Neuroscience 258 (2014) 47–61

INGESTION OF BACTERIAL LIPOPOLYSACCHARIDE INHIBITS PERIPHERAL TASTE RESPONSES TO SUCROSE IN MICE

X. ZHU,[a,b] L. HE[a] AND L. P. MCCLUSKEY[a]*

[a] *Institute of Molecular Medicine and Genetics, Georgia Regents University, Augusta, GA, United States*

[b] *Department of Orthopedic Surgery, Zhongnan Hospital of Wuhan University, Wuhan 430071, China*

Abstract—A fundamental role of the taste system is to discriminate between nutritive and toxic foods. However, it is unknown whether bacterial pathogens that might contaminate food and water modulate the transmission of taste input to the brain. We hypothesized that exogenous, bacterially-derived lipopolysaccharide (LPS), modulates neural responses to taste stimuli. Neurophysiological responses from the chorda tympani nerve, which innervates taste cells on the anterior tongue, were unchanged by acute exposure to LPS. Instead, neural responses to sucrose were selectively inhibited in mice that drank LPS during a single overnight period. Decreased sucrose sensitivity appeared 7 days after LPS ingestion, in parallel with decreased lingual expression of Tas1r2 and Tas1r3 transcripts, which are translated to T1R2 + T1R3 subunits forming the sweet taste receptor. Tas1r2 and Tas1r3 mRNA expression levels and neural responses to sucrose were restored by 14 days after LPS consumption. Ingestion of LPS, rather than contact with taste receptor cells, appears to be necessary to suppress sucrose responses. Furthermore, mice lacking the Toll-like receptor (TLR) 4 for LPS were resistant to neurophysiological changes following LPS consumption. These findings demonstrate that ingestion of LPS during a single period specifically and transiently inhibits neural responses to sucrose. We suggest that LPS drinking initiates TLR4-dependent hormonal signals that downregulate sweet taste receptor genes in taste buds. Delayed inhibition of sweet taste signaling may influence food selection and the complex interplay between gastrointestinal bacteria and obesity.

Key words: neuro-immune interactions, chorda tympani nerve, taste receptor cell, Tas1r1, Tas1r2, Tas1r3.

INTRODUCTION

Recent studies demonstrate the detection of bacterial pathogens by mammalian chemosensory systems. Solitary chemosensory cells (SCCs), scattered throughout the respiratory epithelium, respond to bacterial quorum-sensing molecules as well as bitter irritants such as denatonium (Finger et al., 2003; Tizzano et al., 2010). Ligand binding to SCCs rapidly decreases respiration, and presumably triggers a local inflammatory response (Tizzano et al., 2010). Sensory neurons in the vomeronasal organ also respond to bacterial pathogens via formyl peptide receptors, and may sense contaminated food or sick conspecifics (Liberles et al., 2009; Riviere et al., 2009). Whether taste receptor cells contribute to the recognition of external bacterial pathogens is currently unknown.

Chemical stimuli representing sweet, bitter, salty, sour, and umami bind protein receptors and channels on the taste cell apical membrane to initiate transduction. Sensory information from taste cells on the anterior two-thirds of the tongue is transmitted to the brain by the chorda tympani (CT) nerves, which are particularly responsive to sweet and salty stimuli in many species, including C57BL/6 (B6) mice (Pfaffman, 1955; Ferrell et al., 1981; Mistretta and Bradley, 1983; Iwasaki and Sato, 1984; Shingai and Beidler, 1985; Gannon and Contreras, 1995; Danilova and Hellekant, 2003). Taste receptor cells face constant environmental and pathogenic challenges, but are replaced every 10 days or so. In keeping with this plasticity, taste cells can regenerate morphologically and functionally after denervation (Olmstead, 1921; Guth, 1971; Cheal and Oakley, 1977; Hill and Phillips, 1994). The peripheral taste system has been a useful model to study nerve and sensory target cell plasticity, and more recently, the impact of the immune system on sensory function.

Lipopolysaccharide (LPS), also known as endotoxin, is derived from the cell wall of gram-negative bacteria (Abbas and Lichtman, 2003). LPS signals through the Toll-like receptor (TLR) 4 complex, resulting in the production of proinflammatory cytokines (Raetz and Whitfield, 2002). Multiple factors (i.e. delivery method, dose) influence the effects of LPS on the taste system. Intraperitoneal LPS enhances macrophage responses to CT nerve sectioning (Cavallin and McCluskey, 2005) and promotes normal taste function in the neighboring, intact CT (Phillips and Hill, 1996). In the absence of neural injury, LPS inhibits taste receptor cell proliferation and stimulates the expression of proinflammatory genes

*Corresponding author. Address: 1120 15th Street, CA-3016, Georgia Regents University, (Formerly the Medical College of Georgia), Augusta, GA 30912, United States. Tel: +1-706-721-5616; fax: +1-706-721-8752.

E-mail address: lmccluskey@gru.edu (L. P. McCluskey).

Abbreviations: aceK, potassium acesulfame; ANOVAs, analyses of variance; CT, chorda tympani; GLP, glucagon-like peptide; LPS, lipopolysaccharide; MPG, L-glutamic acid monopotassium salt; QHCl, quinine hydrochloride dehydrate; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction; SCCs, solitary chemosensory cells; SPF, specified pathogen-free; TLR, Toll-like receptor; TRP, transient receptor potential.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.10.072

in taste buds (Wang et al., 2007, 2009; Cohn et al., 2010). Subcutaneous, lingual injections of LPS impair sodium taste function by stimulating neutrophil infiltration of the sensory field (Steen et al., 2010).

Here, we determine whether taste input to the CNS is modulated by orally delivered LPS used to mimic exposure to bacterially-contaminated food or water. Taste function is unchanged by acute, lingual exposure to LPS. However, neural responses to sucrose are inhibited in mice that ingest the endotoxin. We report the TLR4-dependence of these effects and resulting changes in Tas1r2 and Tas1r3 transcripts that form subunits which mediate sweet taste transduction (Nelson et al., 2001; Li et al., 2002). These studies suggest a novel link between ingested LPS and gustatory input to the brain.

EXPERIMENTAL PROCEDURES

Animals

The Animal Care and Use Committee at Georgia Regents University approved all protocols, which follow guidelines set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). Female, specified pathogen-free (SPF) B6 mice (Stock No. 000664, Jackson Laboratory, Bar Harbor, ME, USA) and female SPF C57BL/10ScNJ mutant mice (Stock No. 003752, Jackson Laboratory) were 6–8 weeks old during experiments. B6 mice were chosen because of their widespread use in immunology and neurobiology, and more specifically because they are responsive to sweet taste stimuli (Lush, 1989; Capeless and Whitney, 1995; Bachmanov et al., 1996). The TLR4 is absent in C57BL/10SCNJ mice, which are unresponsive to LPS. Moreover, this strain is derived from a ''sweet taster'' strain. Pilot studies revealed no consistent differences in CT responses from male vs. female B6 control mice. Thus, we chose to use females to be consistent with previous work in our laboratory (McCluskey, 2004; Steen et al., 2010). Mice were group-housed in polyurethane shoebox cages for at least one week before experiments began, when they were moved to single housing. Humidity, temperature, and light (12:12-h light:dark with lights on at 6:00 am) were automatically controlled in a barrier environment. Cages were bedded with autoclaved 1/8″ cut corncob (Harlan Laboratories, Dublin, VA, USA). Mice had free access to irradiated Teklad rodent chow (#2918; Harlan) and autoclaved distilled water, other than a 39-h period when mice were water-deprived (described below) but provided with LPS or vehicle alone.

Neurophysiology

Mice were anesthetized with ketamine (0.5 mg/g, i.p.) and sodium pentobarbital (0.5 mg/g, i.p.). Supplemental doses of these drugs were administered as needed to maintain a surgical level of anesthesia. Body temperature was maintained with a water-circulating heating pad (Gaymar T pump, Kent Scientific Corporation, Torrington, CT, USA) throughout the surgical and recording period. Hypoglossal nerves were sectioned to stop tongue movement, and mice tracheotomized and secured in an atraumatic head holder (Erickson, 1966). The left CT nerve was dissected using a mandibular approach, as previously described in rat (Hill and Phillips, 1994; Wall and McCluskey, 2008). After removing overlying bone and tissue, the CT nerve was dissected near the tympanic bulla, cut centrally, and placed on a platinum electrode. In mice, neural activity could be recorded without desheathing the nerve. Another platinum electrode was grounded in adjacent muscle, and the surgical cavity filled with warm mineral oil.

Whole nerve activity was amplified with a P511 AC Preamplifier (bandpass 1–3000 Hz, Grass Instruments, Warwick, RI, USA) and noise subtracted (Humbug 50/60 Hz Noise Eliminator, Quest Scientific, North Vancouver, BC, Canada). Amplified activity was summated with a time constant of 1.5–2.0 s using an integrator built specifically for whole-nerve recordings (Harper and Knight, 1987). Integrated responses were recorded and analyzed off-line with Power Lab 4S/P (AD Instruments). Tastants were mixed in distilled water and ~3 ml applied to the anterior two-thirds of the tongue with a syringe. The following stimuli were presented (in this order) at room temperature with an inter-stimulus interval of $\geqslant$1 min: 0.1 and 0.5 M NaCl; 0.1 and 0.5 M L-glutamic acid monopotassium salt (MPG); 0.1, 0.5 and 1.0 M sucrose; 16% polycose (Abbott Laboratories, Chicago, IL, USA); 1.0 M D-(+)-glucose; 0.01 N hydrochloric acid (HCl; Fisher Scientific, Waltham, MA, USA); 0.01 M quinine hydrochloride dehydrate (QHCl); and 0.01 M denatonium benzoate. A more limited stimulus panel [i.e. in order, 1 M sucrose, polycose, glucose, HCl, 0.05 and 0.10 M sodium saccharin; and 0.025 and 0.05 M potassium acesulfame (aceK)] was used to test additional non-caloric sweeteners in a subset of mice at day 7 after drinking LPS (n = 6) or sucrose vehicle (n = 6). Stimuli remained on the tongue for 30 s before rinsing for at least 1 min with dH_2O. Chemicals used in taste solutions were purchased from Sigma (St. Louis, MO, USA) unless noted above. The magnitude of integrated responses was measured 20 s after onset. Taste responses were normalized to 0.5 M ammonium chloride (NH_4Cl) responses at the beginning and end of each concentration series as in previous work (Hill and Phillips, 1994; Wall and McCluskey, 2008). Bracketing NH_4Cl responses were averaged, and taste responses were expressed as a ratio of this mean. We analyzed stable series bracketed by NH_4Cl responses within 10% of each other. At the end of the recording, mice were euthanized with sodium pentobarbital (80 mg/kg, i.p.) followed by thoracotomy.

Acute LPS treatment

Phenol-extracted LPS from *Escherichia coli* 026:B6 (#L8274, Sigma) was made daily from lyophilized powder in all experiments. We first tested the effects of LPS applied to the anterior tongue to determine acute effects on neural responses. Baseline CT responses to all tastants were recorded before applying LPS (10 μg/