

Taste Receptor Tas2r5 and Tas1r3 is Expressed in Sublingual Gland

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Abstract

Type 2 taste receptors (TAS2Rs), a large family of GPCRs, were first discovered in the gustatory system, and are co-expressed in a subset of taste receptor cells and detect bitter-tasting compounds. Type 1 taste receptors (TAS1R3) function as an obligate partner for both the umami receptor and the sweet receptor. Recently, it has become clear that taste receptors are also expressed outside the gustatory system. Here, with Tas2r5-Cre/GFP and Tas1r3-Cre/GFP transgenic mice, the expression of taste receptors (Tas1r3 or Tas2r5) is observed in serous gland and mucous gland of tongue. Taste signal transduction cascade (Gnat3 and PLC- β 2) is also detected in serous gland and mucous gland of tongue. After DTA expression in Tas2r5+ and Tas1r3+ cells, the expression of taste receptors and taste signal transduction cascade is ablated in serous and mucous gland of tongue.

Keywords: Serous gland; Mucous gland; Taste receptor; Tongue; Transgenic mice

Introduction

It is believed that taste is the sensory modality that guides organisms to recognize and consume digestible material and avoid toxins. For humans, the so-called “basic” taste is thought as of identifying and distinguishing sweet, umami, sour, salty, and bitter. Gustatory signals come from sensory end organs in the oral cavity-taste bud [1]. Taste buds are clusters of up to 100 polarized neuroepithelial cells that localized to specializations of the tongue epithelium called fungiform (FuP), foliate (FoP), and circumvallate papillae (CV) [2,3]. Sweet, umami, and bitter compound each activate different taste GPCRs that are expressed in discrete sets of receptor cells [4]. For example, the TAS2Rs family of GPCRs is expressed in the special sets of Type II cells, and sense bitter compounds. In mice, 20-35 separate genes encode members of the TAS2Rs family. Based on the analysis of *in situ* hybridizations with mixed probes on rodent taste buds, the TAS2Rs were reported either to be expressed as overlapping subsets of taste bud cells or co-expressed in a single population of taste bud cells [1,5,6]. This pattern of TAS2Rs expression, along with polymorphisms across the gene family, is thought to allow human and animals to detect the enormous range of potentially toxic bitter compounds found in nature [1,7].

The TAS1Rs are dimeric Class III GPCRs, with large N-terminal ligand binding domains. Three different TAS1Rs have been reported, including Tas1r1, Tas1r2 and Tas1r3. So far, it is always believed that these receptors have the biological function *in vivo* only as heterodimers, with Tas1r3 serving as an obligate partner for both the umami receptor (Tas1r1+Tas1r3) and the sweet receptor (Tas1r2+Tas1r3) [8-12]. Furthermore, knockouts of those genes have been used to identify the role of Tas1rs in taste transduction. The genetic modification of Tas1r3 abolished responses to most sweet and umami compounds [9-12]. The cloning of taste signalling effectors and the production of several lines of transgenic mice expressing Green Fluorescent Protein (GFP) from their promoters, particularly Gnat3, Trmp5 and Tas1r3, has revealed an extensive expression in the airways, from the upper airways to the lungs. Furthermore, Tas1rs signalling has been most extensively studied in indigestive system. The sweet receptor is selectively expressed in enteroendocrine L cells, which can release GLP-1 after sugar ingestion, and GLP-1 in turn augments insulin release from the pancreas [13-17]. Furthermore, the expression of the Tas1rs and

their associated G-protein genes has also been reported in mammalian brain, indicating that the Tas1r2/Tas1r3 is a candidate membrane-bound brain glucosensor [18,19].

In previous study, we generate two transgenic mice with the modified BAC-DNA, in which a fusion protein of GFP/Cre is driven by Tas2r5 or Tas1r3 promoter. In Tas2r5-GFP/Cre transgenic mice, the coding sequence of Tas2r5 is replaced by that of GFP/Cre gene [20]. However, in Tas1r3-GFP/Cre transgenic mice, the DNA fragment containing a Cre/GFP fusion protein is exactly inserted between the stop codon and poly (A) tail of Tas1r3 gene [21]. When we separately cross those transgenic mice with R26: lacZbpA^{lox}DTA transgenic mouse [22], the double transgenic mice will be obtained, in which the A chain of diphtheria toxin (DTA) is specifically expressed in Tas1r3+ [21] or Tas2r5+ [20] cells, and catalyzes the ADP-ribosylation of the eukaryotic elongation factor 2, resulting in inhibition of translation, which finally leads to cell death [22]. Here, we observed the expression of taste receptors (Tas1r3 or Tas2r5) and signal transduction cascade (Gnat3 and PLC- β 2) in sublingual gland with transgenic mice system and immunohistological analysis.

Materials and Methods**BAC-Tas2r5-Cre/GFP and BAC-Tas1r3-Cre/GFP construction and generation of transgenic mouse lines**

The generation and set-up of the transgenic mouse line has been described in previous study [20,21]. Briefly, the BAC-Tas2r5-Cre/GFP and BAC-Tas1r3-Cre/GFP construct was purified and microinjected into the pronuclei of B6 (C57BL/6J) mouse zygotes, which were implanted into pseudo pregnant foster mothers using standard techniques. The transgenic founder mice and their progeny were identified by PCR with Cre/GFP-specific primers and Tas2r5/Tas1r3-specific primers.

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R26: ^{LoxP}stop^{LoxP} LacZbpA mice were purchased from the Jackson Laboratory, Bar Harbor, USA. The R26: lacZbpA^{fllox} diphtheria toxin A (DTA) line was a gift from Dr. D. Brockschneider [22].

Histology and immunostaining procedure

For immunocytochemistry, mice were perfused transcardially with 2-4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS; pH 7.2-7.4). The tongue or testis tissues were dissected, post-fixed in PFA for 2-12 hours and cryoprotected in 30% sucrose in PBS at 4°C overnight. After sectioning on a cryostat, 10-12 µm sections were collected onto Superfrost Plus Microscope slides (Fisher Scientific, Loughborough, UK). The polyclonal primary antibodies used were specific for GFP (goat ab-5450, rabbit ab-6556; Abcam, Cambridge, UK), PLC-β2 (rabbit sc-206; Santa Cruz Biotechnology, Santa Cruz, USA), Tas1r3 (rabbit sc-22459; Santa Cruz Biotechnology, Santa Cruz, USA), and β-Galactosidase (chicken, ab9361; Abcam) and Gnat3 (rabbit sc-395; Santa Cruz Biotechnology).

Staining against GFP or Tas1r3 was performed using the standard immunocytochemical procedure. Cryosections were washed in PBS (3 × 10 min), placed into blocking solution [1% bovine serum albumin (BSA), 1% normal horse serum, and 0.3% Triton X-100 in PBS] for 1-2 hr, and then incubated in a mixture of the polyclonal, primary antisera: rabbit anti-GFP (1:500-1000 dilution) and rabbit anti-Tas1r3 (1:300-500 dilution) in blocking solution. Primary antibody incubation lasted for 36-48 hr at 4°C, and then sections were washed in PBS 3 × 10 min and incubated for 2-18 hr in a mixture of secondary antibodies: Alexa568 goat anti-rb (1:400; Molecular Probes, USA). The slides then were washed one time for 10 minutes in 0.1 M PB and two times for 10 minutes in 0.1 M PBS before cover slipping slides with Fluormount G (Southern Biotechnology Associates, USA).

Staining against GFP, PLCβ2 and Gnat3 was performed using the standard immunocytochemical procedure according to the manufacturer's instructions (VECTASTAIN Elite ABC Kit; Vector Laboratories, Burlingame, USA). The standard immunocytochemical procedure used an avidin-biotin-peroxidase complex (ABC; Vector Laboratories, Burlingame, CA) and 3,38-diaminobenzidine (DAB, Sigma). Cryosections were washed in four 4-minute washes of 0.1 M PBS, pH 7.4, containing 0.3% Triton X-100. The slides subsequently were incubated in blocking solution [1% bovine serum albumin (BSA), 1% normal horse serum, and 0.3% Triton X-100 in PBS] for 1-2 hr, followed by a 24 h incubation with the primary antibody at 4°C. The slides were washed with PBS four times followed by a 45-minute application of the biotin-conjugated secondary antibody. Three additional washes in PBS preceded both the 30-minute application of ABC and the 10-minute incubation with a PBS solution containing 0.5 mg/ml DAB, and 0.01% H₂O₂ to tint the reaction product blue. Counterstaining is carried out with standard hematoxylin staining and bright field images of the sections were captured digitally.

LacZ staining

Animals were perfused with 2% PFA in PBS. The tongues were removed and fixed in 2% PFA for 1 h, after which it was cryoprotected in 30% sucrose in PBS at 4°C overnight. The following morning, the tongue tissue was cryosectioned at 20 µm thickness. The sections were then washed three times (3 × 20 min) in PBS, followed by staining in X-gal solution (5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM MgCl₂, 0.02% NP-40, 0.01% Na deoxycholate, 1 mg/ml X-gal) at 37°C overnight. The stained sections were washed three times (3 × 20 min) in PBS and counterstained with nuclear fast red. Brightfield images were captured using a SPOT digital

camera (Diagnostic Instruments, Inc) attached to an Olymopus X51 microscope.

Results

DTA expressions in Tas2r5+ or Tas1r3+ cells ablate GFP expression in taste bud

In our previous study, it is observed that Tas2r5 gene is expressed in testis, and ablation of Tas2r5+ cells lead to the loss of spermatid in most of seminiferous tubules in the double transgenic mouse Tas2r5-Cre/GFP-DTA [20]. Two bottle preference test revealed the loss of bitter taste to the cycloheximide [23], which is specific compound to Tas2r5 receptor after ablation of Tas2r5+ cells in the double transgenic mouse [20]. After directly observing the section of CV papillae, GFP expression was found in taste bud (Figure 1A and 1B). After DTA expression in Tas2r5+ cells, GFP expression was absent from taste bud (Figure 1C). DTA expression in Tas2r5+ cells failed to ablate the Tas1r3 expression in taste bud (Figure 1D), which agreed with previous study [24].

In order to further investigate the expression of taste receptors in sublingual gland, we employed another transgenic mouse, in which Tas1r3 gene drive the expression of Cre/GFP fusion protein. Confocal analysis with anti-GFP showed GFP expression in taste bud (Figure 2A and 2B). After DTA expression in Tas1r3+ cells, most of GFP expression was deleted in CV papillae (Figure 2C). Meanwhile, DTA expression in Tas1r3+ cells ablated Tas1r3 expression in taste bud (Figure 2D).

In short, the current results show the expression of Tas2r5 and Tas1r3 in taste bud of CV papillae. DTA expression in Tas2r5+ and Tas1r3+ cells ablated the expression of GFP in taste bud.

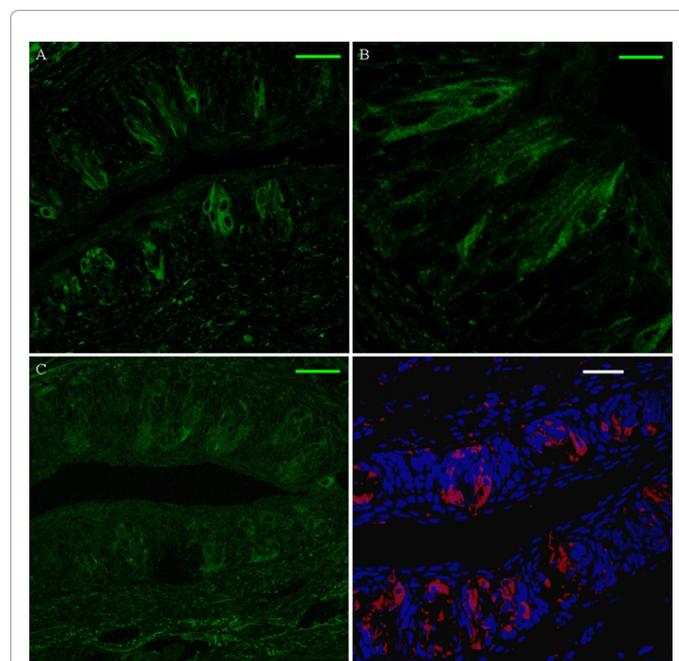


Figure 1: GFP expression in taste bud of CV papillae in Tas2r5-Cre/GFP transgenic mice. GFP expression is observed in CV papillae (A and B). After DTA expression in Tas2r5+ cells, it is hard to observe GFP expression in CV papillae (C). Confocal analysis with anti-Tas1r3 reveals that DTA expression in Tas2r5+ cells does not ablate the expression of Tas1r3 in taste bud. Scale bar A C D 50 µm, B 20 µm.

Tas2r5 and Tas1r3 expression is observed in serous gland and DTA expression in Tas2r5+ or Tas1r3+ cells ablate GFP expression in serous gland

In order to trace the characteristics of Tas2r5+ cells, we cross this mouse with Rosa26-^{LoxP}stop^{LoxP}-LacZ transgenic mouse. In previous study, it has reported that the blue staining is observed in testis [20], kidney [25] and gut [26]. As expected, we also observed the blue staining in CV (Figure 3A) and foliate (Figure 3B) papillae, and mucous gland located at the posterior tongue as well (Figure 3C and Figure S1A-S1C). In addition, it was interesting to note that the blue staining was not detected in serous gland (Figure 3D and S1D).

Furthermore, we employed the immunochemistry methods to analyze GFP expression in serous gland. Immunochemistry analysis with anti-GFP showed that GFP expression was observed in the taste bud of CV papillae (Figure 3E). Meanwhile, positive staining was also detected in serous gland (Figure 3G) and mucous gland (Figure S2A). After checking the section from the double transgenic mouse (ablation of Tas2r5+ cells) with anti-GFP, we fail to observe the positive cells in taste bud of CV papillae and foliate papillae (Figure 3F). More importantly, positive staining was also undetectable in serous gland (Figure 3H).

In order to further investigate Tas1r3 expression in serous gland, immunohistochemistry analysis was implemented in tongue section from the double transgenic mouse (Tas1r3—Cre/GFP—Rosa26—

^{LoxP}stop^{LoxP}—LacZ). GFP expression was detected in papillae including CV papillae (Figure 4A), foliate papillae (Figure 4B), and serous gland (Figure 4C). Immunohistochemistry with anti-β-Gal revealed the β-Gal expression in taste bud of CV papillae (Figure 4D), foliate papillae (Figure 4E) and serous gland (Figure 4F). After DTA expression in Tas1r3+ cells, immunohistochemistry analysis with anti-GFP revealed that GFP expression was undeleted in taste bud of CV papillae (Figure 4G), foliate papillae (Figure 4H), and serous gland (Figure 4I).

In short, the current study suggests that Tas2r5 and Tas1r3 is expressed both in taste bud and sublingual gland.

DTA expression in Tas2r5+ or Tas1r3+ cells result in the loss of Gnat3+ and PLC-β2+ cells in serous gland

It is always believed that Gnat3 and PLC-β2 is two important cascades in bitter taste transduction [4]. Immunochemistry with anti-GNAT3 revealed that Gnat3 was expressed in taste bud of CV papillae (Figure 5A) in the control mouse. We also detected the expression of Gnat3 in serous gland (Figure 5C) and mucous gland (Figure S2B). After analyzing the section from the double transgenic mouse (ablation of Tas2r5+ cells) with immunochemistry, positive staining was still detected in taste bud of CV papillae (Figure 5B). It should be noted that positive staining was undetectable in serous gland (Figure 5D). In addition, immunochemistry analysis with anti-PLC-β2 showed that PLC-β2 was detected in taste bud of CV papillae (Figure 5E). Meanwhile, positive staining was also observed in serous gland (Figure

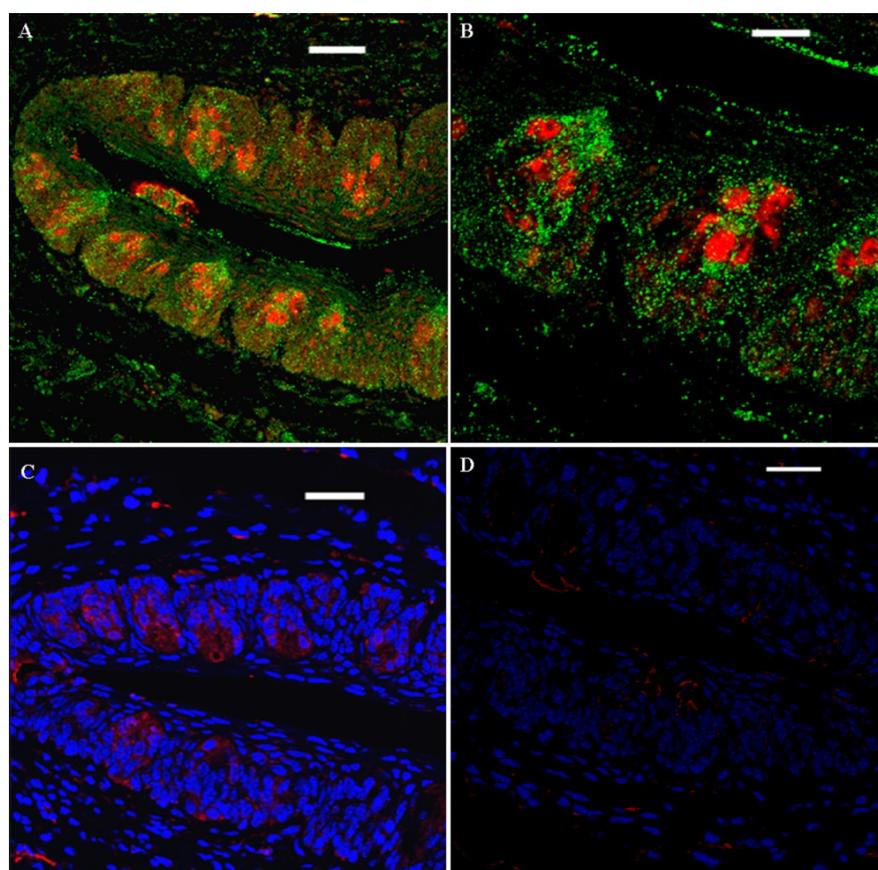


Figure 2: GFP expression in taste bud of CV papillae in Tas1r3-Cre/GFP transgenic mice. (A) Double staining with anti-GFP (Green) and Caspase2L (Red) reveals the expression of GFP in taste bud. (B) High magnification from (A). (C) DTA expression ablates the expression of GFP in taste bud of CV papillae. (D) DTA expression in Tas1r3+ cells ablates Tas1r3 expression in taste bud of CV papillae. Scale bar A C D 50 μm, B 10 μm.

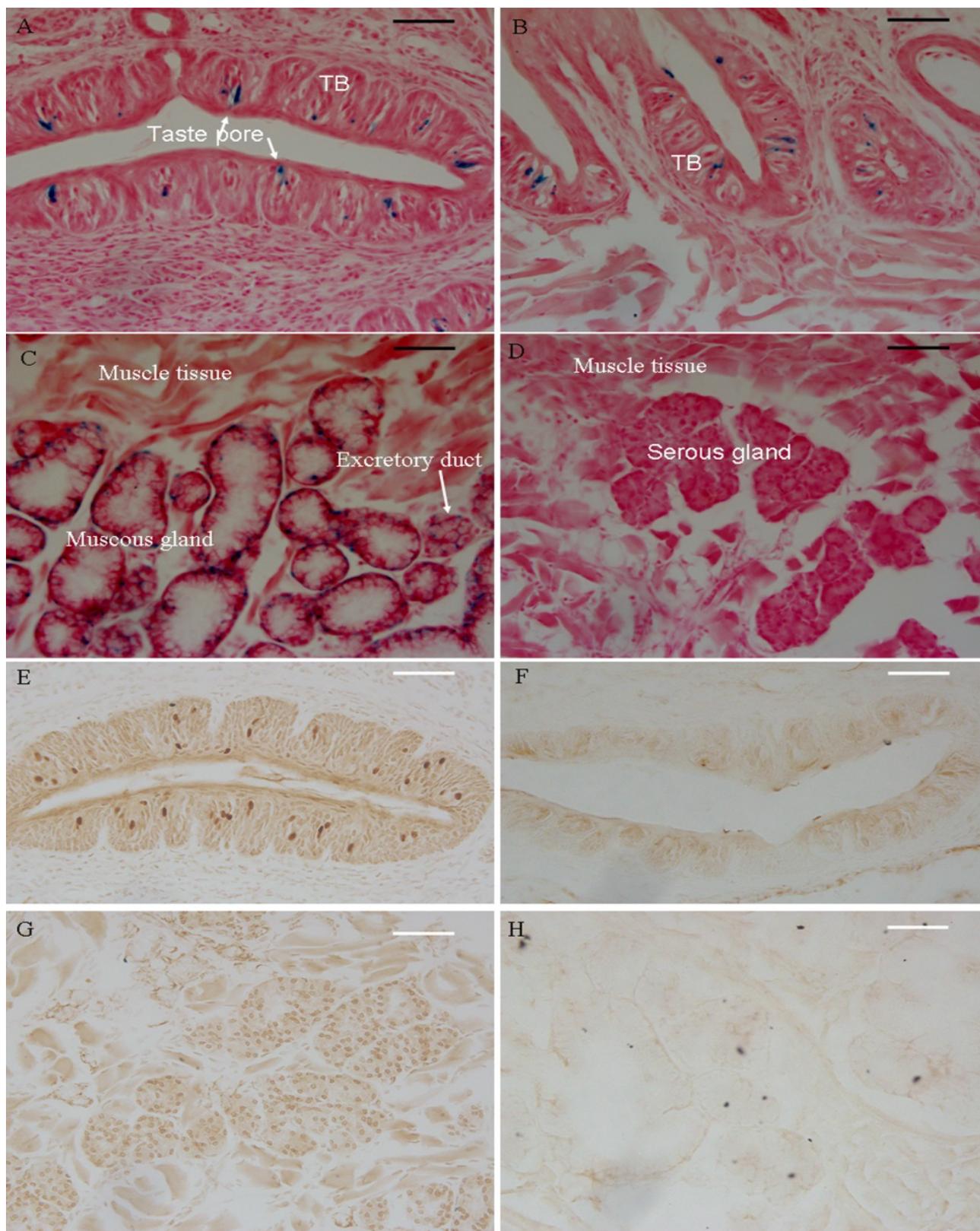


Figure 3: LacZ staining is detected in taste bud and mucous gland, and DTA expression in Tas2r5+ cells ablates GFP expression in taste bud and mucous gland. LacZ staining is detected in taste bud of CV (A) and foliate papillae (B). LacZ staining is detected in mucous gland (C), not in von Ebner's serous glands (D). GFP expression is detected in taste bud (E) and serous gland (G). DTA expression deletes GFP expression in taste bud (F) and serous gland (H). Scale bar 50 μm.

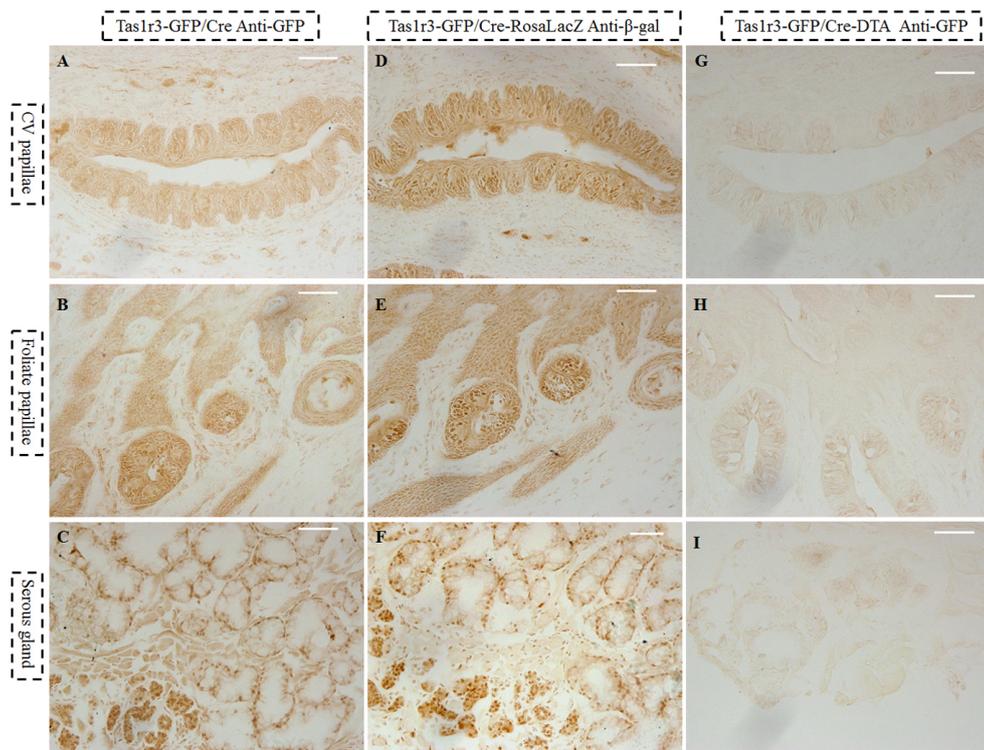


Figure 4: DTA expression in Tas1r3+ cells ablates GFP expression in taste bud and mucous gland. In the Tas1r3-Cre/GFP-Rosa 26-^{LoxP}stop^{LoxP}-LacZ double transgenic mouse, immunohistochemistry with anti-GFP reveal the GFP expression in CV papillae (A), foliate papillae (B) and serous gland (C). Immunohistochemistry with anti-βGal show the LacZ expression in CV papillae (D), foliate papillae (E) and serous gland (F). After DTA expression in the Tas1r3-Cre/GFP-Rosa 26-^{LoxP}stop^{LoxP}-DTA double transgenic mouse, immunohistochemistry with anti-GFP reveal the ablation of GFP expression in CV papillae (G), foliate papillae (H) and serous gland (I). Scale bar 50 μm.

5G) and mucous gland (Figure S2B), which showed similar expression pattern with Gnat3. After DTA expression in Tas2r5+ cells, PLC-β2 expression was still observed in taste bud of CV papillae (Figure 5F). However, PLC-β2 expression was ablated in serous gland (Figure 5H).

In order to investigate whether the ablation of Tas1r3+ cells influence on the expression of Gnat3 and PLC-β2, the sensitive immunohistochemistry was further employed to confirm the distribution of taste signal transduction cascades in tongue. In control mice, Gnat3 expression was observed in taste bud of CV (Figure 6A) and serous gland (Figure 6C). After DTA expression in Tas1r3+ cells, the expression of Gnat3 was still observed in taste bud of CV (Figure 6B). However, the expression of Gnat3 was ablated in serous gland (Figure 6D). In control mice, we observed the expression of PLC-β2 in taste bud of CV papillae (Figure 6E) and serous gland (Figure 6G). After DTA expression in Tas1r3+ cells, the expression of PLC-β2 was ablated in serous gland (Figure 6H). However, the expression of PLC-β2 was still detected in taste bud of CV papillae (Figure 6F).

In a word, the current data collectively suggests that Gnat3 and PLC-β2 expression is observed in serous and mucous gland and DTA expression in Tas2r5+ and Tas1r3+ cells ablate the Gnat3 and PLCβ2 expression in sublingual gland.

Discussion

It is always believed that taste receptors are expressed in taste bud [1,5,9,12,23]. In previous study, we have revealed the expression of taste receptors and signal transduction in testis [20,21]. Here, it is

shown further that taste receptor (Tas2r5 and Tas1r3) and taste signal transduction cascade is expressed in sublingual gland. In fact, taste receptors and taste transduction cascades have been reported in many epithelia including the digestive system [13,17,26], respiratory system [27-29], urinary bladder [30], pancreas [31], heart [32-34], thyroid [35], brain [18,36] and testis [20,37-41]. Although taste receptors, found in human lung and gut, is thought as of carrying out protective functions against inhalation or ingestion of potential harmful compounds, the real sensory status of those taste receptors *in vivo* remains unclear, rather than by a traditional taste transduction mechanism. Several studies have further shown that taste receptors (Tas2rs and Tas1rs) and taste transduction cascades were expressed in the three stages of spermatogenesis and somatic cells (Leydig cell and Sertoli cell) in mouse testis [20,37-40]. Another transgenic line, Tas2r131^{BLiG/BLiG}, hrGFP expression is driven by Tas2r131 gene promoter, which is located at another locus, that does not contain Tas2r5 gene. Interestingly, hrGFP expression is also observed both in taste bud and testis. In addition, hrGFP+ cells are observed in the vomeronasal organ, the respiratory epithelium, epididymis, thymus and ovary [39,40]. In short, the current results collectively shows that G-protein-coupled receptors implicated in taste (Tas1r and Tas2rs), first discovered in the gustatory system, are also expressed outside the gustatory system, and have specific functions beyond the oral cavity.

In previous study, we have shown that DTA expression ablate the expression of Tas2r5 in testis [20,21], kidney [25,42] and intestine [26]. With those mice models, it is revealed that Tas1r3 and Tas2r5 is expressed in serous gland and mucous gland of tongue, and DTA

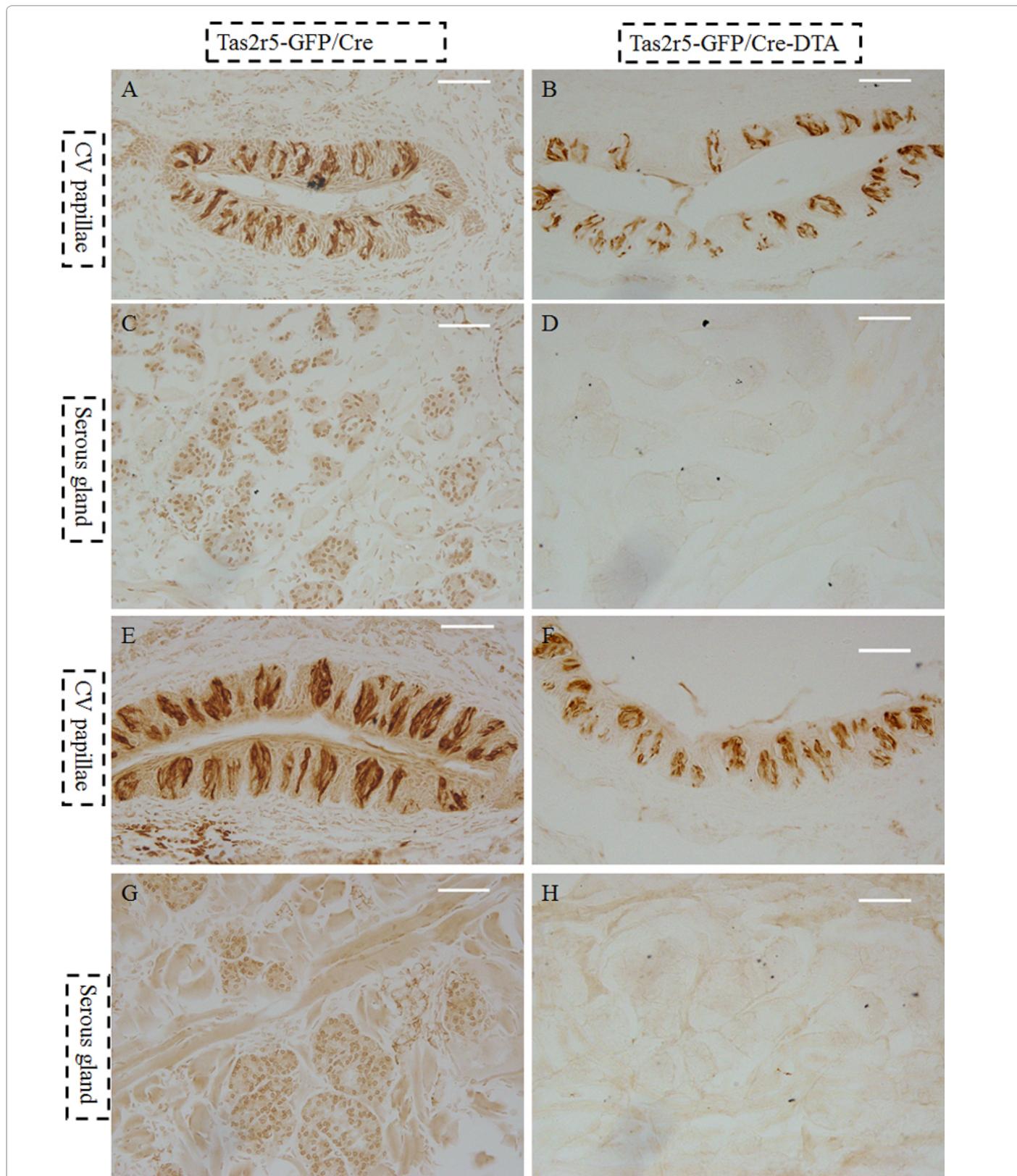


Figure 5: DTA expression in *Tas2r5*⁺ cells ablates the *Gnat3* and *PLC-β2* expression in serous gland. *Gnat3* expression is detected in taste bud (A) and serous gland (C). DTA expression deletes *Gnat3* expression in serous gland (D), not taste bud (B). *PLC-β2* expression is detected in taste bud (E) and serous gland (G). DTA expression does not delete *PLC-β2* expression in taste bud (F). DTA expression deletes *PLC-β2* expression in serous gland (H). Scale bar 50 μm.

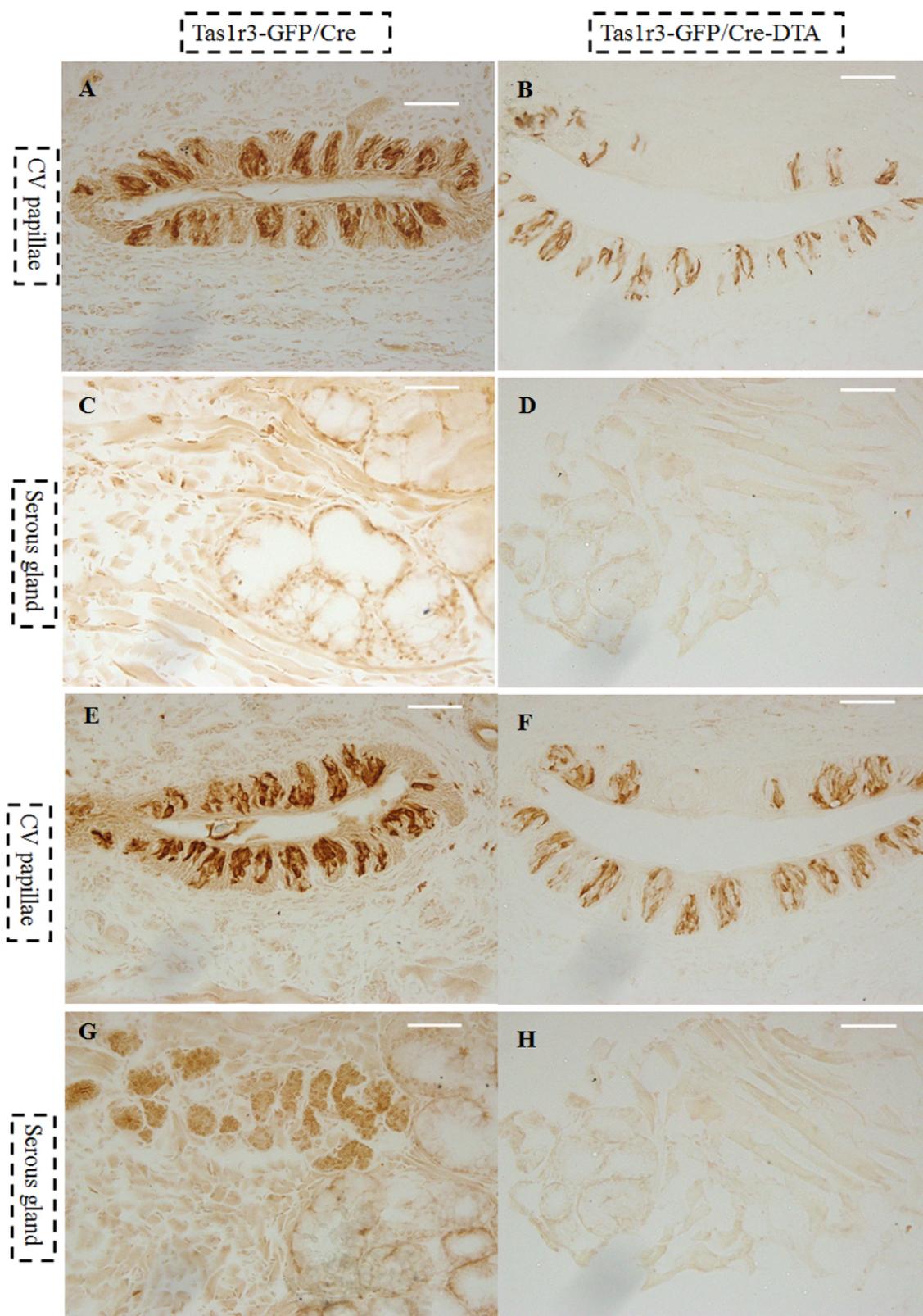


Figure 6: DTA expression in Tas1r3+ cells ablates the Gnat3 and PLC-β2 expression in serous gland. Gnat3 expression is detected in taste bud (A) and serous gland (C). DTA expression fails to delete Gnat3 expression in taste bud (B). DTA expression deletes Gnat3 expression in serous gland (D). PLC-β2 expression is detected in taste bud (E) and serous gland (G). DTA expression does not delete PLC-β2 expression in taste bud (F). DTA expression deletes PLC-β2 expression in serous gland (H). Scale bar 50 μm.

expression ablate the expression of Tas1r3 and Tas2r5 in serous gland and mucous gland of tongue. So far, the function of Tas1r3 and Tas2r5 in serous and mucous gland remains unknown. Recently, it has shown that human thyrocytes, which gets no direct access to the external environment, express Tas2rs and Gnat3. TAS2Rs have a direct role in the regulation of thyroid hormone production, and Tas2r activation modulates TSH-dependent iodide efflux from thyrocytes. Furthermore, TAS2R42 polymorphism is related with lower circulating levels of thyroid hormones in a human cohort [35]. It is well known that ligand mediate activation of Tas2rs. It is still a question where these ligands originate from and how they get to organs such as heart, kidney, testis, thyroid and lingual glands. So far, inhaled irritants and toxins are the best candidates for activating Tas2rs outside the gustatory system. There is an alternative possibility that endogenous Tas2rs agonist is generated within the circulation tissues. In fact, metabolite ligands have been reported to associate with a variety of receptor system including GPCRs and may have potential therapeutic applications in a variety of metabolic diseases and in pain. It is still unclear whether these taste receptors can functions by monitoring the level of metabolite ligands *in vivo*.

Conclusion

In conclusion, we show the expression of Tas2r5 and Tas1r3 in taste bud of CV papillae and sublingual gland with Tas2r5-Cre/GFP and Tas1r3-Cre/GFP transgenic mice. DTA expression in Tas2r5+ and Tas1r3+ cells ablated the expression of GFP in taste bud and sublingual gland. On the other hand, the expression of taste signal transduction cascade, Gnat3 and PLC- β 2, is observed in serous and mucous gland, and DTA expression in Tas2r5+ and Tas1r3+ cells ablate the Gnat3 and PLC β 2 expression in sublingual gland.

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