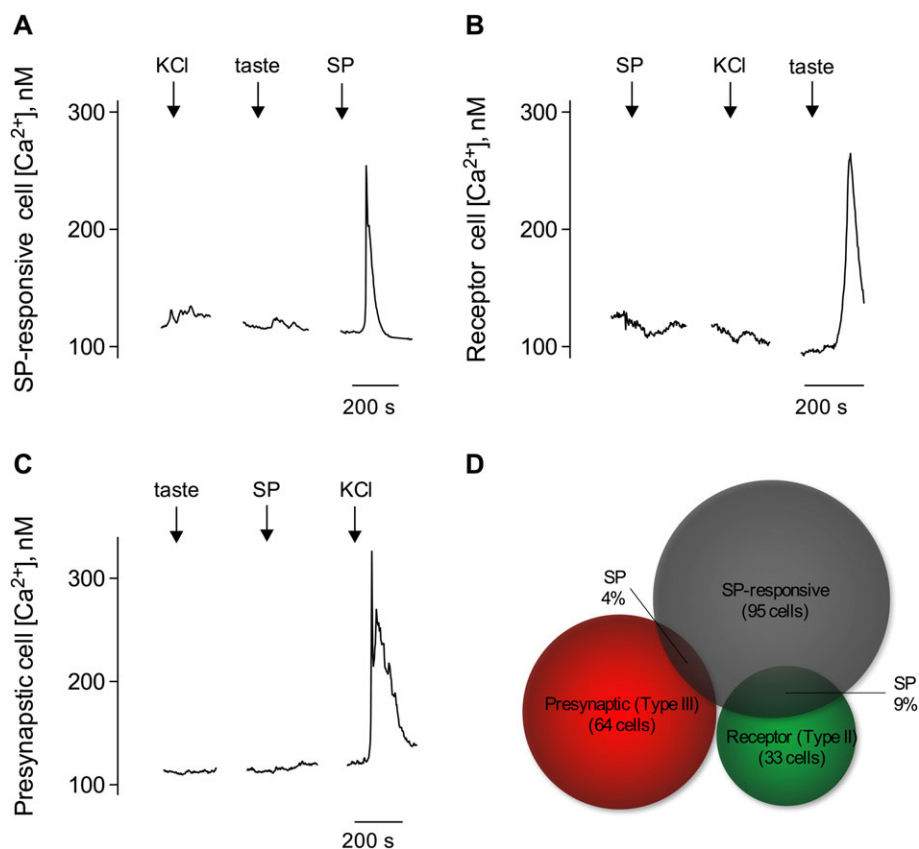


Figure 1

SP elicits intracellular Ca^{2+} transients in taste cells. Taste cells isolated from vallate taste buds were loaded with Fura 2, and their responses to stimuli were recorded by Ca^{2+} imaging. Sequentially, stimuli, such as taste mix (\downarrow , taste), 50 mM KCl (\downarrow , KCl) and 10 nM SP (\downarrow , SP), were applied to the bath medium in each experiment. (A) Traces show Ca^{2+} recordings from an SP-responsive taste cell, which did not respond to either taste mix or KCl depolarization. Immunofluorescence confocal microscopy revealed that SP acts on Type I cells *via* NK_1 receptors (also named SP receptors) (see Figure 4A). (B) Individual receptor (Type II) cells showing robust taste-evoked responses did not respond to SP. KCl depolarization did not elicit Ca^{2+} transients in receptor (Type II) cells. (C) Isolated presynaptic (Type III) cell showed KCl depolarization-elicited Ca^{2+} responses but absence of intracellular transients in response to SP. (D) Venn diagrams representing the correlation between SP-responsive taste cells (95 cells counted from five mice). Specifically, Type I cells and relative proportions of presynaptic (Type III) (4 of 64 cells) and receptor (Type II) (9 of 33 cells) taste cells that responded to SP.

significant. Data presented in graphs show means \pm 95% confidence interval. Significance is set at $P < 0.05$. GraphPad Prism (version 6.0, GraphPad Software Inc., San Diego, CA, USA) was used to perform the statistical analysis of *in vitro* experiments and to generate figures.

Materials

Drugs. All stimuli and pharmacological agents were made in Tyrode's buffer, and bath-applied. SP, RP67580 and CGP55845 were obtained from Tocris (Park Ellisville, MO, USA). ATP, **thapsigargin**, **U73122**, **bicuculline** and GABA were purchased from Sigma.

Chemicals. The taste mix consisted of two bitter compounds, cycloheximide (10 μ M) and denatonium (1 mM), as well as two sweet compounds, sucralose (1 mM) and SC45647 (0.1 mM). All compounds were purchased from Sigma.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise

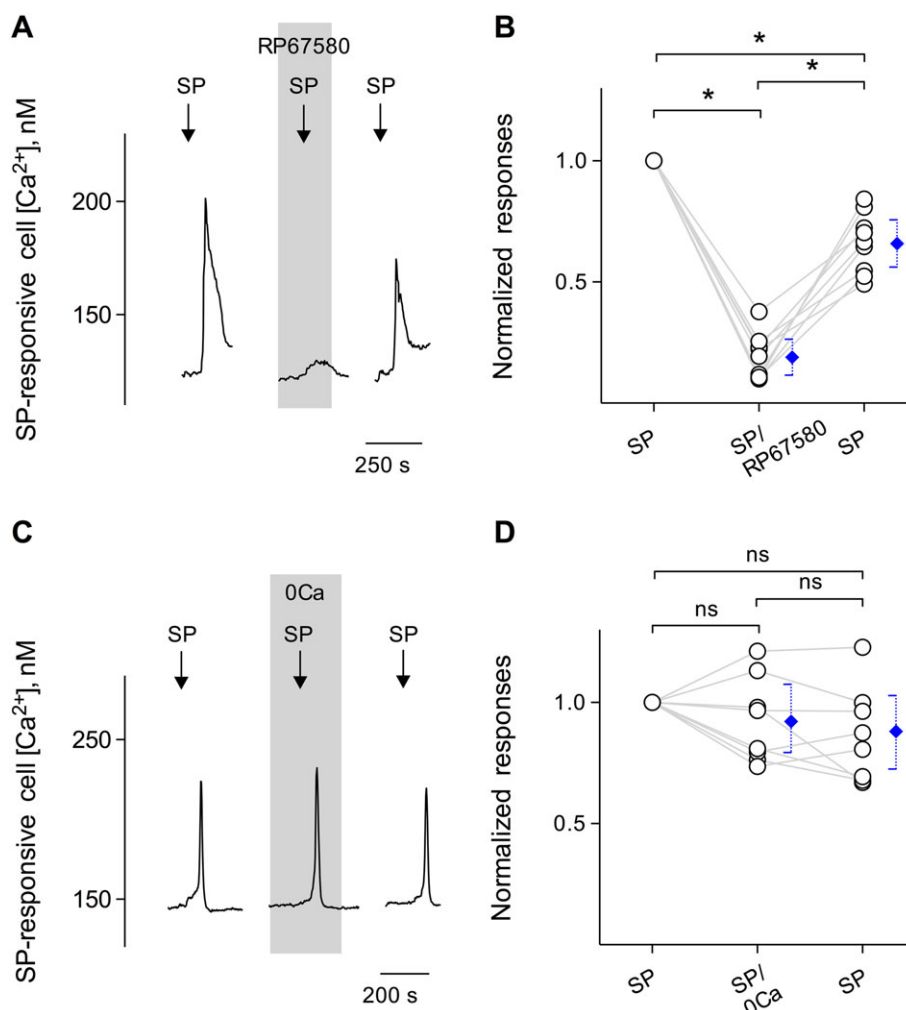


Figure 2

SP stimulates its receptors on Type I cells and induces Ca²⁺ release from intracellular stores. (A) Bath-application of 10 nM SP elicits Ca²⁺ mobilization in Fura 2-loaded taste cells (\downarrow , SP). RP67580 (0.1 μ M), an NK1 receptor antagonist (present throughout the shaded area), reversibly inhibited SP-induced Ca²⁺ responses. (B) Summary of SP-elicited Ca²⁺ responses before, during and after the presence of RP67580, plotted as in (A). Points represent normalized peak taste cell responses. Blue symbols show mean \pm 95% confidence interval (95% CI). * $P < 0.05$; significantly different as indicated; repeated measures ANOVA; $n = 9$ cells from five mice. (C) SP-elicited Ca²⁺ mobilization in an isolated taste cell was not affected when Ca²⁺ was eliminated from the bathing solution (0Ca, present throughout the shaded area). These findings indicate that SP-elicited responses in taste cells were generated by the release of the intracellular Ca²⁺, consistent with the excitation of GPCRs. (D) Summary of experiments testing sources of SP-elicited Ca²⁺ mobilization, plotted as in (C). Points indicate normalized peak taste cell responses. Blue symbols show mean \pm 95% CI. ns, not significant; repeated measures ANOVA; $n = 8$ cells from five mice.

Results

SP mobilizes intracellular Ca²⁺ responses in taste cells

We tested whether SP excites taste bud cells to mobilize intracellular Ca²⁺ transients and which type(s) of taste cells are targets for the stimulation. Isolated taste cells were loaded with

Fura 2, a Ca²⁺-sensitive dye, and tested with bath-applied SP. Stimuli, such as ATP, taste mix, KCl and SP, were applied sequentially to the bath medium in each experiment. Bath-applying the lower concentration of SP, for example, 10 nM induced robust Ca²⁺ responses in a subset of taste cells (Figure 1A). Approximately 19% of isolated live taste cells (95 of 525) exhibited Ca²⁺ responses when stimulated with 10 nM SP. Taste cells were identified as Type II (receptor) or Type III (presynaptic) cells based on their responses to taste mix or KCl depolarization respectively (Figures 1B, C) (DeFazio *et al.*, 2006; Huang *et al.*, 2007; Vandenbeuch *et al.*, 2010; Huang and Wu, 2015). We reasoned, however, that if

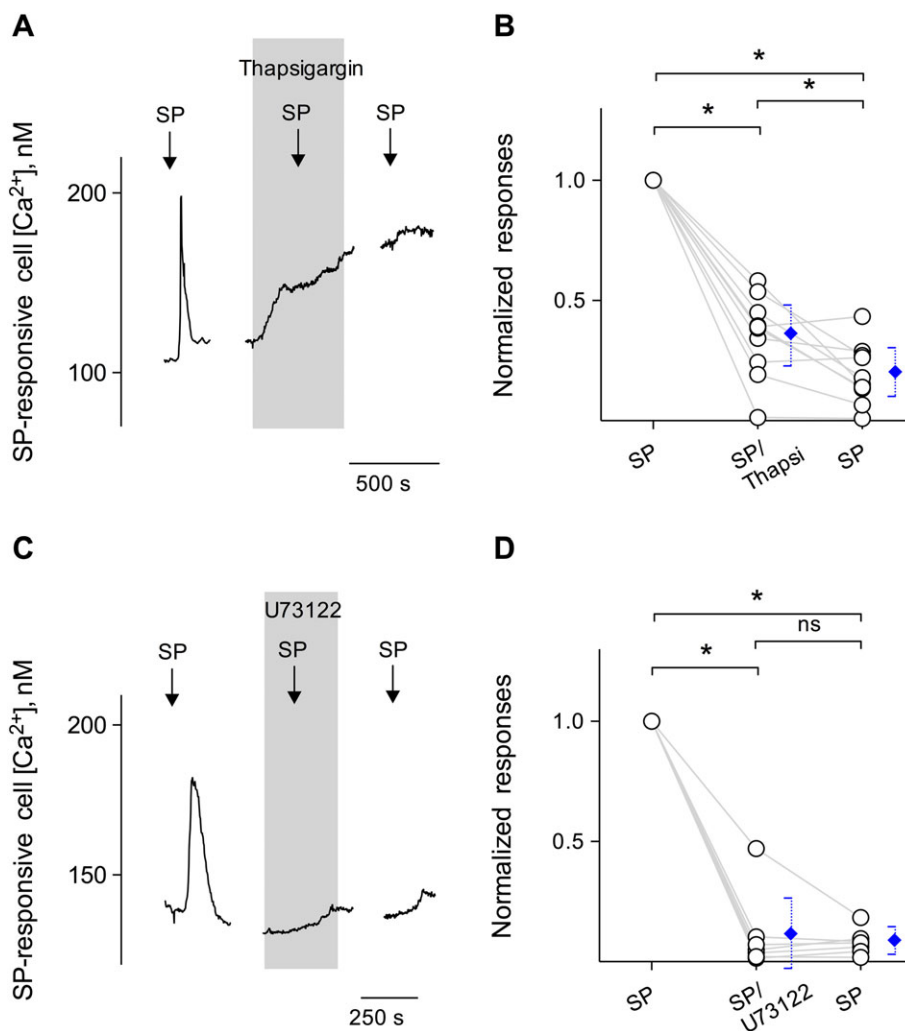


Figure 3

Intracellular Ca²⁺ release in Type I cells is via a PLC-mediated pathway. (A) Bath-application of 10 nM SP elicits Ca²⁺ mobilization in the taste cell (↓, SP). Treating Type I cells with thapsigargin (1 μM), a SERCA inhibitor (present throughout the shaded area), irreversibly reduced Ca²⁺ responses evoked by SP, consistent with Ca²⁺ store release mechanisms for this stimulus. (B) Summary of SP-elicited Ca²⁺ responses before, during and after the incubation of thapsigargin, plotted as in (A). Points represent normalized peak taste cell responses. Blue symbols show mean ± 95% confidence interval (95% CI). **P* < 0.05; significantly different as indicated; repeated measures ANOVA; *n* = 8 cells from five mice. (C) SP (10 nM)-elicited Ca²⁺ mobilization in an isolated Type I cell was blocked when U73122 (10 μM) (present throughout the shaded area) was present in the bathing solution. Consistent with the blockage of the PLC-mediated cascade, these findings indicate that SP-elicited responses in Type I cells were due to Ca²⁺ release from intracellular stores. (D) Summary of SP-elicited Ca²⁺ responses before, during and after the incubation of U73122, plotted as in (C). Points represent normalized peak taste cell responses. Blue symbols show mean ± 95% CI. **P* < 0.05; significantly different as indicated; repeated measures ANOVA; *n* = 7 cells from five mice.

the Type I taste cells were responsible for SP excitation, one might not necessarily observe significant taste- and KCl-evoked Ca^{2+} oscillations. Unexpectedly, two independent subpopulations, 9 and 4% of SP-responsive taste cells, also showed Ca^{2+} transients in response to taste mix (9 of 95) and 50 mM KCl (4 of 95) respectively. Nevertheless, our results suggest that SP acts on taste bud cells *via* the SP receptor, that is, the NK_1 receptor, most likely on Type I cells, but also possibly on other types of taste cells, including Type II and Type III cells. Using the double-immunostaining technique that evaluates Ca^{2+} imaging data, we refined these findings to examine whether Type I cells express NK_1 receptors (as indeed the case, shown below). Figure 1D depicts the summary

of these series of experiments and shows the relative proportions of taste cells that respond to SP.

SP causes Ca^{2+} transients through release from internal stores

RP67580, an NK_1 receptor antagonist, significantly and reversibly reduced Ca^{2+} responses elicited by SP (10 nM), corroborating that the Ca^{2+} signals were caused by activation of NK_1 receptors, also known SP receptors (Figures 2A, B). Because the intracellular Ca^{2+} transients in taste cells initiate from two different sources, influx from the extracellular Ca^{2+} and/or release from internal Ca^{2+} stores, we conducted

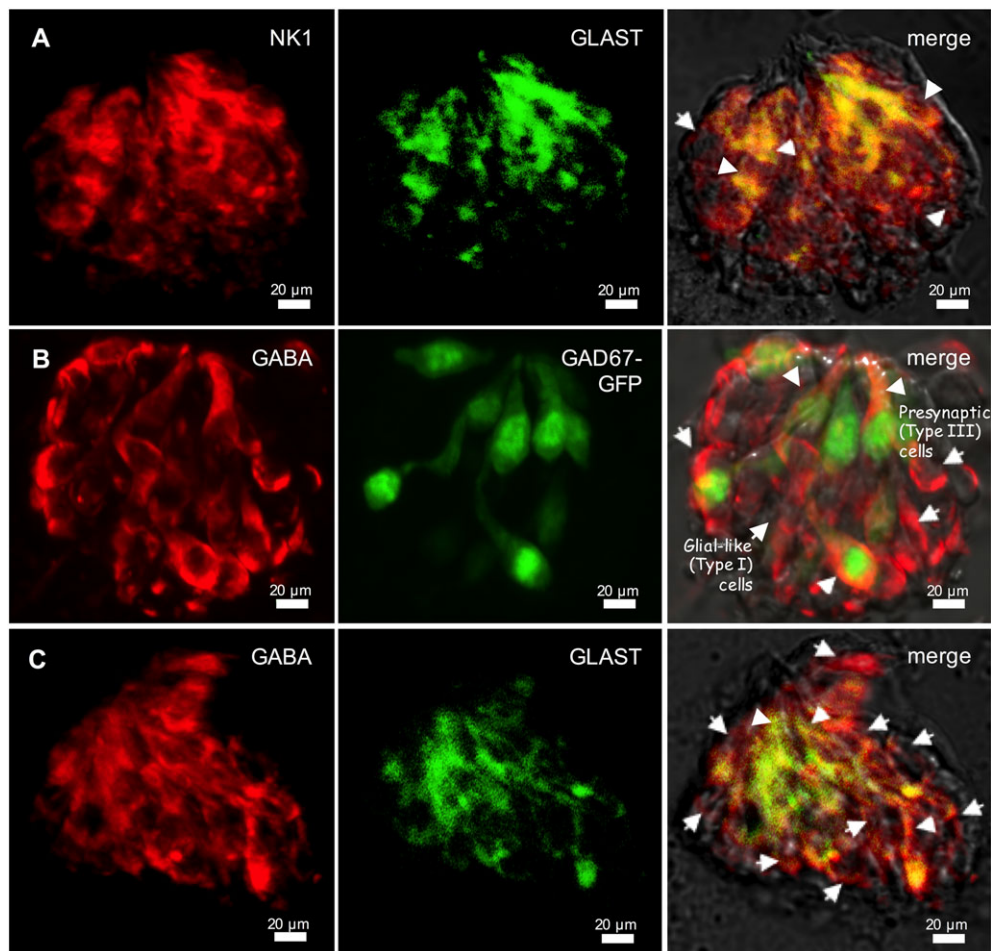


Figure 4

Type I taste cells express NK_1 receptors and synthesize GABA. Nomarski optics images showing the view of individually isolated taste buds were merged with immunofluorescent confocal micrographs for these micrographs. (A) Double-immunostaining of NK_1 receptors (NK_1 ; red) and GLAST, a marker of Type I cells (green), was performed on isolated and fixed taste buds. Several taste cells exhibit double labelling, suggesting that Type I cells express NK_1 receptors (arrowheads). In addition to those obtaining double immunoreactivities, NK_1 -immunoreactive taste cells without GLAST immunoreactivity were also revealed (arrow). The optical thicknesses (z-stack) of confocal images are 12 μm . Bar = 20 μm . (B) GABA immunostaining of an isolated and fixed taste bud from a GAD67-GFP mouse. Many GABA immunoreactive taste cells express GFP (arrowheads), but some do not. This latter category may be Type I cells (arrows), which express GAD65, a GABA synthetic enzyme (Dvoryanchikov *et al.*, 2011). Bar = 20 μm . (C) Double-immunostaining of GABA (red) and GLAST (green) on isolated and fixed taste buds. GABA immunoreactivity is in cells that express GLAST (arrowheads). In addition to those obtaining double immunoreactivities, Presynaptic (Type III) cells, GABA-immunoreactive taste cells without GLAST immunoreactivity, were also observed (arrows). The optical thicknesses (z-stack) of confocal images are 15 μm . Bar = 20 μm .

a series of experiments to examine the source of Ca^{2+} responses during the stimulation. First, we stimulated taste cells with SP in the absence of extracellular Ca^{2+} . Taste cells showed robust Ca^{2+} transients in response to SP when Ca^{2+} in the medium was replaced with Mg^{2+} (0 Ca), suggesting that SP-elicited Ca^{2+} responses were caused by the release from internal Ca^{2+} stores (Figures 2C, D). The endoplasmic reticulum is a major intracellular organelle involved in the internal Ca^{2+} storage. Thus, we explored whether thapsigargin, a **sarco/endoplasmic reticulum Ca^{2+} -ATPase** (SERCA) inhibitor which depletes intracellular Ca^{2+} stores, abolished Ca^{2+} transients in the SP stimulated taste cells. As we expected, thapsigargin (1 μM , 5–10 min) significantly and irreversibly reduced SP-elicited Ca^{2+} responses (Figures 3A, B). The PLC signalling cascade plays a crucial role in releasing the intracellular calcium from internal stores into the cytosol of taste cells (Huang *et al.*, 2005; 2011b; Simon *et al.*, 2006).

We tested the effects of U73122, a broad-spectrum PLC blocker, on SP-elicited Ca^{2+} responses. Ca^{2+} imaging revealed that pretreating individual taste cells with U73122 (10 μM) significantly and irreversibly reduced SP-elicited Ca^{2+} responses (Figures 3C, D). These results unambiguously indicated that the SP-elicited Ca^{2+} transients were involved in the PLC-mediated cascade. This clearly differs from the frank Ca^{2+} influx stimulated by KCl depolarization described in previous studies (Huang *et al.*, 2007; 2009; 2012; Vandenbeuch *et al.*, 2010; Huang and Wu, 2015). Collectively, these findings indicated that SP activates NK_1 receptors and subsequently elicits PLC/ IP_3 -mediated Ca^{2+} release from intracellular stores.

Type I cells express NK_1 receptors

We used immunofluorescent confocal microscopy to investigate the expression of NK_1 receptors on taste bud cells.

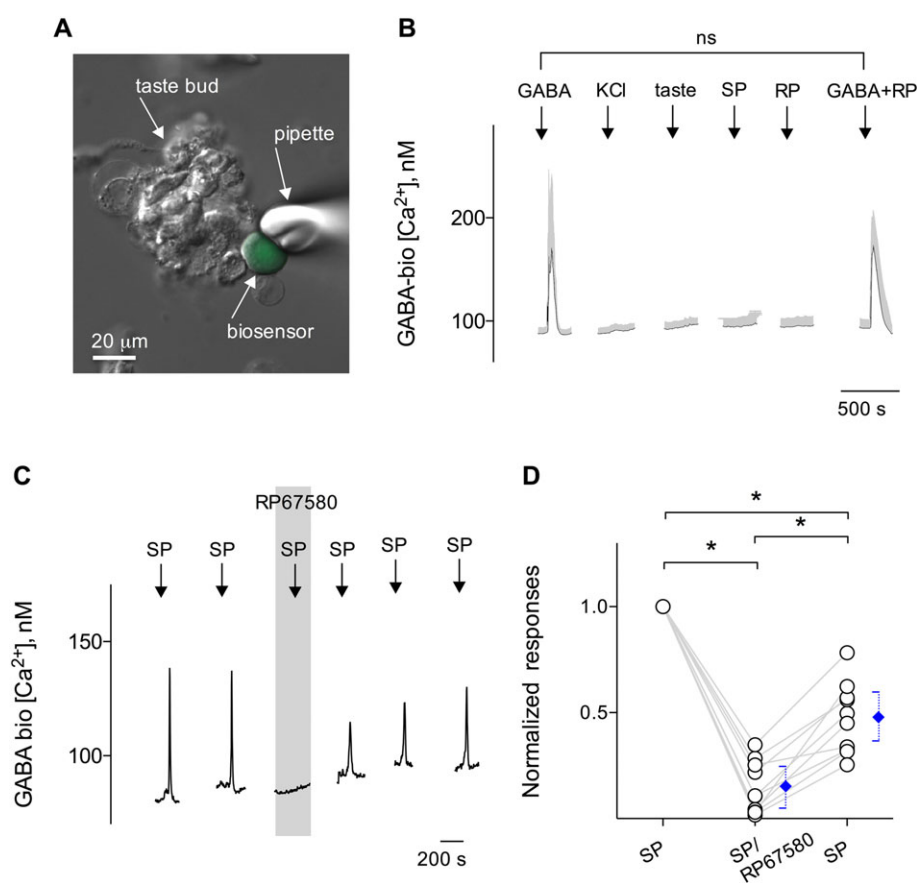


Figure 5

SP elicits GABA secretion from taste buds. CHO/GABA_B cells (hereafter called GABA biosensors) were positioned against isolated taste buds to measure SP-elicited transmitter release. (A) Micrograph of a biosensor cell abutted against an isolated taste bud in a living preparation. A Nomarski optics image and a fluorescence microscopy image were merged. (B) Sequential Ca^{2+} mobilization in CHO/GABA_B cells was elicited by repeated applications of 100 nM GABA (\downarrow , GABA) but not by depolarization with 50 mM KCl (\downarrow , KCl), taste mix (\downarrow , taste), SP (up to 100 nM) (\downarrow , SP) or 100 nM RP67580 (\downarrow , RP). Trace shows mean \pm SEM (grey areas) of Ca^{2+} responses from GABA biosensor cells. Importantly, GABA biosensor cells (13 cells) retain robust Ca^{2+} responses to GABA in the presence of RP67580 (\downarrow , GABA + RP). ns, not significant. (C) Traces showed robust responses from the biosensor cell positioned against a taste bud stimulated with 10 nM SP. RP67580 (0.1 μM) (present throughout the shaded area) reversibly reduced the biosensor responses evoked by stimulating taste buds repeatedly with SP. These results are consistent with SP evoked GABA release from taste buds. (D) Summary of SP-elicited GABA secretion before, during and after the incubation of RP67580, plotted as in (C). Points represent normalized peak biosensor cell responses. Blue symbols show mean \pm 95% confidence interval. * $P < 0.05$; significantly different as indicated; repeated measures ANOVA; $n = 10$ cells recorded in 10 experiments from nine mice.

Several laboratories have shown previously that GLAST is expressed only in Type I taste cells and thus a marker for Type I cells (Lawton *et al.*, 2000; Vandenbeuch *et al.*, 2013; Yoshida *et al.*, 2015). To verify the expression of NK₁ receptors on Type I taste cells, we double-immunostained isolated and fixed vallate taste buds with antibodies directed against NK₁ receptors and GLAST. As shown in Figure 4A, NK₁-immunoreactive taste cells were also immunostained with GLAST, indicating that these are Type I taste cells express NK₁ receptors. Some NK₁-immunoreactive taste cells lack GLAST

immunoreactivity, suggesting that other types of taste cells may express NK₁ receptors as well. In this study, no specific immunoreactivity in taste cells was observed in these controls with the omission of primary antibodies. Nevertheless, confirmation that Type I cells prominently express NK₁ receptors is in good correspondence with the finding of Type I cells that showed Ca²⁺ transients in response to SP in the Ca²⁺ imaging studies. Next, we refined these findings to examine whether SP elicits transmitter secretion from mouse vallate taste buds.

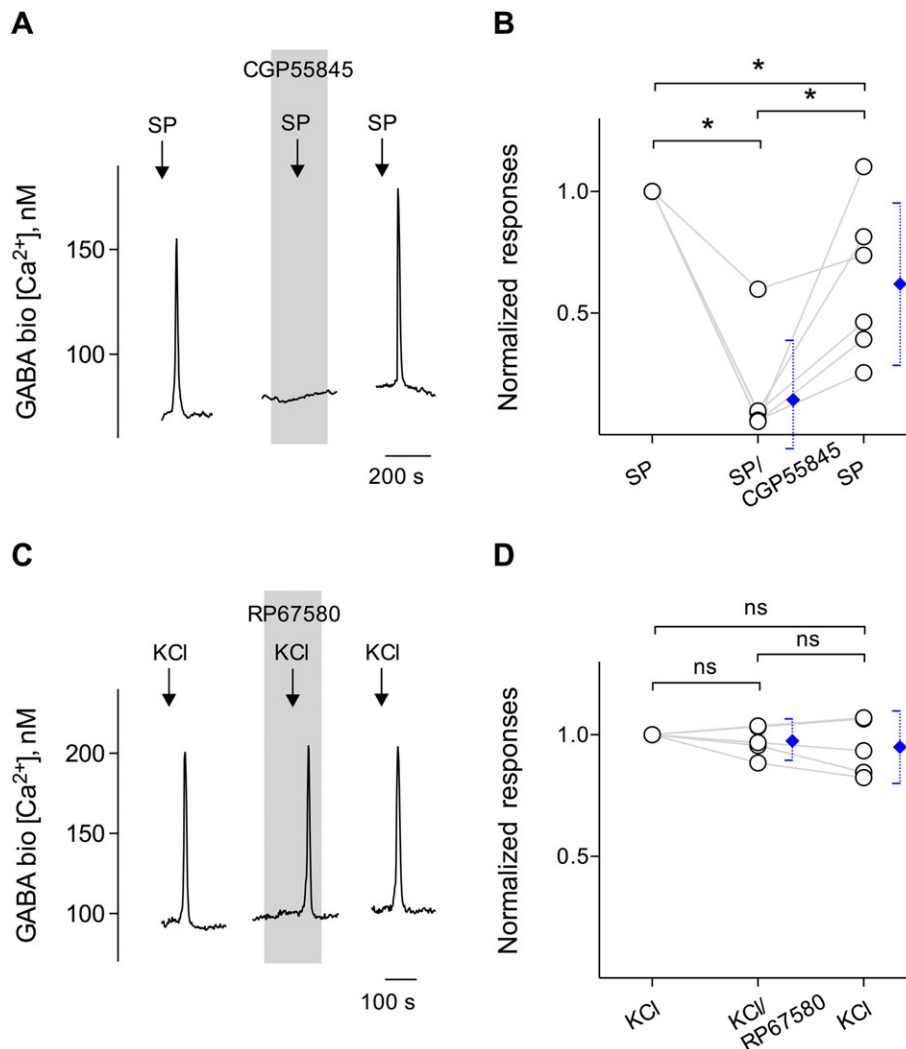


Figure 6

Stimulating taste buds with SP evokes GABA release. (A) Traces show robust responses from a biosensor positioned against a taste bud when the taste bud was stimulated with 10 nM SP (↓, SP). CGP55845 (10 μM), a GABA_B receptor antagonist (present throughout the shaded area), reversibly abolished SP-evoked biosensor responses. Withdrawing the biosensor from the taste bud eliminated all responses to SP (data not shown). (B) Summary of SP-elicited GABA secretion before, during and after the incubation of CGP55845, plotted as in (C). Points represent normalized peak biosensor cell responses. Blue symbols show mean ± 95% confidence interval (95% CI). **P* < 0.05; significantly different as indicated; repeated measures ANOVA; *n* = 6 cells recorded in six experiments from six mice. (C) In separate experiments, traces represent robust responses from the biosensor cell positioned against a taste bud when the taste bud was depolarized by 50 mM KCl (↓, KCl), that is, GABA secretion from presynaptic (Type III) cells. RP67580 in the bath medium (present throughout the shaded area) did not affect Ca²⁺ mobilization in the biosensor in response to KCl, consistent with the finding that RP67580 only blocked SP-elicited GABA secretion from Type I cells. (D) Summary of KCl-elicited GABA secretion before, during and after the incubation of RP67580, plotted as in (C). Points represent normalized peak biosensor cell responses. Blue symbols show mean ± 95% CI. ns, not significant; repeated measures ANOVA.; *n* = 5 cells recorded in five experiments from five mice.

Type I cells synthesize GABA

In GAD67-GFP mice, GFP is detected in about 75% of Type III cells of taste buds (Tomchik *et al.*, 2007; Dvoryanchikov *et al.*, 2011). GABA-immunoreactive taste cells were either GAD65 expressing (i.e. Type I) or GAD67 expressing (i.e. Type III) (Dvoryanchikov *et al.*, 2011; Huang *et al.*, 2011a). In the present study, we observed GABA immunofluorescence in isolated taste buds in which Type III cells were marked with

GFP and determined that some GABA immunoreactive cells lacked GFP, indicating these cells were most likely Type I cells (Figure 4B). To test the underlying assumption, we immunostained isolated and fixed vallate taste buds with antibodies directed against GABA and GLAST. As shown in Figure 4C, GABA-immunoreactive taste cells were also immunostained with GLAST, indicating that these are Type I taste cells synthesizing GABA.

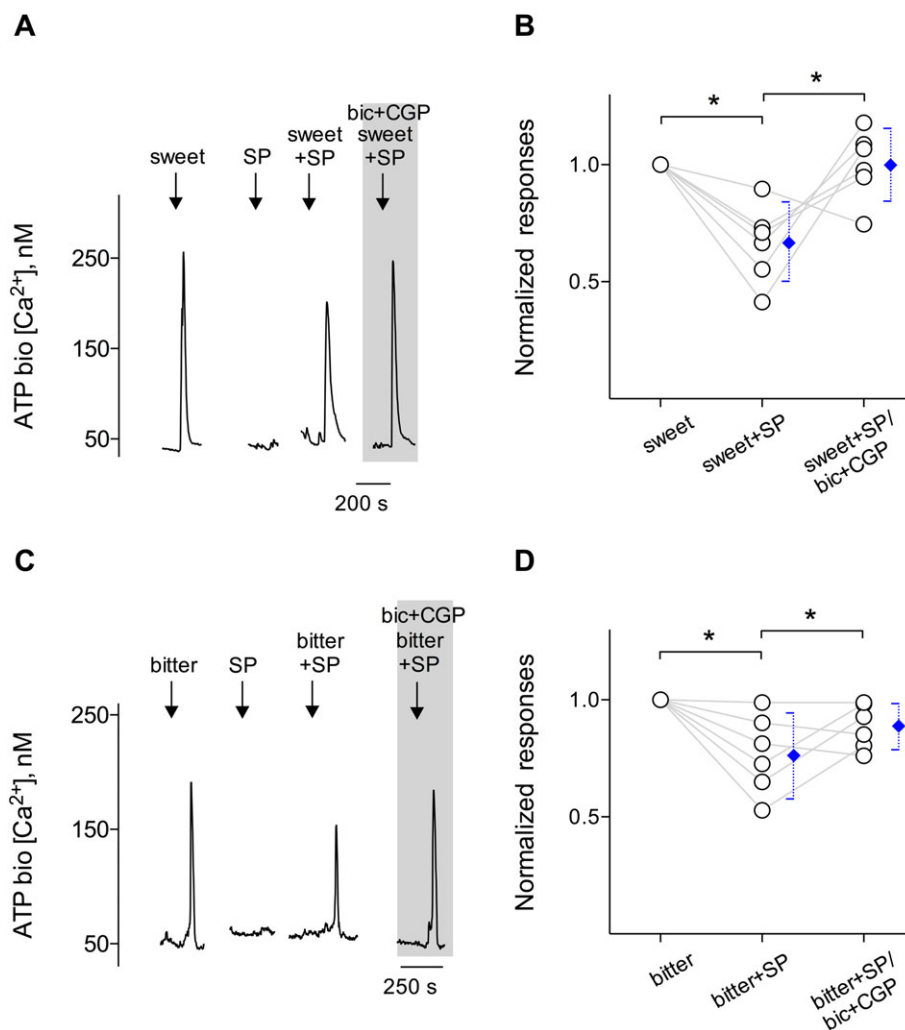


Figure 7

SP inhibits taste-evoked ATP secretion from taste buds. CHO/ATP_{P2X2/P2X3} cells (hereafter called ATP biosensors) were used to monitor taste-evoked ATP secretion from isolated taste buds. The extracellular GABA inhibits taste-evoked ATP secretion from receptor (Type II) cells (Dvoryanchikov *et al.*, 2011). We tested whether SP-elicited GABA secretion from Type I cells depresses taste-evoked ATP secretion. Whole taste buds were isolated from mouse vallate papillae to retain cell-to-cell communication between receptor (Type II) and Type I cells. Traces show robust responses from the biosensor positioned against an isolated taste bud when the taste bud was stimulated by sweet stimuli [A (↓, sweet)] or bitter stimuli [C (↓, bitter)], indicating ATP secretion from the taste bud. SP (10 nM) (↓, SP) itself did not evoke ATP secretion from taste buds. Taste-evoked ATP secretion was moderately reduced by adding 10 nM SP with taste stimuli [A (↓, sweet+SP); C (↓, bitter+SP)]. Restoration of ATP secretion was observed by adding 10 μM bicuculline and 10 μM CGP55845, GABA_A and GABA_B receptor antagonists ('bic + CGP', present throughout the shaded area), to the bath. As expected, blocking GABA receptors restored SP-inhibited ATP secretion, suggesting that SP plays as an inhibitory transmitter *via* the GABAergic signalling in taste buds. (B, D) Summary of SP inhibited sweet- or bitter-evoked ATP secretion before and during the presence of 'bic + CGP' in the bath medium, plotted as in (A) and (C) respectively. Points represent normalized peak taste cell responses. Blue symbols show mean ± 95% confidence interval. **P* < 0.05; significantly different as indicated; repeated measures ANOVA. (B) *n* = 6 cells recorded in six experiments from six mice; (D) *n* = 6 cells recorded in six experiments from six mice.

SP triggers GABA release from taste buds

We investigated whether transmitter release from SP-stimulated taste buds is possible. Using Ca^{2+} imaging with GABA biosensor cells (Figure 5A), we tested whether SP evokes taste buds to release GABA. Parenthetically, in the absence of taste buds, GABA biosensor cells do not respond either to depolarization with bath-applied KCl (50 mM; substituted for NaCl) or to bath-applied SP (up to 100 nM) except GABA (100 nM). GABA biosensor cells maintained their responses to bath-applied GABA when Ca^{2+} in the medium was replaced with Mg^{2+} . This is consistent with the coupling of GABA_B receptors and the G-protein α subunit, $G_{\alpha_{q/o/s}}$, to intracellular Ca^{2+} release mechanisms (Huang *et al.*, 2011a). GABA biosensor cells retained full receptor agonist sensitivity in the presence of RP67580, an NK_1 receptor antagonist (Figure 5B).

Subsequently, we isolated taste buds from vallate papillae of the mouse tongue. GABA cells could be observed by immunostaining taste buds isolated for recording, verifying that their GABA content was maintained throughout the isolation procedure (see Figure 4B, C). As shown in Figure 5C, we attempted to detect GABA secretion upon SP stimulation each time after the release site was located (i.e. GABA biosensors abutted against taste buds showed Ca^{2+} transients while

being stimulated). Figure 5C also showed that 10 nM SP was sufficient to trigger GABA secretion from taste buds. To verify that SP activated its cognate receptors on taste cells and subsequently elicited transmitter release, and thus GABA, we applied the selective NK_1 receptor antagonist, RP67580 (100 nM) in the bath solution, and retested with SP. RP67580 significantly and reversibly blocked SP-elicited biosensor responses (Figures 5C, D), indicating that the activation of NK_1 receptors elicited GABA secretion.

In separate experiments, biosensor responses to SP were reversibly blocked by 10 μM CGP55845, a GABA_B receptor antagonist (Figures 6A, B). Because NK_1 receptors are targets of SP and because CGP55845 specifically blocks Ca^{2+} responses in GABA biosensors, as described in Huang *et al.* (2011a), these observations corroborated that the biosensor cell was detecting SP-evoked GABA secretion from taste buds. To date, we have obtained successful biosensor recordings from 20 of 99 taste buds when testing SP stimulation. The success rate of measuring GABA release in these experiments was 20%, in good correspondence with the value described above that a limited number of individual Type I cells responded to SP. Lastly, we tested whether RP67580 reduced the GABA secretion from Type III cells, that is, KCl-elicited GABA secretion. We detected no reduction of biosensor responses in the presence of RP67580 in the bath

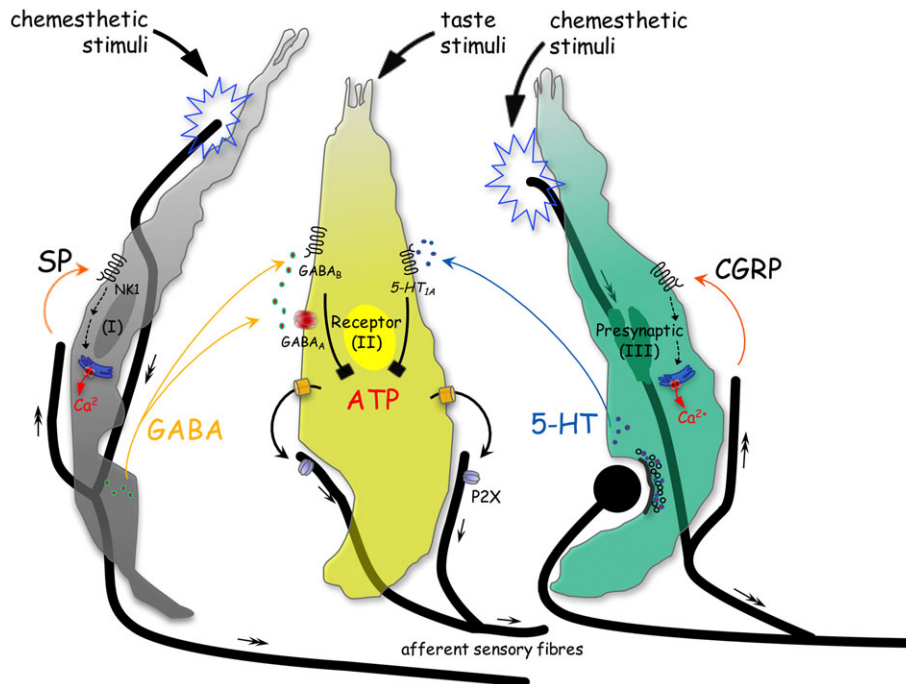


Figure 8

Schematic drawing shows the postulated scenario of SP, a putative efferent transmitter, in taste buds. Three distinct types of taste cells are shown, Type I (I), Type II (receptor) (II) and Type III (presynaptic) (III) cells. During sweet or bitter taste stimulation, ATP activates gustatory afferent fibres that propagate taste signals (small arrows) centrally (Finger *et al.*, 2005; Huang *et al.*, 2007; Jaber *et al.*, 2014; Vandenbeuch *et al.*, 2015). Chemesthetic stimuli activate sensory afferent fibres that propagate signals centrally (double-headed arrows) and release SP and/or CGRP (orange curved arrows). The activation of the CGRP receptors triggers presynaptic (Type III) cells to elevate intracellular Ca^{2+} transients and to secrete 5-HT, which inhibits ATP release from receptor (Type II) cells via serotonergic signalling pathways (black symbol) (Huang and Wu, 2015). Moreover, in the present study, the activation of NK_1 receptors (NK_1) triggers Type I cells to elevate intracellular Ca^{2+} transients and to secrete GABA, which reduces taste-evoked ATP secretion through GABAergic signalling pathways. Collectively, combined with recent findings that CGRP suppresses taste signal transmission, an interpretation of the peptidergic actions within taste buds is that it appears to be an important chemesthetic regulation of gustatory signal transmission, as theoretically it may relate to the processing of the gustatory information.

medium (Figures 6C, D). Collectively, these observations were fully consistent with the conclusion that SP triggered the secretion of GABA *via* the NK₁ receptor, most likely on Type I cells, but also possibly on Type III cells.

SP-induced GABA secretion inhibits ATP signalling in taste buds

Finally, we examined the role of SP during taste stimulation in whole taste buds where cell-to-cell communication remains intact. We hypothesize that GABA evoked by SP mediates inhibitory interactions within the taste bud during gustatory stimulation. Previous studies showed that taste stimulation elicits ATP release from intact taste buds isolated from lingual epithelium (Huang *et al.*, 2007; 2011b; Murata *et al.*, 2010; Dvoryanchikov *et al.*, 2011; Huang and Wu, 2015; 2016). ATP is believed to be an important excitatory transmitter between taste buds and gustatory sensory afferent fibres (Finger *et al.*, 2005; Huang *et al.*, 2011b; Vandenbeuch *et al.*, 2015). Additionally, GABA released from Type III cells provides the negative paracrine feedback onto Type II cells by activating GABA_A as well as GABA_B receptors and reducing taste-evoked ATP secretion in taste buds (Dvoryanchikov *et al.*, 2011; Huang *et al.*, 2011a). We therefore measured taste-evoked ATP secretion from intact taste buds using ATP biosensors (Huang *et al.*, 2007) and tested whether SP-induced GABAergic transmission alters taste-evoked ATP secretion, as follows:

Taste stimulation → Receptor (Type II) cells → ATP secretion → Afferent sensory fibres
 \uparrow
 SP stimulation → Type I cells → GABA secretion (from GAD 65)

If so, this would provide proof that SP decreases gustatory responses in taste buds. Suramin, a broad-spectrum P2 receptor antagonist, was used to verify the responses in ATP biosensor cells that were generated by activation of the P2X receptors expressed on biosensors (Huang *et al.*, 2007; Huang and Wu, 2015). The success rate of measuring taste-evoked ATP secretion in these experiments was 12% (18 out of 147), corresponding with rates found in previous studies (Huang *et al.*, 2007; Huang and Wu, 2015). Figure 7 showed that 10 nM SP itself did not evoke ATP secretion from taste buds. Sweet/bitter-evoked ATP secretion was significantly reduced when SP (10 nM) was added to the sweet or bitter taste stimuli (arrows, sweet+SP or bitter+SP in Figure 7A, C, respectively), consistent with SP-evoked GABAergic inhibition. Repeating sweet/bitter plus SP stimulation in the presence of CGP55845 and bicuculline restored taste-evoked ATP secretion. This is consistent with SP-evoked GABA secretion contributing to depressed ATP output during taste stimulation. Our findings suggest that GABA indirectly mediated the inhibitory actions of SP on taste-evoked ATP secretion.

Discussion and conclusions

Our results document that (i) SP leads to Ca²⁺ release from internal stores *via* the activation of the PLC signalling cascade; (ii) a prominent action of SP is to evoke GABA release from

Type I cells by acting on NK₁ receptors expressed on these cells; and (iii) the net effect of SP stimulation alters taste-evoked ATP secretion from taste buds.

Possible sources of SP

Previous studies indicated that lingual epithelia are rich in SP (Nagy *et al.*, 1982; Nishimoto *et al.*, 1982; Yamasaki *et al.*, 1984; Astbäck *et al.*, 1997; Ishida *et al.*, 2002) as well as CGRP (Astbäck *et al.*, 1997; Ishida *et al.*, 2002; Huang and Wu, 2015) containing nerve fibres, especially in those areas where taste buds can be expected. The most likely source of peptidergic fibres in taste buds is as polymodal nociceptors, many of which express both SP and CGRP (Ishida *et al.*, 2002). These sensory fibres mostly express TRPV1 receptors for chemesthetic activators such as capsaicin and a variety of other strong oral irritants (Osada *et al.*, 1997; Ishida *et al.*, 2002; Roper, 2014). Much evidence is now available that sensory neurons, which are excited by the environmental stimuli, can release stored transmitters *via* their terminals in the lingual epithelium (Maggi and Meli, 1988; Wang *et al.*, 1995; Ishida *et al.*, 2002; Simon *et al.*, 2003; Sato *et al.*, 2012). We anticipated that peptides may be released from peripheral axon terminals onto taste cells (Nagai *et al.*, 1996; Finger and Simon, 2002; Simon *et al.*, 2003; Sato *et al.*, 2012). Their actions on the modulation of taste transmission have been studied recently. Specifically, our previous study showed that the action of CGRP on taste bud cells implies an inhibitory role in taste sensitivity (Huang and Wu, 2015). In the present study, our current findings indicated that SP modulates the signal output from taste buds.

SP-evoked Ca²⁺ responses are associated with the PLC signalling cascade

Tissue-specific signalling events are associated with the specific intracellular coupling and receptor phenotypes for the SP receptors, because SP operates *via* NK₁ receptors (Douglas and Leeman, 2011; Diandong *et al.*, 2014). Activating NK₁ receptors triggers G_{αs}-mediated activation of AC, with a subsequent increase in cAMP (Diandong *et al.*, 2014). Alternatively, NK₁ receptors can couple to G_{αq}-mediated PLC, an enzyme responsible for the IP₃-induced Ca²⁺ release from intracellular stores as a second messenger (Miyano *et al.*, 2010; Douglas and Leeman, 2011). Nevertheless, our Ca²⁺ imaging and immunostaining data revealed that Type I cells in vallate taste papillae express functional NK₁ receptors. Our results are consistent with those of the previous work, which stated that the predominant secondary messenger system in Type I cells is represented by PLC (Grant, 2012). Type II and/or Type III cells may also express NK₁ receptors because some taste cells showing NK₁ immunoreactivity do not express GLAST. It remains to be seen whether providing this staining pattern by immunostaining the corresponding cell marker makes sense of that overlapping with NK₁ receptors.

Previous studies have shown that there are two classes of taste bud cells directly involved in gustatory signalling. Type II cells detect and transduce sweet, bitter and umami compounds, and Type III cells respond directly to sour taste stimuli (Tomchik *et al.*, 2007; Huang *et al.*, 2008b).

Although Type II and/or Type II cells only rarely respond to SP, the possibility that SP has a role in taste modalities cannot be ruled out. Intriguingly, Grant (2012) reported that activation of NK₁ receptors could have an additive effect on umami responses in Type II cells, suggesting a possible mechanism by which SP-mediated enhancement of taste may occur. Our data indicated that SP acts specifically on Type I cells, not globally on the taste bud. It is likely that peptidergic efferent input acts directly and prominently on paracrine transmission within taste buds, which has the potential to shape the final signals that taste buds transmit to the brain (see below).

SP shapes the signal output that taste buds transmit to the brain

Previous studies, using single cell RT-PCR and immunofluorescence confocal microscopy, showed the distinct expression patterns of GABA synthetic enzymes in mouse vallate taste buds. For example, Type III cells express GAD67 and Type I cells express GAD65 (DeFazio *et al.*, 2006; Starostik *et al.*, 2010; Dvoryanchikov *et al.*, 2011). Sour taste stimulation and KCl depolarization cause Type III taste bud cells to secrete GABA (Huang *et al.*, 2011a). Recent studies reported that Type I cells may transduce the salt (Na⁺) taste (Vandenbeuch *et al.*, 2008; Roper, 2015). We have no information on whether Type I cells contribute to the GABAergic transmission in taste buds during salt stimulation, but the unique feature of SP is that it appears to primarily affect Type I cells and to elicit the secretion of a signalling molecule, namely, GABA. Parenthetically, Vandenbeuch *et al.* (2008) reported that Type I cells lack voltage-gated Ca²⁺ currents. In contrast to the requirement for Ca²⁺ influx for GABA secretion in Type III cells (Huang *et al.*, 2011a), GABA secretion from Type I cells may be associated with the intracellular distribution of the Ca²⁺ responses to SP, which blocks Ca²⁺-activated K⁺ channels leading to membrane depolarization, as is the case in airway smooth muscles (Deshpande *et al.*, 2010). The effective mechanism for such secretion remains to be determined.

ATP is now widely acknowledged as the main gustatory transmitter within taste buds, acting on P2X receptors on afferent nerve fibre terminals (Bo *et al.*, 1999; Finger *et al.*, 2005; Huang *et al.*, 2011b; Vandenbeuch *et al.*, 2015). The combined actions of membrane depolarization, mediated by TRPM5, concurrent with an increased intracellular Ca²⁺ release from stores, mediated by a **PLCB2**-IP₃-signalling cascade, are required to trigger ATP release from Type II cells (Pérez *et al.*, 2002; Huang *et al.*, 2005; Huang and Roper, 2010; Kinnamon, 2011). Type I cells themselves synthesize GABA, and thus, one might speculate that GABA released from Type I cells might also exert paracrine inhibitory feedback within taste buds. GABAergic inhibition in taste buds has been documented showing that GABA stabilizes or even hyperpolarizes taste bud cells (Cao *et al.*, 2009) and reduces taste-evoked ATP secretion from Type II cells by acting on GABA_A and GABA_B receptors expressed by these cells (Dvoryanchikov *et al.*, 2011). We found that SP reduced sweet and/or bitter stimuli-evoked ATP secretion from whole taste buds, which maintain intact cell-to-cell communication. Our data indicated SP-elicited GABA secretion, which

inhibits taste-evoked ATP secretion from Type II cells. The significance of such inhibition in the intact system supports the notion that chemesthesis-induced GABAergic inhibition is used in shaping responses to sweet and/or bitter qualities *per se*.

In summary, our results are consistent with a role for SP as an inhibitory transmitter that shapes taste signals *via* GABAergic signalling during the processing of gustatory information in taste buds. Collectively, combined with recent findings that CGRP depresses taste signals *via* serotonergic signalling (Huang and Wu, 2015), an interpretation of the peptidergic actions within taste buds is that it appears to be an important chemesthetic regulation of gustatory signal transmission, as theoretically it may relate to the processing of the gustatory information. Figure 8 summarizes this postulated scenario in the schematic taste bud.

Acknowledgements

This work was supported by SIUSOM research seed grant and Fellows Grant Award Program (FGAP) of American Association of Anatomists (AAA-3419) to A.Y.H.

Author contributions

A.Y.H. and S.Y.W. designed research; A.Y.H. and S.Y.W. performed research; A.Y.H. and S.Y.W. analysed data; A.Y.H. wrote the paper. All authors read and approved the final paper.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organisations engaged with supporting research.

References

- Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Marrion NV, Peters JA *et al.* (2017a). The concise guide to PHARMACOLOGY 2017/18: G protein-coupled receptors. *Br J Pharmacol* 174: S17–S129.
- Alexander SPH, Peters JA, Kelly E, Marrion NV, Faccenda E, Harding SD *et al.* (2017b). The concise guide to PHARMACOLOGY 2017/18: Ligand-gated ion channels. *Br J Pharmacol* 174: S130–S159.
- Alexander SPH, Striessnig J, Kelly E, Marrion NV, Peters JA, Faccenda E *et al.* (2017c). The concise guide to PHARMACOLOGY 2017/18: Voltage-gated ion channels. *Br J Pharmacol* 174: S160–S194.

- Alexander SPH, Fabbro D, Kelly E, Marrion NV, Peters JA, Faccenda E *et al.* (2017d). The concise guide to PHARMACOLOGY 2017/18: Enzymes. *Br J Pharmacol* 174: S272–S359.
- Alexander SPH, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD *et al.* (2017e). The concise guide to PHARMACOLOGY 2017/18: Transporters. *Br J Pharmacol* 174: S360–S446.
- Astbäck J, Arvidson K, Johansson O (1997). An immunohistochemical screening of neurochemical markers in fungiform papillae and taste buds of the anterior rat tongue. *Arch Oral Biol* 42: 137–147.
- Bo X, Alavi A, Xiang Z, Oglesby I, Ford A, Burnstock G (1999). Localization of ATP-gated P2X2 and P2X3 receptor immunoreactive nerves in rat taste buds. *Neuroreport* 10: 1107–1111.
- Cao Y, Zhao FL, Kolli T, Hivley R, Herness S (2009). GABA expression in the mammalian taste bud functions as a route of inhibitory cell-to-cell communication. *Proc Natl Acad Sci U S A* 106: 4006–4011.
- Chang RB, Waters H, Liman ER (2010). A proton current drives action potentials in genetically identified sour taste cells. *Proc Natl Acad Sci U S A* 107: 22320–22325.
- Curtis MJ, Bond RA, Spina D, Ahluwalia A, Alexander SP, Giembycz MA *et al.* (2015). Experimental design and analysis and their reporting: new guidance for publication in *BJP*. *Br J Pharmacol* 172: 3461–3471.
- DeFazio RA, Dvoryanchikov G, Maruyama Y, Kim JW, Pereira E, Roper SD *et al.* (2006). Separate populations of receptor cells and presynaptic cells in mouse taste buds. *J Neurosci* 26: 3971–3980.
- Deshpande DA, Wang WC, McIlmoyle EL, Robinett KS, Schillinger RM, An SS *et al.* (2010). Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat Med* 16: 1299–1304.
- Diandong H, Kefeng S, Weixin F, Moran W, Jiahui W, Zaifu L (2014). The role of Gas in activation of NK92-MI cells by neuropeptide substance P. *Neuropeptides* 48: 1–5.
- Douglas SD, Leeman SE (2011). Neurokinin-1 receptor: functional significance in the immune system in reference to selected infections and inflammation. *Ann N Y Acad Sci* 1217: 83–95.
- Dvoryanchikov G, Tomchik SM, Chaudhari N (2007). Biogenic amine synthesis and uptake in rodent taste buds. *J Comp Neurol* 505: 302–313.
- Dvoryanchikov G, Huang YA, Barro-Soria R, Chaudhari N, Roper SD (2011). GABA, its receptors, and GABAergic inhibition in mouse taste buds. *J Neurosci* 31: 5782–5791.
- Finger TE, Simon SA (2002). The cell biology of lingual epithelia. In: Finger TE, Silver WL, Restrepo D (eds). *The Neurobiology of Taste and Smell*. Wiley-Liss: New York, pp. 287–314.
- Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L *et al.* (2005). ATP signaling is crucial for communication from taste buds to gustatory nerves. *Science* 310: 1495–1499.
- Green BG (2012). Chemesthesis and the chemical senses as components of a “chemofensor complex”. *Chem Senses* 37: 201–206.
- Grant J (2012). Tachykinins stimulate a subset of mouse taste cells. *PLoS One* 7: e31697.
- Gryniewicz G, Poenie M, Tsien RY (1985). A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J Biol Chem* 260: 3440–3450.
- Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S *et al.* (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucl Acids Res* 46: D1091–D1106.
- Holzer P (1988). Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience* 24: 739–768.
- Huang AY, Wu SY (2015). Calcitonin gene-related peptide reduces taste-evoked ATP secretion from mouse taste buds. *J Neurosci* 35: 12714–12724.
- Huang AY, Wu SY (2016). The effect of imiquimod on taste bud calcium transients and transmitter secretion. *Br J Pharmacol* 173: 3121–3133.
- Huang YJ, Maruyama Y, Lu KS, Pereira E, Plonsky I, Baur JE *et al.* (2005). Mouse taste buds use serotonin as a neurotransmitter. *J Neurosci* 25: 843–847.
- Huang AL, Chen X, Hoon MA, Chandrashekar J, Guo W, Trankner D *et al.* (2006). The cells and logic for mammalian sour taste detection. *Nature* 442: 934–938.
- Huang YJ, Maruyama Y, Dvoryanchikov G, Pereira E, Chaudhari N, Roper SD (2007). The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. *Proc Natl Acad Sci U S A* 104: 6436–6441.
- Huang YA, Maruyama Y, Roper SD (2008a). Norepinephrine is coreleased with serotonin in mouse taste buds. *J Neurosci* 28: 13088–13093.
- Huang YA, Maruyama Y, Stimac R, Roper SD (2008b). Presynaptic (Type III) cells in mouse taste buds sense sour (acid) taste. *J Physiol* 586: 2903–2912.
- Huang YA, Dando R, Roper SD (2009). Autocrine and paracrine roles for ATP and serotonin in mouse taste buds. *J Neurosci* 29: 13909–13918.
- Huang YA, Roper SD (2010). Intracellular Ca²⁺ and TRPM5-mediated membrane depolarization produce ATP secretion from taste receptor cells. *J Physiol* 588: 2343–2350.
- Huang YA, Pereira E, Roper SD (2011a). Acid stimulation (sour taste) elicits GABA and serotonin release from mouse taste cells. *PLoS One* 6: e25471.
- Huang YA, Stone LM, Pereira E, Yang R, Kinnamon JC, Dvoryanchikov G *et al.* (2011b). Knocking out P2X receptors reduces transmitter secretion in taste buds. *J Neurosci* 31: 13654–13661.
- Huang YA, Grant J, Roper S (2012). Glutamate may be an efferent transmitter that elicits inhibition in mouse taste buds. *PLoS One* 7: e30662.
- Ishida Y, Ugawa S, Ueda T, Murakami S, Shimada S (2002). Vanilloid receptor subtype-1 (VR1) is specifically localized to taste papillae. *Brain Res Mol Brain Res* 107: 17–22.
- Jaber L, Zhao FL, Kolli T, Herness S (2014). A physiologic role for serotonergic transmission in adult rat taste buds. *PLoS One* 9: e112152.
- Kapaun CL, Dando R (2016). Deconvoluting physical and chemical heat: temperature and spiciness influence flavor differently. *Physiol Behav* 170: 54–61.
- Karagiannides I, Torres D, Tseng YH, Bowe C, Carvalho E, Espinoza D *et al.* (2008). Substance P as a novel anti-obesity target. *Gastroenterology* 134: 747–755.
- Kaya N, Shen T, Lu SG, Zhao FL, Herness S (2004). A paracrine signaling role for serotonin in rat taste buds: expression and localization of serotonin receptor subtypes. *Am J Physiol Regul Integr Comp Physiol* 286: R649–R658.

- Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: reporting *in vivo* experiments: the ARRIVE guidelines. *Br J Pharmacol* 160: 1577–1579.
- Kinnamon SC (2011). Taste receptor signaling – from tongues to lungs. *Acta Physiol* 204: 158–168.
- Lawton DM, Furness DN, Lindemann B, Hackney CM (2000). Localization of the glutamate-aspartate transporter, GLAST, in rat taste buds. *Eur J Neurosci* 1122: 3163–3171.
- Maggi CA, Meli A (1988). The sensory-efferent function of capsaicin-sensitive sensory neurons. *Gen Pharmacol* 19: 1–43.
- McGrath JC, Lilley E (2015). Implementing guidelines on reporting research using animals (ARRIVE etc.): new requirements for publication in *BJP*. *Br J Pharmacol* 172: 3189–3193.
- Miyano K, Morioka N, Sugimoto T, Shiraishi S, Uezono Y, Nakata Y (2010). Activation of the neurokinin-1 receptor in rat spinal astrocytes induces Ca²⁺ release from IP₃-sensitive Ca²⁺ stores and extracellular Ca²⁺ influx through TRPC3. *Neurochem Int* 57: 923–934.
- Montavon P, Hellekant G, Farbman A (1996). Immunohistochemical, electrophysiological, and electron microscopical study of rat fungiform taste buds after regeneration of chorda tympani through the non-gustatory lingual nerve. *J Comp Neurol* 367: 491–502.
- Muñoz M, Bernabeu-Wittel J, Coveñas R (2011). NK-1 as a melanoma target. *Expert Opin Ther Targets* 15: 889–897.
- Murata Y, Yasuo T, Yoshida R, Obata K, Yanagawa Y, Margolskee RF *et al.* (2010). Action potential-enhanced ATP release from taste cells through hemichannels. *J Neurophysiol* 104: 896–901.
- Nagai T, Kim DJ, Delay RJ, Roper SD (1996). Neuromodulation of transduction and signal processing in the end organs of taste. *Chem Senses* 21: 353–365.
- Nagy JI, Goedert M, Hunt SP, Bond A (1982). The nature of the substance P-containing nerve fibers in taste papillae of the rat tongue. *Neuroscience* 7: 3137–3151.
- Nishimoto T, Akai M, Inagaki S, Shiosaka S, Shimizu Y, Yamamoto K *et al.* (1982). On the distribution and origins of substance P in the papillae of the rat tongue: an experimental and immunohistochemical study. *J Comp Neurol* 207: 85–92.
- Oka Y, Butnaru M, von Buchholtz L, Ryba NJ, Zuker CS (2013). High salt recruits aversive taste pathways. *Nature* 494: 472–475.
- Osada K, Komai M, Bryant BP, Suzuki H, Goto A, Tsunoda K *et al.* (1997). Capsaicin modifies responses of rat chorda tympani nerve fibers to NaCl. *Chem Senses* 22: 249–255.
- Roper SD (2014). TRPs in taste and chemesthesis. *Handb Exp Pharmacol* 223: 827–871.
- Roper SD (2015). The taste of table salt. *Pflügers Arch* 467: 457–463.
- Pérez CA, Huang L, Rong M, Kozak JA, Preuss AK, Zhang H *et al.* (2002). A transient receptor potential channel expressed in taste receptor cells. *Nature Neurosci* 5: 1169–1176.
- Sato T, Nishishita K, Okada Y, Toda K (2012). Efferent fibers innervate gustatory and mechanosensitive afferent fibers in frog fungiform papillae. *Chem Senses* 37: 315–324.
- Simon SA, de Araujo IE, Gutierrez R, Nicolelis MA (2006). The neural mechanisms of gustation: a distributed processing code. *Nat Rev Neurosci* 7: 890–901.
- Simons CT, O'Mahony M, Carstens E (2002). Taste suppression following lingual capsaicin pretreatment in humans. *Chem Senses* 27: 353–365.
- Simons CT, Boucher Y, Carstens E (2003). Suppression of central taste transmission by oral capsaicin. *J Neurosci* 23: 978–985.
- Simon SA, Liu L, Erickson RP (2003). Neuropeptides modulate rat chorda tympani responses. *Am J Physiol Regul Integr Comp Physiol* 284: R1494–R1505.
- Starostik MR, Rebello MR, Cotter KA, Kulik A, Medler KF (2010). Expression of GABAergic receptors in mouse taste receptor cells. *PLoS ONE* 5: e13639.
- Talavera K, Yasumatsu K, Voets T, Droogmans G, Shigemura N, Ninomiya Y *et al.* (2005). Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. *Nature* 438: 1022–1025.
- Taruno A, Vingtdoux V, Ohmoto M, Ma Z, Dvoryanchikov G, Li A *et al.* (2013). CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes. *Nature* 495: 223–226.
- Tomchik SM, Berg S, Kim JW, Chaudhari N, Roper SD (2007). Breadth of tuning and taste coding in mammalian taste buds. *J Neurosci* 27: 10840–10848.
- Vandenbeuch A, Clapp TR, Kinnamon SC (2008). Amiloride-sensitive channels in type I fungiform taste cells in mouse. *BMC Neurosci* 9: 1.
- Vandenbeuch A, Tizzano M, Anderson CB, Stone LM, Goldberg D, Kinnamon SC (2010). Evidence for a role of glutamate as an efferent transmitter in taste buds. *BMC Neurosci* 11: 77.
- Vandenbeuch A, Anderson CB, Parnes J, Enjyoji K, Robson SC, Finger TE *et al.* (2013). Role of the ectonucleotidase NTPDase2 in taste bud function. *Proc Natl Acad Sci U S A* 110: 14789–14794.
- Vandenbeuch A, Larson ED, Anderson CB, Smith SA, Ford AP, Finger TE *et al.* (2015). Postsynaptic P2X₃-containing receptors in gustatory nerve fibres mediate responses to all taste qualities in mice. *J Physiol* 593: 1113–1125.
- Wang Y, Erickson RP, Simon SA (1995). Modulation of rat chorda tympani nerve activity by lingual nerve stimulation. *J Neurophysiol* 73: 1468–1483.
- Yamasaki H, Kubota Y, Takagi H, Tohyama M (1984). Immunoelectron-microscopic study on the fine structure of substance-P-containing fibers in the taste buds of the rat. *J Comp Neurol* 227: 380–392.
- Yoshida R, Noguchi K, Shigemura N, Jyotaki M, Takahashi I, Margolskee RF *et al.* (2015). Leptin suppresses mouse taste cell responses to sweet compounds. *Diabetes* 64: 3751–3762.