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INFORMATION PROCESSING IN BRAINSTEM BITTER TASTE-RELAYING NEURONS DEFINED BY GENETIC TRACING

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Abstract—Bitter reception is mediated by taste receptor cells that coexpress multiple T2Rs, a family of G-protein-coupled receptors. However, it remains elusive how bitter taste information is translated in the brain into appropriate behavioral responses. Here we used a combination of genetic tracing and electrophysiological and immunohistochemical analyses in mice to functionally characterize the neurons in the solitary tract nuclei of the medulla, which receive input from mT2R5-expressing cells. The neurons defined by a transneuronal tracer originating from mT2R5-expressing cells receive glutamatergic synaptic input via the AMPA receptor. The satiety peptide cholecystokinin increases glutamatergic transmission, suggesting an interaction between information processing of taste and the homeostatic control of feeding. Nevertheless, the tracer-labeled neuron types are heterogeneous, and can be classified into catecholamine and pro-opiomelanocortin neurons. Our data reveal that the architectural solution in the first-order central relay that processes information from mT2R5-expressing cells uses unique ensembles of neurons with different neurotransmitters.

Key words: bitter taste, the solitary tract nuclei, catecholamine neuron.

INTRODUCTION

The taste sensory system is responsible for evaluating the quality of food, and plays a critical role in the life and nutritional status of organisms. Taste perception in mammals is mediated by taste receptor cells that are arranged in the taste buds of the oral epithelium, and that express receptors responsible for detecting sweet, bitter, salty, sour, and umami taste stimuli (Lindemann, 2001). The five basic taste qualities are mediated by separate taste receptor cells each expressing a given receptor class and thus tuned to a single taste modality (Zhang et al., 2003; Zhao et al., 2003; Mueller et al., 2005; Huang et al., 2006; Chandrashekar et al., 2010). T2Rs and T2R-expressing cells are necessary and sufficient for the detection and perception of bitter compounds (Mueller et al., 2005). The taste receptor cells are innervated by sensory neurons (geniculate, petrosal, and nodose ganglion neurons) in branches of the facial, glossopharyngeal and vagus nerves. Each sensory neuron contacts multiple taste receptor cells within a taste bud, and relays taste information to neurons in the nucleus of the solitary tract (NTS) of the medulla (Hamilton and Norgren, 1984). From the NTS, taste information is transferred to the gustatory cortex through synapses in the pontine parabrachial nuclei (PBN) and the thalamus in rodents (Norgren, 1995).

In contrast to sweet taste, bitter taste detection provides mammals with a sensory input to warn against the ingestion of noxious substances, and to evoke aversive and displeasing responses. Activation of specific populations of sweet and bitter taste receptor cells and their innervating neurons can elicit contrastive behavioral responses (Zhao et al., 2003; Mueller et al., 2005). Although gustatory neurons in the NTS appear to be more broadly tuned across taste qualities than peripheral fibers, they are also reported to be differentially activated by bitter versus sweet taste stimuli (Lemon and Smith, 2005). Recently, *in vivo* two-photon calcium imaging simultaneously from large numbers of neurons showed that each of the bitter, sweet, umami and salty tastes is represented in the primary taste cortex by the activation of finely tuned cells that are clustered in its own separate field (Chen et al., 2011). This cortical map reveals the potential neuronal bases that underlie cognition and discrimination of the basic taste qualities. Nevertheless, it remains elusive how taste information evokes specific emotional and behavioral responses. To identify and prove which taste-transmitting neurons play critical roles in taste-evoked behavioral and emotional responses or taste cognition, it is necessary to visualize the neuronal circuitries transmitting specific taste information, to clarify the cellular characteristics of the labeled neurons, and to examine the roles of specific neurons by manipulating the activities of cognate neuronal populations.

We recently applied a genetic approach to delineate the neuronal circuitries of bitter and sweet tastes by

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Abbreviations: AITC, allyl isothiocyanate; α-MSH, alpha-melanocyte-stimulating hormone; BSA, bovine serum albumin; CCK, cholecystokinin; CSF, cerebral spinal fluid; DIC, differential interference contrast; EGFP, enhanced green fluorescent protein; EGTA, ethylene glycol tetraacetic acid; EPSC, excitatory postsynaptic currents; GFP, green fluorescent protein; GLP-1, glucagon-like peptide 1; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LiCl, lithium chloride; NTS, nucleus of the solitary tract; PBN, parabrachial nuclei; PBS, phosphate-buffered saline; POMC, pro-opiomelanocortin; RF, reticular formation; TH, tyrosine hydroxylase.

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selectively expressing the fluorescent transneuronal tracer, tWGA-DsRed, in either bitter- or sweet-responsive taste receptor cells in mice, and then visualizing the spatial distribution of tWGA-DsRed-labeled neurons in the brain (Sugita and Shiba, 2005). The genetic approach using the WGA transgene, expressed in a specific population of neurons, had been developed as an effective tool for tracing selective multisynaptic neural pathways originating from them (Yoshihara et al., 1999). When tWGA-DsRed is expressed in taste receptor cells, it reaches the neurons in the NTS by passing through two intercellular junctional structures with the aid of the machineries of exocytosis and endocytosis. The locations of tWGA-DsRed-labeled neurons suggested the topographic segregation of taste representation in the NTS where the gustatory neurons are organized with sweet/umami inputs located rostral and with bitter inputs located caudal (Sugita and Shiba, 2005). Thus, genetic tracing revealed a topographic map of bitter taste representation in the brain that was distinct from sweet taste. To predict the roles of the tracer-labeled neurons, it will be necessary to determine the neuron types, their mechanisms of synaptic transmission, and the heterogeneity of neurons that convey specific taste information at a central relay.

Here we combined genetic tracing with electrophysiological and immunohistochemical approaches to functionally define the bitter taste-relaying neurons in the NTS, fluorescently labeled by the tracer tWGA-DsRed originating from T2R-expressing cells. Analyses of the cellular characteristics of tWGA-DsRed-labeled NTS neurons revealed that catecholamine and pro-opiomelanocortin (POMC) neurons relay and process bitter taste information, suggesting the neuronal bases underlying interaction between the processing of taste information and the homeostatic control of feeding at a level of the NTS.

EXPERIMENTAL PROCEDURES

Breeding, housing, and genotyping of transgenic mice

Transgenic mice expressing mT2R5-green fluorescent protein (GFP) and tWGA-DsRed under the control of the promoter element of the mT2R5 gene, a receptor for the bitter tastant cycloheximide, were maintained on a C57BL/6 background (Sugita and Shiba, 2005) and genotyped for the presence of an enhanced green fluorescent protein (EGFP) by polymerase chain reaction (PCR). They were housed on a 12-h light/dark cycle. This study was approved by the Committee of Research Facilities for Laboratory Animal Science, Hiroshima University. The animals were treated in accordance with the Guide for Animal Experiment, Hiroshima University.

Direct fluorescence detection

The spatial distribution of tWGA-DsRed originating from mT2R5-expressing bitter taste receptor cells was examined in coronal or horizontal cryostat sections, obtained by the film-transfer method which enabled us to cut the samples including the soft tissues and the hard calcified tissues (Kawamoto and Shimizu, 2000; Sugita and Shiba, 2005). Mice (3–6 months old) were anesthetized with sodium pentobarbital and then perfused transcardially with 2% formaldehyde solution (1× phosphate buffered saline (PBS), 2% formaldehyde). The mouse head or brain was isolated, and rapidly frozen in 4% carboxymethyl cellulose. The frozen block was sectioned either from the posterior part of the head for coronal sections, or from the ventral part of the brain for horizontal sections, using a tungsten carbide blade attached to the cryomicrotome at −25 °C. During sectioning, each section surface was covered with a polyvinylidene chloride film (10 μm thick) coated with synthetic rubber cement, and then the sample was cut at a speed not exceeding 4 mm/s. The coronal or horizontal sections (30 μm) were fixed with 3.7% formaldehyde solution (1× PBS, 3.7% formaldehyde, 0.18% Triton X-100) for 10 min. The distribution of tWGA-DsRed was directly visualized by DsRed fluorescence, excited using a Hg-lamp, and collected using the recommended band-pass filter sets. Fluorescence images were taken with the UPlanSApo 40×/0.95 NA or LCPlanFl 40×/0.60 NA objective lens at room temperature using an inverted microscope (IX 71; Olympus (Tokyo, Japan)) equipped with a charge-coupled device camera (CoolSNAP HQ; Photometrics (Tucson, AZ, USA)). The images were acquired using MetaMorph imaging software (v7.0; Molecular Devices (Sunnyvale, CA, USA)). Brain structures were identified microscopically by reference to a mouse brain atlas (Paxinos and Franklin, 2001). The anterior–posterior lengths of brain structures and the locations of the tWGA-DsRed-labeled neurons were calculated from the number of sections multiplied by 30 μm. For each coronal section, the distance to the obex was calculated and denoted in Fig. 1A, while referring to the anterior part as plus (+), and the posterior part as minus (−). Three-dimensional images of the brain were reconstructed by assembling the brain structures extracted from the serial coronal section images, using TRI/3D-SRF2 software (RATOC (Tokyo, Japan)).

Immunohistochemistry

The horizontal or coronal cryostat sections (30 μm) were obtained using the film-transfer method. The samples were fixed and permeabilized with 3.7% formaldehyde solution (1× PBS, 3.7% formaldehyde, 0.18% Triton X-100) for 10 min. Nonspecific binding sites were blocked with 1% bovine serum albumin (BSA) for 30 min. The sections were treated with primary antibodies against tyrosine hydroxylase (TH) (Chemicon (Millipore) (Temecula, CA, USA), AB152; 1:600), POMC (Phoenix Pharmaceuticals (Burlingame, CA, USA), H-029-30; 1:100) and glucagon-like peptide 1 (GLP-1) (Peninsula Laboratories, LLC (San Carlos, CA, USA), T-4362; 1:300), which were pre-bound with the Alexa-488- or Alexa-647-labeled Fab fragments of secondary antibodies in the Zenon labeling system (Invitrogen (Carlsbad, CA, USA)). The subcellular distributions of TH, POMC, and GLP-1 were visualized by