Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres
Brain Research

Research Report

Neuronal expression of bitter taste receptors and downstream signaling molecules in the rat brainstem

Ozra Dehkordi[a,b,c,*], Jed E. Rose[d], Mehrnaz Fatemi[c], Joanne S. Allard[b], Kannan V. Balan[e], John K. Young[f], Sabiha Fatima[c], Richard M. Millis[b,c], Annapurni Jayam-Trouth[c]

[a]Department of Surgery, Howard University Hospital, Washington, DC 20060, United States
[b]Department of Physiology & Biophysics, Howard University College of Medicine, Washington, DC 20059, United States
[c]Department of Neurology, Howard University Hospital, Washington, DC 20060, United States
[d]Department of Psychiatry, Duke University Medical Center, Durham, NC 27705, United States
[e]Department of Pediatrics, Case Western Reserve University, School of Medicine, Cleveland, OH 44106-6010, United States
[f]Department of Anatomy, Howard University College of Medicine, Washington, DC 20059, United States

ARTICLE INFO

Article history:
Accepted 18 July 2012
Available online 24 July 2012

Keywords:
α-gustducin
PLC-β2
Tryptophan hydroxylase
Brainstem
Bitter taste receptors

ABSTRACT

Previous studies have shown that molecules of the taste transduction pathway may serve as biochemical markers for chemoreceptive cells in respiratory and gastrointestinal tracts. In this study, we tested the hypothesis that brainstem neurons contain signaling molecules similar to those in taste buds which may sense the chemical composition of brain extracellular fluids. We used the reverse transcription polymerase chain reaction (RT-PCR), Western blot and immunohistochemical techniques to evaluate presence of different bitter-responsive type 2 taste receptors (T2Rs), their associated G-protein α-gustducin, the downstream signaling molecules phospholipase C isoform β2 (PLC-β2) and transient receptor potential melastatin 5 (TRPM5) in the brainstem of rats. RT-PCR confirmed the mRNA coding for α-gustducin, PLC-β2, TRPM5 and rT2R1 but not that of rT2R16, rT2R26 and rT2R38 in the medulla oblongata. Western blotting confirmed the presence of α-gustducin at the protein level in rat brainstem. Immunohistochemistry identified cells expressing α-gustducin and PLC-β2 at multiple cardiorespiratory and CO_2/H^+ chemosensory sites, including rostral ventral medulla, facial, parapyramidal, solitary tract, hypoglossal and raphe nuclei. In the medullary raphe, α-gustducin and PLC-β2 were colocalized with a subpopulation of tryptophan hydroxylase (TPH)-immunoreactive serotonergic neurons, a subset of which has respiratory CO_2/H^+ chemosensitivity. Presence of the T2R1 gene and other genes and proteins of the bitter taste transduction pathway in the brainstem implies additional functions for taste receptors and their effector molecules apart from their gustatory function.

*Corresponding author at: Division of Neurosurgery, Howard University Hospital, Washington, DC 20060, United States. Fax: +1 202 865 1977.
E-mail address: odehkordi@howard.edu (O. Dehkordi).

0006-8993/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2012.07.038

1. Introduction

The ability to taste the chemical composition of foods is important for recognition of nutrients and rejection of toxins to maintain energy balance and homeostasis. Binding of tastant substances to different receptors in taste receptor cells initiates signaling pathways leading to taste perception that humans describe as sweet, bitter, salt, umami and sour (Palmer, 2007; Ishimaru and Matsunami, 2009). The sensory responses to bitter, sweet and umami compounds are initiated by G-protein coupled receptors (GPCRs) which are transduced via G-protein signaling cascades (Palmer, 2007; Ishimaru and Matsunami, 2009). α-Gustducin, the alpha subunit of a heterotrimeric G-protein, is thought to be the main intracellular partner of the sweet and bitter taste receptors T1Rs and T2Rs (Wong et al., 1996; Matsunami et al., 2000; Zhao et al., 2003; Mueller et al., 2005; Ishimaru and Matsunami, 2009). Although α-gustducin was originally believed to be expressed exclusively in taste receptor cells, evidence is emerging that the taste transduction molecules α-gustducin, its effector molecules phospholipase C isoform β2 (PLC-β2) and transient receptor potential melastatin 5 (TRPM5), as well as GPCRs T2R and T1R are expressed in tissues outside the oral cavity. These sites include epithelial cells of gastrointestinal (Wu et al., 2005; Bezençon et al., 2007; Rozengurt and Sternini, 2007; Sternini et al., 2008), pancreatic (Höfer et al., 1998) and solitary chemosensory cells (SCCs) of nasal and airway epithelia (Finger et al., 2003; Tizzano et al., 2006; Lin et al., 2008; Dehkordi et al., 2010; Rose et al., 2012). Functional studies have shown that SCCs of nasal epithelium utilize the molecules of the taste transduction pathway to sense and respond to bitter and odorous irritants (Finger et al., 2003; Gulbransen et al., 2008). These findings suggest that taste sensing molecules may have broader functions than acting as chemosensors for taste. Chemosensitive neurons possessing various neurochemical profiles and functions are known to be widely distributed in the brainstem (Nattie, 2000; Douglas et al., 2001). Bitter tasting chemicals and nutrients such as nicotine, alcohol and caffeine can easily cross the blood-brain barrier. Bitter tasting di- and tri-peptides derived from food proteins can also enter the brain through high-affinity peptide transporters located at the choroid plexus (Shu et al., 2002; Upadhyaya et al., 2010 The presence of these bitter-tasting ligands in the CNS suggests the possibility that bitter taste receptors may be present in the CNS and may have physiological roles apart from their involvement in the conscious perception of taste. The present study was, therefore, designed to test the hypothesis that taste signaling molecules may be found in neurons at multiple brainstem sites of known chemosensitivity (Nattie, 2000; Douglas et al., 2001). This hypothesis was tested by first evaluating the expression of the T2Rs and their downstream signaling components α-gustducin, PLC-β2 and TRPM5 in the brainstem of rats at the mRNA and protein levels. Sequential double labeling immunofluorescence studies were then performed to demonstrate the presence of these molecules in serotonergic neurons of the medullary raphe that are known to be chemosensitive to acid (Richerson et al., 2001; Wang et al., 2001).

2. Results

2.1. *Expression of bitter taste receptor genes, their effector molecule genes and their proteins in the brainstem*

We first determined the presence of rT2R1, rT2R16, rT2R26, rT2R38, α-gustducin, PLC-β2 and TRPM5 in the brainstem by evaluating the expression of their mRNA using RT-PCR. With the exception of rT2R16, rT2R26 and rT2R38, the PCR products of all molecules were present in the brainstem RNA extract (Fig. 1). All primers yielded products of the expected sizes. No signal was detected in the negative reverse transcription controls (RT-) suggesting PCR specificity and absence of genomic amplification. The immunoblotting results demonstrated that rabbit polyclonal antibody specific for α-gustducin recognized a protein band of molecular weight of 40 kDa in medulla oblongata and tongue (positive control) but not in liver (negative control) (Fig. 2 Panel C).

2.2. *Immunohistochemical localization of α-gustducin and PLC-β2 IR cells in the brainstem*

Fig. 2 (Panels A and B) demonstrates α-gustducin immunoreactivity (IR) in taste buds of circumvallate papillae (CP: positive

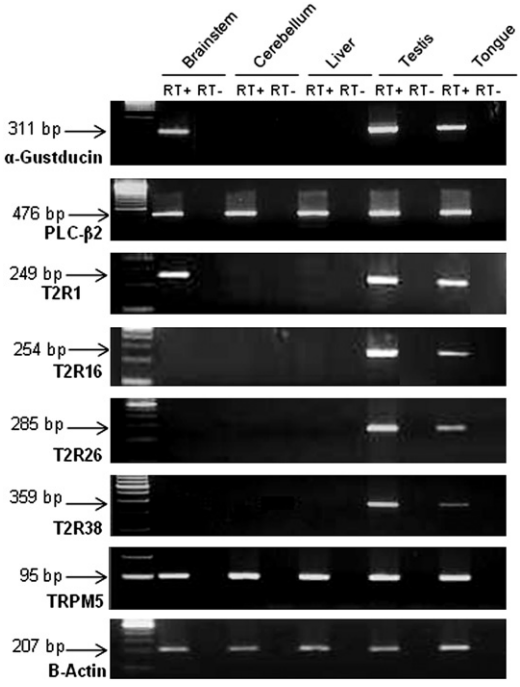

Fig. 1 – RT-PCR demonstrating taste transduction molecules in the rat brainstem. The templates were cDNA prepared from RNA isolated from brainstem, as well as from the following control tissues: Cerebellum, liver, testis and tongue. RT+=Amplified product of the expected sizes were obtained for α-gustducin (311 bp), PLC-β2 (476 bp), T2R1 (249 bp), T2R16. (254 bp), T2R26 (285 bp), T2R38 (359 bp) and TRPM5 (95 bp) and the housekeeping gene β-actin (207 bp). RT−=Negative control reactions without reverse transcriptase. The size for PCR products are indicated by arrows.