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ASSESSING EXPRESSION OF TAS2R16 RECEPTOR ON THE TONGUE OF ELDERLY PERSONS

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ABSTRACT

In conducted study, we assessed expression of TAS2R16 receptor gene on the tongue of elderly persons. The TAS2R16 receptor belongs to family of G-protein coupled bitter taste receptors and is expressed in type 2 taste cells, which are a part of taste buds. The taste buds are distributed across the tongue's surface on the specialised structures called papillae. The TAS2R16 receptor mediates bitter taste in response to β-glucopyranosides such as salicin. The purpose of conducted study was to examine, whether the ageing process influence gene expression and hence the perception of taste at the molecular level. Ageing process is often related to either decreased or total lost perception of taste qualities. It is due to physiological changes in the oral cavity. The changes in taste cell membranes involve altered function of ion channels and receptors, which ultimately lead to decreased tasting ability of elderly people. In addition, various causes, such as oral and systemic diseases, drug administration, lifestyle (i.e. smoking) and some oral conditions (wearing dentures, dental caries and coated tongue), may extracerbate this issue. Loss of taste may become a large factor in reduction of appetite, which may lead to malnutrition. To accomplish the objective of this study, we recruited ten elderly persons. One 25-year old human was used as calibrator. We used non-invasive scrapping method for collecting taste cells from fungiform papillae of each subject. A multiplex TaqMan real-time PCR was performed to amplify cDNA of TAS2R16 and PGK1 genes, whereas the last one served as housekeeping gene. The TAS2R16 gene expression for elderly persons relative to that of young one was calculated according to the $2^{-\Delta C_T}$ formula. Results pointed out to increased expression of TAS2R16 gene by 2-fold in 5th and 8th seniors. It is assumed that they perceive more intense bitterness from salicin at the molecular level than 25-year old person. The 2nd, 3rd, 7th and 10th elderly persons have had decreased expression level about 70%, whereas in case of 6th one that was even about 90%. It is supposed that these subjects, in particular last one, respond to salicin very weakly. This data may show evidence of almost total loss of taste. The causes and consequences are discussed in more detail.

Keywords: TAS2R16; elderly person; bitter taste

INTRODUCTION

Humans can distinguish fife basic taste qualities, which are bitter, sweet, sour, umami and salty, as well as newly discovered and potentially accepted taste qualities, for instance, metallic, electrical, fatty and watery **(Liman et al., 2014; Fábián et al., 2015; Chaudhari and Roper, 2010)**. Detection of taste stimuli (compounds) in the oral cavity is provided by thousands of taste buds, which are arranged on the tongue papillae **(Gravina et al., 2013)**. Additionally, the taste buds are on the soft palate, larynx, and pharynx **(Behrens et al., 2007)**.

Each taste bud consists of approximately 50 – 100 taste receptor cells (TRCs) **(Dotson et al., 2012)**. There are four types of TRCs: type 1, 2, 3 and 4 (basal cells) **(Bachmanov et al., 2014)**. Type 4 cells are round, are located at the bottom of the taste buds, and are considered to be progenitor cells of other types of TRCs **(Yamamoto and Ishimaru, 2013)**. Types 1, 2 and 3 (also referred to as dark, light and intermediate, respectively) are mature TRCs possessing microvilli at the apical ends **(Chandrashekar et al., 2006**; **Cvijanovic et al., 2015)**.

These TRCs express proteins that participate in taste transduction. Some of these proteins are inserted into the cell membrane to form taste receptors **(Reed et al., 2006)**. The microvilli of TRCs project trough „taste pore" into the oral cavity, where interact with taste stimuli via taste receptors **(Chandrashekar et al., 2006**; **Cvijanovic et al., 2015)**. TRCs convert chemical stimulus into an output signal that is sent to the brain via the afferent gustatory neurons to evoke taste perception **(Medler, 2015)**.

Type 2 cells express type of G-protein coupled receptors for detecting sweet, bitter, and umami tastes **(Yamamoto and Ishimaru, 2013**; **Niki et al., 2010)**, whereas type 3 cells are supposed to express channel type receptors, involved in mediating of sour taste **(Chaudhari and Roper, 2010**; **Behrens and Meyerhof, 2011)**. However, it is not yet known which TRCs are specifically responsible for the salty taste sensation. According to **Fábián et al., (2015)** it is likely that type 2 cells also do that in a specific way.

Up to now, the criteria for accepting any protein as a receptor, have been fulfilled by that one for sweet and umami (T1R), bitter (T2R) and salty (ENaC) taste. The sour taste receptors are still unknown, although some channel-type candidates have been proposed (ASICs,

PKDs, etc.) **(Bachmanov et al., 2014; Fábián et al., 2015)**.

In contrast to sweet and umami taste, which have evolved to recognize a limited subset of nutrients, in particular sources of energy such as saccharides and proteins, bitter taste has the onerous task of preventing the ingestion of a large number of structurally distinct toxic compounds **(Chandrashekar et al., 2006)**. This is supported by fact that while just three genes exist in the TAS1R gene family (which is responsible for the receptors for both sweet and umami taste) **(Feeney et al., 2011)** over 25 genes from TAS2R family encode functional bitter taste receptors **(Behrens and Meyerhof, 2013)**.

On the other hand, bitter sensations are also mediated by number of phytonutrients found in fruit, vegetable, coffee and green tea (i.e. phenols, flavonoids, isoflavones, terpenes, glucosinolates, isothiocyanates), which are reported to have antioxidant and anticancer properties and a wide spectrum of tumor-supressing activities **(Drewnowski and Gomez-Carneros, 2000; Trembecká et al., 2013)**.

As regard the ligands, the bitter taste receptors exhibit heterogeneous molecular receptive ranges. Some of them are narrowly tuned to 2 or 4 bitter-tasting compounds, whereas others are promiscuously activated by numerous ligands **(Chaudhari and Roper, 2010)**. However, ligands for some receptors (TAS2R41, -42, -45, -48 and -60) remain still unknown **(Niki et al., 2010)**.

In this study, we performed TaqMan real-time PCR in order to examine the expression level of TAS2R16 receptor gene on the tongue of elderly persons, in relation to the young human. The purpose was to find out, whether the ageing process influence gene expression level and hence the perception of taste at the molecular level. The TAS2R16 receptor mediates bitter taste in response to β-glucopyranosides such as salicin **(Bufe et al., 2002)**.

The studies has shown that sensitivities to salty and bitter tastes show more substantial decreases in ageing process than do sensitivities to sour and sweet tastes **(Feng et al., 2013)**. Taste disorders, in particular loss of taste, are often underestimated, but can have an unfavourable fallout on the health of older people, such as loss of appetite, changes in food preferences, anorexia, weight loss and malnutrition, consequently exacerbating their chronic diseases and related morbidity and mortality **(Imoscopi et al., 2012)**.

MATERIAL AND METHODOLOGY

Participants

Ten elderly persons (mix of males and females) were recruited to participate in the study. One 25-year old healthy volunteer (male) was employed as control (i.e. calibrator – C). After a complete explanation of the study to the subjects, written informed consent was obtained from every participant.

Sample collection

We adopted a scraping method to collect tissue samples for this study. It is so minimally invasive technique that it is often used to collect tissue samples from the oral cavity. Using a tongue scraper, we obtained the epithelium specimen from tip, dorsum and foliate papilla in the tongue. None of the participants had anything to eat or drink for at least 90 min before scraping the tongue surface. The mixture of scrapped taste cells from each subject (cca 50 μL) was labelled with identifying code Sample from 25-year old human was labelled with number 0, whereas samples from elderly persons were labelled with numbers from 1 to 10. The samples were inserted into eppendorf tube with pre-pipetted volume (150 μL) of stabilising solution RNA*Later* (Sigma-Aldrich).

Preparation of cDNA

Total RNA was isolated using Nucleospin RNA II (Macherey-Nagel) with on-column DNA digestion according to the manufacturer's instructions. RNA concentration and quality was assessed spectrophotometrically using DS-11 FX+ device (DeNovix). Totally 100 ng of RNA was reverse transcribed using a mixture of random hexamer and oligo-dT oligonucleotide primers with ImProm-II Reverse Transcription System (Promega) following the recommendations of the manufacturer. Identical reactions omitting reverse transcriptase were performed to generate negative control templates.

Quantitative real-time PCR

Gene specific primers and TaqMan probe were used to amplify TAS2R16 gene (forward, 5'-CTGGCCTCCACCATCTTTC-3'; reverse, 5'-TGCAGTGACCAGTGC TATGAT-3'; TaqMan probe, 5'-TCATGGCATCACTGACCAAGCAGA-3') and housekeeping gene –phosphoglycerate kinase 1 (PGK1) (forward, 5'-GGTGCTCAACAACATGGAGATTG-3'; reverse, 5'-GCTTTGGACATTAGGTCTTTGACA-3'; TaqMan probe, 5'-TCTCTGTTTGATGAAGAGGGAGCCA-3'), in a same tube (duplex reaction). Sequences for both genes were designed using Web programme RealTime PCR Design Tool (Biosearch Technologies) and synthesized by Generi Biotech (Czech Republic).

Amplification was performed on TOptical Gradient 96 device (Analytik Jena, Germany). The reaction mixture contained both primers for PGK1 at a concentration of 0.3 pmol.μL^{-1} and both primers for TAS2R16 at a concentration of 0.5 pmol.μL^{-1}. In all cases, the probes for both genes were used at a final concentration of 0.1 pmol.μL^{-1}.

For the real-time PCR reaction, 100 ng of cDNA template was incubated with qPCR ProbesMaster with UNG/lowROX clear mix containing dNTPs with dUTP and hot start DNA polymerase (Jena Bioscience, Germany) in a final volume of 25 μL. Cycling parameters were as follows: 50 °C for 2 min for UNG treatment, followed by 95 °C for 2 min for initial denaturation, followed by 45 cycles of 15 s at 95 °C, 45 s at 60 °C. Each cDNA (+RT) and RNA (-RT) sample was tested in triplicate. As negative controls, reactions were performed using water or products of the cDNA reaction performed in the absence of the reverse transcriptase enzyme. Raw data were acquired and processed with the qPCR Software 3.0 (Analytik Jena, Germany) and further analysed with Microsoft Excel. Gene expression relative to that of PGK1

was calculated according to the $2^{-\Delta C_T}$ formula **(Livak and Schmittgen, 2001)**.

RESULTS AND DISCUSSION

The C_T (obtained in real-time PCR) and relative gene expression values are shown in Table 1. The last ones are also presented graphically on Figure 1. The TAS2R16 expression level of calibrator was set to unity and the relative expression levels of all the other samples were given in relation to the calibrator sample (i.e. x-fold either the increase or decrease in relation to the calibrator).

Increased expression of TAS2R16 gene around by 2-fold has been observed in 5th and 8th elders. It is assumed that they perceive more intense bitterness from salicin at the molecular level than 25-year old human. This may suggest that either density of taste buds was not decreased, or involvement some mechanism, contributing to up-regulated expression of TAS2R16 gene on the tongue.

Since the phytochemicals taste bitter, people with higher expression level of bitter taste receptors might not prefer food with their higher content **(Drewnowski and Gomez-Carneros, 2000)**. Thus, such people must involuntary refuse the food with numerous beneficial effects. For instance, salicin is precursor of acetylsalicylic acid (aspirin) and has pharmacological effects on treatment of fever, pain, and inflammation **(Kim et al., 2015)**.

On the other hand, in some cases (2nd, 3rd, 7th and 10th elders) the gene expression decreased about 70%. Moreover, we have noticed even more than 90% reduction of TAS2R16 gene expression (6th elder). He expressed only 0.08-fold amount of TASR16 gene, compared to calibrator. We suppose that these seniors, in particular last one, are responding on salicin very weakly. This data may show evidence of almost total loss of taste, the causes of which are discussed below.

Table 1 Recorded $C_T$values for TAS2R16 and PGK1 gene, calculated relative expression of TAS2R16 gene.

Sample	C_T TAS2R16	C_T PGK1	ΔC_T	ΔC_T Expression	Mean ΔC_T Expression	*SD** ΔC_T Expression	$\Delta\Delta C_T$ Expression	*SD** $\Delta\Delta C_T$ Expression	% KD**
							Normalised to calibrator		
C	23.22	26.14	-2.92	7.57	7.17	0.55	1.00	0.08	0.00
	23.29	26.18	-2.89	7.41					
	23.39	26.10	-2.71	6.54					
1	30.1	32.00	-1.90	3.73	4.04	0.51	0.56	0.07	-43.71
	30.01	32.22	-2.21	4.63					
	30.2	32.11	-1.91	3.76					
2	29.11	30.70	-1.59	3.01	2.03	1.25	0.28	0.17	-71.67
	29.19	28.51	0.68	0.62					
	29.2	30.50	-1.30	2.46					
3	30.5	32.69	-2.19	4.56	4.43	0.23	0.62	0.03	-38.23
	30.41	32.60	-2.19	4.56					
	30.4	32.46	-2.06	4.17					
4	28.95	28.41	0.54	0.69	0.84	0.25	0.12	0.03	-88.27
	29.1	28.61	0.49	0.71					
	28.74	28.91	-0.17	1.13					
5	27.39	31.12	-3.73	13.27	14.02	0.99	1.95	0.14	95.35
	27.26	31.03	-3.77	13.64					
	27.27	31.19	-3.92	15.14					
6	31.88	31.65	0.23	0.85	0.54	0.39	0.08	0.05	-92.48
	32.83	32.25	0.58	0.67					
	32.21	28.85	3.36	0.10					
7	34.02	34.39	-0.37	1.29	1.58	0.41	0.22	0.06	-77.96
	33.05	34.09	-1.04	2.06					
	33.55	34.03	-0.48	1.39					
8	24.36	28.46	-4.10	17.15	16.71	1.20	2.33	0.17	132.89
	24.55	28.49	-3.94	15.35					
	24.43	28.57	-4.14	17.63					
9	22.44	24.83	-2.39	5.24	4.69	0.49	0.65	0.07	-34.68
	22.64	24.81	-2.17	4.50					
	22.28	24.39	-2.11	4.32					
10	28.41	26.97	1.44	0.37	1.24	0.79	0.17	0.11	-82.65
	28.24	28.80	-0.56	1.47					
	27.08	28.00	-0.92	1.89					

* Standard deviation.

** Percent knockdown of TAS2R16 expression.

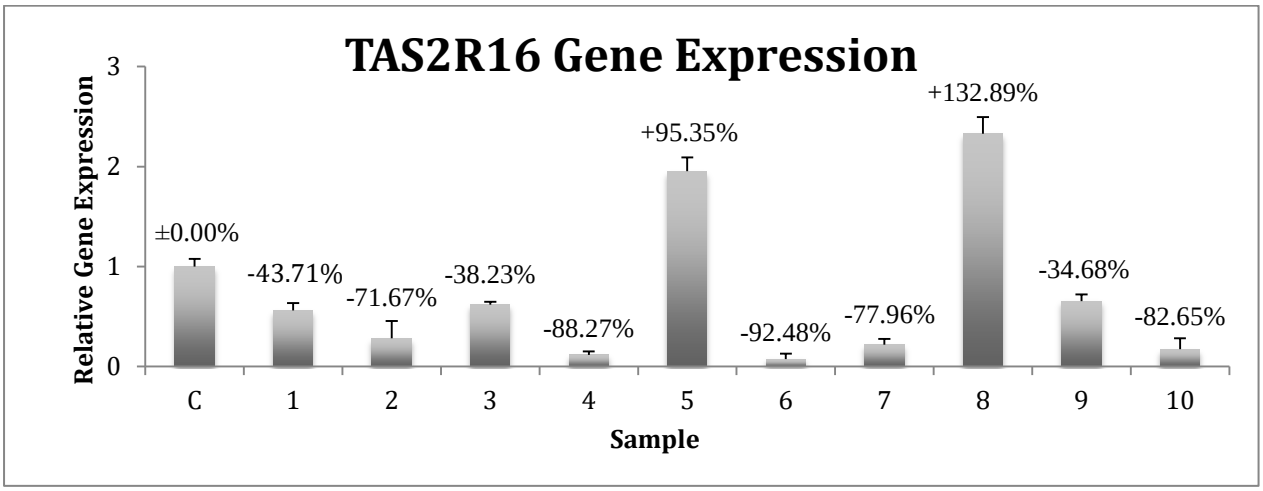

Figure 1 Expression level of TAS2R16 receptor on the tongue of elderly persons in relation to young human.

The issue of taste lost has been associated with normal ageing, drug administration, oral (dental caries, stomatitis) and systemic diseases. The last ones include neurological (Alzheimer, epilepsy), cardiovascular (hypertension), endocrinal (diabetes mellitus types 1 and 2, hypothyroidism), gastrointestinal, kidney, liver, respiratory and viral diseases, as well as some kind of cancer (lung, breast, stomach) **(Boyce and Shone, 2006; Feng et al., 2013; Imoscopi et al., 2012; Ikeda et al., 2008)**.

As regards ageing process, numerous physiological changes in the oral cavity are observed that contribute to taste loss. These include: a) thinning and drying of oral mucosa due to declining keratinisation, b) thinning of the epithelial structure, c) atrophying of salivary glands or disappearing of acini (replaced by adipose and fibrous connective tissue) and e) diminishing of taste buds' density **(Imoscopi et al., 2012)**. Therefore, the changes in taste cell membranes involve altered function of ion channels and receptors, which ultimately lead to decreased tasting ability of elderly people **(Boyce and Shone, 2006)**.

Further, some oral conditions, such as wearing dentures, dry mouth and coated tongue, may cause taste impairment. Many elderly persons have poor oral health, characterized by heavy plaque accumulation, mucosal inflammation, and high dental caries activity **(Solemdal et al., 2012; Imoscopi et al., 2012)**.

In addition to ageing process and diseases, the proper lifestyle is also important to maintain taste sensitivity. For instance, **Aoki et al., (2014)** compared expression of TAS2R genes (including TAS2R16) between group of elderly smokers and non-smokers. They demonstrated significantly lower expression levels of TAS2Rs in individuals who smoked cigarettes. Furthermore, asignificant positive correlation (p = 0.496) between age and expression of TAS2R was observed in non-smokers. This study revealed that smoking in seniority lead to decreased sensitivity to bitter-tasting food.

Besides the smoking, alcohol also interferes into taste perception, because impaires intestinal absorption of zinc and vitamin A, both of which are essential to gustatory function **(Imoscopi et al., 2012)**.

A loss of taste reduces the joy of eating nutritious, flavoursome foods. Taste disorders may become a large factor in reduction of appetite, which may lead to malnutrition **(Ikeda et al., 2008)**. Nutritional deficiencies of vitamin B12 also contribute to the taste dysfunction **(Boyce and Shone, 2006; Feng et al., 2013)**. Consequently, protein malnutrition and deficits of zinc, selenium and vitamin B6 aggravate dysregulation of the immune system among older individuals. These seniors are more frequently predisposed to infectious diseases, with serious health implications, compared to healthy ones **(Brownie, 2006)**.

CONCLUSION

To conclude, we demonstrated TAS2R16 gene expression on the tongue of elderly persons. In majority of tested subjects we found out decrease in expression level of this gene. This may point out to inability to detect salicin, i.e. to partial loss of taste function. This disorder might be caused by several factors such as normal ageing process and diseases. Taste loss is serious issue, which may have psychological implications, resulting in malnutrition and impaired health status.

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