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Regulation of bitter taste responses by tumor necrosis factor

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ABSTRACT

Inflammatory cytokines are important regulators of metabolism and food intake. Over production of inflammatory cytokines during bacterial and viral infections leads to anorexia and reduced food intake. However, it remains unclear whether any inflammatory cytokines are involved in the regulation of taste reception, the sensory mechanism governing food intake. Previously, we showed that tumor necrosis factor (TNF), a potent proinflammatory cytokine, is preferentially expressed in a subset of taste bud cells. The level of TNF in taste cells can be further induced by inflammatory stimuli. To investigate whether TNF plays a role in regulating taste responses, in this study, we performed taste behavioral tests and gustatory nerve recordings in TNF knockout mice. Behavioral tests showed that TNF-deficient mice are significantly less sensitive to the bitter compound quinine than wild-type mice, while their responses to sweet, umami, salty, and sour compounds are comparable to those of wild-type controls. Furthermore, nerve recording experiments showed that the chorda tympani nerve in TNF knockout mice is much less responsive to bitter compounds than that in wild-type mice. Chorda tympani nerve responses to sweet, umami, salty, and sour compounds are similar between TNF knockout and wild-type mice, consistent with the results from behavioral tests. We further showed that taste bud cells express the two known TNF receptors TNFR1 and TNFR2 and, therefore, are potential targets of TNF. Together, our results suggest that TNF signaling preferentially modulates bitter taste responses. This mechanism may contribute to taste dysfunction, particularly taste distortion, associated with infections and some chronic inflammatory diseases.

1. Introduction

Taste is the sensory system for detecting nutrients and potentially harmful substances in food and drink and, therefore, plays important roles in guiding food intake. Among the five basic taste modalities, sweet and umami tastes detect sugars and amino acids, respectively, and are generally preferred. Bitter taste recognizes toxins and noxious compounds and elicits avoidance behavior. Acids and salts are detected by sour and salt taste mechanisms. Recent research has made rapid progress in understanding taste receptors and signaling pathways, particularly for sweet, umami, and bitter tastes (Breslin and Huang, 2006; Chandrashekar et al., 2006; Liman et al., 2014). What remain largely unclear, however, are the regulatory mechanisms that modulate taste responses or taste bud structure under diverse physiological and pathological conditions.

Inflammation is likely one of such regulatory mechanisms. Many diseases with underlying inflammation, such as infections and autoimmune ailments, are associated with taste alterations (Bromley and Doty, 2003; Pribitkin et al., 2003; Schiffman, 1983). Taste alterations can occur as taste loss (lacking or reduced taste reception) or taste distortion (e.g. persistent bitter or metallic taste in the mouth) (Brand, 2000; Bromley, 2000). In animal models, induced inflammation has been shown to affect taste responses and taste bud structure (Cavallin and McCluskey, 2005; Cohn et al., 2010; Phillips and Hill, 1996). How inflammation exerts its effects on taste reception or taste bud structure has not been fully elucidated. Inflammation is an immune response to infection, tissue damage, and stress. In addition to its roles in regulating immunity and tissue repair, inflammation can strongly affect metabolism and food intake (Forsythe et al., 2008; Hotamisligil, 2006). The various effects of inflammation are often mediated by

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inflammatory cytokines, a group of signaling proteins that are highly induced during inflammatory responses. A number of inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-6, and interferons, are pleiotropic and play important parts in regulating immunity, metabolism, and food intake (Cannon, 2000; Dantzer, 2001; Plata-Salaman, 1998).

Our studies have found that several inflammation-associated cytokines are preferentially expressed in taste bud cells compared to nontaste lingual epithelial cells (Cohn et al., 2010; Feng et al., 2012, 2014a,b; Kim et al., 2012; Wang et al., 2007), suggesting that these cytokines may have special functions in the peripheral taste system. In particular, we have found that TNF is specifically expressed in a subset of taste bud cells even in healthy mice (Feng et al., 2012; Kim et al., 2012). Immunocolocalization experiments showed that TNF is colocalized with the sweet and umami taste receptor subunit T1R3, indicating that TNF is expressed specifically in the sweet and umami taste bud cells (Feng et al., 2012). Moreover, TNF expression level and its secretion in taste buds can be further augmented by inflammation, such as lipopolysaccharide (LPS)-induced inflammation (Cohn et al., 2010; Feng et al., 2012; Kim et al., 2012).

TNF was thought to be produced primarily by macrophages, but it is also produced by a broad variety of cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, fibroblasts, adipocytes, and neurons (Hotamisligil et al., 1993; Niu et al., 2009; Walsh et al., 1991). TNF is known to activate a variety of cellular signaling pathways that are important not only for fighting against certain pathogens but also for regulating stress responses and metabolism (Cabal-Hierro and Lazo, 2012; Silke, 2011; Wajant et al., 2003). TNF contributes to behavioral changes associated with various illnesses (i.e. sickness behavior) which include fatigue, malaise, depression, and anorexia. It has been shown that administration of recombinant TNF induces significant reduction in food intake both in rodents and in humans (Bernstein et al., 1991; Michie et al., 1989; Spiegelman and Hotamisligil, 1993). How TNF regulates food intake remains incompletely understood. Both peripheral and brain mechanisms are likely involved (Bernstein et al., 1991; Dantzer, 2001; Plata-Salaman, 1998).

The taste system plays an important role in regulating food intake. Considering the specific expression of TNF in taste bud cells, it is conceivable that TNF may be involved in modulating taste responses under physiological and pathological conditions. In this study, we used TNF knockout mice and their wild-type controls to investigate the role of TNF in the taste system. We conducted gustatory nerve recording and taste behavioral testing using these mice. Our results show that TNF-deficient mice are significantly less responsive to bitter compounds than control mice, whereas their responses to sweet, umami, sour, and salty compounds did not differ significantly from those of control mice. Our results suggest that TNF is involved in the regulation of bitter taste reception.

2. Materials and methods

2.1. Animals

TNF knockout mice (stock number 005540) and wild-type control mice (C57BL/6J, stock number 000664) were purchased from the Jackson Laboratory (Bar Harbor, ME) and then bred and maintained at the Monell Chemical Senses Center. Generation of TNF knockout mice was described by Pasparakis et al. (1996). In these TNF knockout mice, the first coding exon (including the ATG translation initiation codon) and a portion of the first intron of the TNF gene were deleted. The mutant mice have been backcrossed to C57BL/6J genetic background for ten generations. All mice were housed at the Monell Chemical Senses Center animal facility under a 12 h/12 h light/dark cycle. Mice were given free access to standard rodent food (8604 Teklad rodent diet, Harlan Laboratories) and water except during the periods of taste behavioral tests (described below). 4–10 months old mice were used for all the experiments described below. Age and gender matched wild-type and TNF-knockout mice were included for the experiments. All procedures were performed according to protocols approved by the Monell Chemical Senses Center Institutional Animal Care and Use Committee.

2.2. Reagents

All taste compounds used in behavioral and electrophysiological tests were purchased from Sigma (St. Louis, MO). Rabbit polyclonal antibody against mouse ecto-nucleoside triphosphate diphosphohydrolase 2 (ENTPDase2) was purchased from Centre de Recherche (Quebec, Canada) (Bartel et al., 2006). Rabbit polyclonal antibodies against phospholipase C-β2 (PLC-β2, sc-206) (Clapp et al., 2001) and gustducin (sc-395), goat polyclonal antibodies against the voltage-gated potassium channel KCNQ1 (sc-10646) (Wang et al., 2009) and TNFR1 (sc-1069), and a blocking peptide (sc-1069p) for the anti-TNFR1 antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). A purified rabbit polyclonal antibody against neural cell adhesion molecule (NCAM) was purchased from Millipore (Billerica, MA). Purified goat polyclonal antibodies against carbonic anhydrase 4 (Chandrashekar et al., 2009) and TNFR2 and a blocking antigen (recombinant mouse sTNFR2, 426-R2-050) for the anti-TNFR2 antibody were purchased from R&D Systems (Minneapolis, MN). Dylight-649 (or Dylight-488)-conjugated donkey anti-rabbit or donkey anti-goat antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

2.3. Taste behavioral tests

Two-bottle preference tests were conducted as previously described (Bachmanov and Beauchamp, 2008; Bachmanov et al., 2001, 2002; Wang et al., 2009). Briefly, TNF-deficient and wild-type mice were individually caged. For the first 2 days, mice were familiarized with the two drinking bottles, both containing deionized water. For the following days, mice were presented with two drinking bottles: one contained deionized water and the other a taste solution. The positions of the bottles were switched after 24 h to minimize positional effect. The volume of consumed liquid from each bottle was recorded at 24 and 48 h. Each concentration of a taste compound was tested for 48 h. The taste compounds were tested in the following order: NaCl (37.5, 75, 150, 300, and 600 mM), quinine hydrochloride (QHCl) (0.003, 0.01, 0.03, 0.1, and 0.3 mM), Saccharin (0.0625, 0.25, 1, 4, and 16 mM), inosine-5′-monophosphate (IMP) (0.3, 1, and 3 mM), and citric acid (1, 3, and 10 mM). Each mouse in the experiment was tested with all the above listed compounds. Between the testing of two different compounds, mice received deionized water in both drinking tubes for at least 3 days. During the experiment mice had free access to food. TNF-deficient mice and wild-type mice were tested at the same time in parallel. Taste preference scores were calculated for each animal by dividing the volume of consumed taste solution during the 48 h test period by the total volume of fluid intake during the same 48 h test period (i.e. preference score = intake of taste solution/(intake of taste solution + intake of water)). The average preference scores were then calculated for each group. By this calculation formula, preference scores between 0.5 and 1 indicate that mice prefer the taste solution over water, whereas preference scores between 0 and 0.5 indicate that mice avoid the taste solution. 8–16 male mice (4–8 months old) in each group were used.

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