Expression and Functional Activity of the Bitter Taste Receptors TAS2R1 and TAS2R38 in Human Keratinocytes

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Abstract
Recent studies have shown that human bitter taste receptors (TAS2Rs) are not only expressed in mucous epithelial cells of the tongue, but also in epithelial cells of the colon, stomach and upper respiratory tract. These cell types come in close contact with external bitter compounds by ingestion or breathing. In the present work we addressed the question whether bitter taste receptors might also be expressed in cornified epithelial cells of the skin. Here, we show for the first time the expression of TAS2R1 and TAS2R38 in human skin. Double staining of HaCaT cells and primary keratinocytes demonstrated the colocalization of TAS2R1 and TAS2R38 with the adaptor protein α-gustducin that is essential for signal transduction upon ligand binding. To test if TAS2Rs in keratinocytes are functional, we stimulated HaCaT cells with diphenidol, a clinically used bitter-tasting antiemetic, or amarogentin, the bitterest plant substance, that binds TAS2Rs, including TAS2R1 and TAS2R38. Diphenidol and amarogentin induced calcium influx. Furthermore, in keratinocytes diphenidol and amarogentin stimulated the expression of the differentiation markers keratin 10, involucrin and transglutaminase. Therefore, apart from the known role in mucous membranes of the gastrointestinal tract, TAS2Rs are expressed in the epidermis and might play a role in keratinocyte differentiation.

Introduction
The 25 human bitter taste receptors are G-protein-coupled receptors (GPCRs) that are expressed in taste buds of the tongue. Although bitter tasting is seen as a code for danger, many bitter compounds also provide important nutrients [1]. The signaling cascade of bitter taste receptors leads via the G protein α-gustducin to the activation of phospholipase C-β2 and the formation of inositoltrisphosphate as well as diacylglycerol. These second messengers lead to the release of internal calcium and subsequent activation of transient receptor potential cation channel 5. Activation of taste receptor cells by a stimulus is then transported via afferent neurons and processed in taste centers in the brain [2]. It was long believed...
that bitter taste receptors (TAS2Rs) are solely expressed in taste buds of the tongue. However, some years ago it could be shown that TAS2Rs are also expressed outside the gustatory epithelium of the tongue and oropharynx. They influence glucose homeostasis in the human gastrointestinal tract [3] and are expressed in bronchial smooth muscle cells where they stimulate the relaxation of airways for improved breathing [4, 5]. Furthermore, the TAS2R38 is expressed in upper respiratory epithelial cells [6] where it is involved in the protection against bacterial infection.

As TAS2Rs are expressed in mucous epithelial cells of the tongue, colon and stomach, and, as recently shown, in upper respiratory epithelial cells, we wondered if these receptors might also be expressed in the cornified squamous epithelium of the skin. Two conserved TAS2Rs, TAS2R1 and TAS2R8, were selected as targets for this analysis [7]. Double staining of keratinocytes revealed co-localization of α-gustducin with TAS2R1 and TAS2R8, and stimulation of these cells with diphenidol or amarogentin demonstrated the functionality of the TAS2Rs by inducing calcium influx. Diphenidol is a synthetic TAS2R agonist which activates 16 TAS2Rs including TAS2R1 and TAS2R38 [8]. Amarogentin, the bitterest substance in nature that is present in higher amounts in Gentiana lutea, can also activate several TAS2Rs (i.e. TAS2R1, -4, -39, -43, -46, -47 and -50). We selected these substances because they activate several TAS2Rs. We could show that diphenidol and amarogentin, respectively, increased the gene expression of keratin 10, involucrin and transglutaminase 1, three genes that are upregulated during the differentiation process of keratinocytes.

**Materials and Methods**

**Antibodies and Reagents**

The following antibodies and dilutions were used for immunohistochemical stainings: the polyclonal rabbit anti-human TAS2R1 antibody (Osensis, Thermo Fisher Scientific GmbH, Schwerte, Germany), 1:1,000; the polyclonal rabbit anti-human TAS2R38 antibody (Abcam, Cambridge, UK), 1:1,000, the polyclonal goat anti-human α-gustducin antibody (Santa Cruz Biotechnology, Heidelberg, Germany), 1:200, and the rabbit immunoglobulin fraction (Dako) as negative control. The secondary antibody multilink biotin, the streptavidin-horseradish peroxidase label and the AEC (Dako) as negative control. The secondary antibody multilink biotin, the streptavidin-horseradish peroxidase label and the AEC (Dako) as negative control.

For immunohistochemistry, skin sections were deparaffinized and subsequently subjected to a 6-min proteinase K (Dako) treatment. Immunostaining was performed with the anti-TAS2R1 and -38 antibodies. Application of the primary antibody (4°C, overnight) was followed by incubation with biotinylated swine anti-goat, anti-mouse and anti-rabbit antibody immunoglobulinGs (1 h, room temperature), streptavidin conjugated to horseradish peroxidase (20 min, room temperature), AEC solution as chromogen and hematoxylin counterstaining. Stainings with the rabbit immunoglobulin fraction served as isotype control. Images were taken with a microscope (Carl Zeiss AG, Oberkochen, Germany) equipped with Axiosvision software.

**Immunofluorescence**

To perform TAS2R1 or TAS2R38 and α-gustducin double labeling, HaCaT cells and human primary keratinocytes (HPKs) were stained with the polyclonal rabbit anti-human TAS2R1 or TAS2R38 antibody and the goat anti-human α-gustducin antibody. The required secondary antibodies (Alexa 488 donkey anti-goat and Alexa 555 donkey anti-rabbit antibodies) were applied for 2 h at room temperature according to the manufacturer’s instructions. The cells were then stained with diamidino phenylindole and mounted in fluorescence mounting medium (Dako). Images were taken with a fluorescence microscope (Zeiss) equipped with the Axiosvision software. For TASR1 and c-Myc double labeling, the cells were stained with the polyclonal rabbit anti-human TAS2R1 and the c-Myc hybridoma supernatant. The required secondary antibodies were Alexa 488 goat anti-mouse and Alexa 555 donkey anti-rabbit antibodies.

**Cell Culture**

HPKs were prepared from adult skin obtained from dermatological surgery and cultured according to the method of Rheinwald and Green [9] in serum-free medium. The human keratinocyte cell line HaCaT was from CLS Cell Lines Service (Heidelberg, Germany) and cultured in Dulbecco’s modified essential medium (Invitrogen) containing 10% fetal calf serum (PAA, Pasching, Austria) and 1.8 mM calcium. All cells were cultured at 37°C in a humidified atmosphere with 5% CO2. For double fluorescence stainings, the cells were seeded in 4-field chamber slides (Thermo Fisher Scientific GmbH; 1 × 10^5 cells/ml). For calcium imaging experiments, cells were seeded on coverslips coated with polylysine (Sigma-Aldrich, Munich, Germany) at a density of 1.5–2 × 10^5. To measure the induction of differentiation markers, HaCaT cells were cultured in Dulbecco’s modified essential medium without calcium for at least 1 week, and HPKs were cultured in serum-free medium with less than 0.1 mM calcium. Then the cells were either incubated with 2 mM calcium or the bitter compounds at a concentration of 100 μM for 72 h before the RNA was isolated. The PAM212 cells (mouse epithelial cell line) were kindly provided by Fabian Gendrisch (University Medical Center, Freiburg, Germany) and the NIH3T3 cells (mouse embryo fibroblast cell line) by Dr. Volker Assmann (University Medical Center, Hamburg-Eppendorf, Germany). The PAM212 cells were cultured in RP10 medium (Invitrogen) containing 10% fetal
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Calf serum (PAA). The NIH3T3 cells were cultured in Dulbecco’s modified essential medium (Invitrogen) containing 10% fetal calf serum (PAA).

Cell Transfection

Human TAS2R1 cDNA from the pCMV6-TAS2R1-GFP vector (OriGene, Rockville, Md., USA) was subcloned in the pCMV6 entry vector with a C-terminal Myc/DDK tag (OriGene) using the Sgfl and Mlu1 restriction sites. As the GFP expression was not detectable after transfection of the pCMV6-TAS2R1-GFP vector, a subcloning was performed to introduce a c-Myc tag that could be used for detection. For transient transfection 5 × 10⁴ PAM212 or NIH3T3 cells were seeded in each well of a 24-well plate in growth medium as described above. After 24 h at 37 °C, cells were transfected with 1 μg pCMV6-TAS2R1 vector and 2 μl TurboFectin 8.0 (Thermo Fisher Scientific GmbH) according to the manufacturer’s instruction. Twenty-four hours after transfection TAS2R1 and c-Myc double labeling was performed.

Western Blot

Cell lysates were prepared from HaCaT cells and HPKs with NP-40 buffer (150 mM NaCl, 1% NP-40, 50 mM Tris-Cl, pH 8.0), and Western blot analysis was performed as described [10]. The anti-TAS2R1 and anti-TAS2R38 antibodies were used at a dilution of 1:750. For antibody preabsorption experiments, the anti-TAS2R38 antiserum was preincubated with a 5-fold excess (v/v) of the immunogenic peptide for 1 h at 37 °C. Then the antibody was applied as primary antibody to the blotting membrane, and the normal Western blot procedure was continued.

RNA Extraction and PCR

Total RNA was extracted with the RNeasy Mini kit (Qiagen, Hilden, Germany) from subconfluent HaCaT cells or HPKs. First-strand cDNA was synthesized from 2 μg total RNA in 20 μl final volume using the Omniscript kit (Qiagen) with random hexamer primers (Invitrogen). Two-microliter aliquots of the reverse transcription solution were used as a template for specific PCR reactions with an annealing temperature of 58 °C, and the PCR product was analyzed by gel electrophoresis. The PCR primers (20 pmol each) used to amplify TAS2R1, TAS2R38, keratin 10, involucrin or transglutaminase and the housekeeping gene β-actin were:

- TAS2R1 forward primer, 5′-gtatcctcttctcctgtctg-3′, reverse primer, 5′-ttaaaattaagatgagagtg-3′; TAS2R38 forward primer, 5′-cgatcctcgaactgtctca-3′, reverse primer, 5′-ggaatctgccttgtgg-3′; keratin 10 forward primer, 5′-ccccggacccagggctgtag-3′, reverse primer, 5′-gggtcttcctggctggctttc-3′.

The PCR primers used to amplify keratin 10, involucrin and transglutaminase were already published [11].

Relative expression levels of keratin 10, involucrin or transglutaminase 1 compared to their normalized expression level in untransfected cells were shown in a histogram prepared with the program Graph Pad Prism. The asterisks denote statistical significance compared to the solvent, with ** p < 0.01, * p < 0.05, b.s. = borderline significant (p ≥ 0.05 and p < 0.07), n.s. = not significant.

Calcium Influx Measurements

Intracellular Ca²⁺ concentration measurements in single cells were carried out using the fluorescence indicator fura-2-am (Invitrogen). Cells were washed with a buffer containing 130 mM NaCl, 5 mM KCl, 10 mM HEPES, 10 mM glucose and 1 mM CaCl₂ adjusted to pH 7.4 and loaded with 2 μM fura-2-am and 0.04% Pluronic F-127 (Invitrogen) for 30 min. After rinsing twice, cells were allowed to de-esterify fura-2-am for 30 min. Measurements were performed with a monochromator-based imaging system attached to an inverted microscope (Axiovert S100, Zeiss) at 37°C. Fluorescence was excited at 340 and 380 nm and emission measured at 510 nm. After 60 s, cells were stimulated with various concentrations of diphenidol, amarogentin or solvent. After correction of background fluorescence, the fluorescence ratio was calculated using Axiovision software.

Results

Expression of TAS2R1 and TAS2R38 in Human Skin

Immunohistochemical stainings were performed to determine the expression of the two conserved bitter taste receptors TAS2R1 and TAS2R38 in human skin. TAS2R1 is the only taste receptor that is located on chromosome 5, and TAS2R38 is the best-characterized bitter taste receptor that is located on chromosome 7 and closely related to the other TAS2Rs.

However, to receive reliable staining results it is essential to have high quality TAS2R-specific antibodies. The human anti-TAS2R38 antibody from Abcam was intensively tested by Behrens et al. [7], and cross-reactivity of the TAS2R38 antiserum with other TAS2Rs could be excluded. To test the specificity of the TAS2R1 antibody used in this study, we constructed a pCMV6-TAS2R1 expression vector coding for TAS2R1 with a C-terminal c-Myc tag. PAM212 cells (mouse epithelial cell line) were transiently transfected with the pCMV6-TAS2R1 vector. As the similarity between human TAS2R1 and the corresponding mouse receptor lies under 46%, it is not expected that the human anti-TAS2R1 antibody recognizes the corresponding mouse receptor [12]. To visualize the receptor proteins, either an antibody against the C-terminal c-Myc tag or the specific TAS2R1 antibody from Osensis were used. Only the PAM212 cells, that express the c-Myc peptide, were also positive in the TAS2R1 staining, whereas these cells showed no TAS2R38 staining. Figure 1a shows one representative staining result in transfected PAM212 cells. As the PAM212 cells showed a rather low transfection efficiency, we repeated the transfection with NIH3T3 cells (mouse embryonic fibroblast cell line) that can more easily be transfected. Again, the c-Myc-positive cells were also TAS2R1-positive but not TAS2R38-positive (fig. 1b). This proves the specificity of the TAS2R1 antibody from Osensis.

Next normal human skin was stained with these anti-TAS2R1 and -38 antibodies. The staining pattern of the epidermis, the outermost layer of the skin mostly com-
posed of keratinocytes, showed TAS2R1 and TAS2R38 expression throughout the epidermis (fig. 2). The TAS2R expression in the basal cells was only weak, and the staining intensity increased from the stratum spinosum to the stratum corneum. Although a membrane staining was expected, the TASR1 and TASR38 expression seemed to be cytoplasmic. This observation was also described by Behrens et al. [7] showing that the localization of the TAS2R38 in gustatory papillae is not restricted to the cell surface but rather evenly distributed over the entire cell

Fig. 1. Comparison of the immunocytochemical costaining patterns of anti-TAS2R1 and c-Myc-specific antibodies. PAM212 (a) and NIH3T3 (b) cells were transiently transfected with an expression vector coding for the human TAS2R1. To visualize the receptor proteins, either an antibody against the C-terminal c-Myc tag (shown in the fluorescein isothiocyanate, FITC channel) or the specific TAS2R1 antibody (shown in the phycoerythrin, PE channel) were used. Overlay pictures of the FITC, PE and diamidine phenylindole (DAPI) channel are shown to visualize the cells that express both the c-Myc tag and the TAS2R1 protein. Scale bar = 50 μm.

Fig. 2. Immunocytochemical stainings against TAS2Rs. Human skin was stained with an isotype control antibody (a) or antibodies against TAS2R1 (b) and TAS2R38 (c). Scale bar = 100 μm.
body. This finding can be explained by the fact that if most of the cell membrane is covered with the antibody, the staining might appear to be intracellular although it sticks more or less to the cell surface. Furthermore, receptors like the TAS2Rs are transported from the endoplasmic reticulum to the Golgi apparatus and processed and packaged in secretory vesicles, before they are inserted in the plasma membrane by a fusion step of these secretory vesicles with the membrane. Therefore TAS2Rs can also be detectable in the cytoplasm. In addition, several modifications of TAS2Rs such as glycosylation, oligomerization and association with G proteins could selectively affect the accessibility and detection efficiency of extra- and intracellular epitopes with the specific anti-TAS2R1 and anti-TAS2R38 antibodies [7]. No staining was seen in the human epidermis with an isotype control antibody (fig. 2a).

**Fig. 3.** Gene and protein expression of TAS2R1 and TAS2R38 in tongue tissue, HaCaT cells and HPKs. **a** RT-PCR with RNA from HaCaT cells, HPKs and commercially available tongue RNA was performed for TAS2R1, TAS2R38 and the housekeeping gene β-actin. The H2O control contains H2O instead of the template. The arrows point to the PCR product. **b** Western blot was performed with protein lysates of HaCaT, HPKs and commercially available tongue lysate visualized by staining with anti-TAS2R1, anti-TAS2R38 and the anti-HSC-70 (housekeeping gene) antibody. The bands correspond to the predicted molecular weight of receptor monomers and oligomers. Preabsorption of the TAS2R38 antibody with the corresponding antigenic peptides (TAS2R38 peptide) for 1 h at 37°C nearly totally abolished the signal with the anti-TAS2R38 antibody. The size of the marker proteins is indicated in kilodaltons.

RNA and Protein Expression of TAS2R1 and TAS2R38 in HaCaT Keratinocytes and Primary Keratinocytes

The expression of TAS2R1 and TAS2R38 was verified in the keratinocyte cell line HaCaT and human primary keratinocytes (HPKs) (fig. 3a). Commercially available tongue RNA was used as positive control, and the expression of the housekeeping gene β-actin demonstrated equal RNA con-
Both HaCaT cells and HPKs as well as the tongue tissue expressed TAS2R1 and TAS2R38. The TAS2R1 and TAS2R38 gene expression was confirmed on the protein level by Western blot with protein lysates from HaCaT cells, HPKs and commercially available human tongue lysate as positive control (fig. 3b). The detection of HSC-70 was used as loading control (fig. 3b, last picture). The bands correspond to the predicted molecular weight of receptor monomers (38 kDa for TAS2R38 and 34 kDa for TAS2R1) and oligomers. It has been observed that TAS2Rs form homo- and heteromers in vitro. Although a physiological significance of the receptor hetero-oligomerization is not obvious, receptor oligomerization might in general be necessary for receptor function [13]. The TAS2R1 monomer was only visible in the lysates from HaCaT cells but not in the tongue tissue lysate and the lysate of HPKs. The TAS2R1 pattern is possibly different in tissue lysates and primary cells as compared to cell line lysates (fig. 3b, first picture). In the Western blot with the anti-TAS2R38 antibody the lysate of HPKs showed bands that corresponded to the predicted molecular weight of receptor monomers and oligomers (fig. 3b, second picture), whereas in HaCaT cells only
the monomer could be detected. However, with the tongue lysate, no band could be shown. This might be due to the fact that in the commercially available tongue lysate no taste buds expressing the TAS2R38 were present. With a previous tongue lysate we received a weak band for the dimeric receptor in the Western blot (data not shown). Therefore, commercially available tongue lysate is not always a reliable positive control for TAS2Rs. The specificity of the TAS2R38 antibody was demonstrated by parallel experiments in which the primary anti-TAS2R38 antiserum has been preabsorbed with the corresponding antigenic peptides. As expected, this antiserum preparation with reduced levels of reactive specific anti-TAS2R38 antibodies did not detect TAS2R38 in the cell lysates (fig. 3b, third picture).

**Coexpression of TAS2R1 or TAS2R38 with α-Gustducin in HaCaT Cells and Primary Keratinocytes**

To perform double stainings of TAS2R1 or TAS2R38 together with the taste-specific G protein α-gustducin, the keratinocyte cell line HaCaT (fig. 4a) and HPKs (fig. 4b) were stained with the human anti-TAS2R1 or anti-TAS2R38 antibody and the human anti-α-gustducin antibody. Then the appropriate secondary antibodies (Alexa 488 donkey anti-goat and Alexa 555 donkey anti-rabbit antibodies) were applied, and the cells were stained with diamidine phenylindole. It could be demonstrated that the TAS2R1- and TAS2R38-positive keratinocytes also expressed α-gustducin. No fluorescence signal was detectable with an isotype control antibody (fig. 4a and b, picture in the last column).

**The Bitter Compounds Diphenidol and Amarogentin Induce Calcium Influx in HaCaT Cells**

Having demonstrated that the human keratinocyte cell line HaCaT expresses α-gustducin and the bitter taste receptors TAS2R1 and TAS2R38, we next analyzed whether these bitter taste receptors were also functional. Therefore, intracellular calcium influx was measured after stimulation with diphenidol. Diphenidol is a synthetic TAS2R agonist which activates 16 TAS2Rs including TAS2R1 and TAS2R38 [8]. Diphenidol medication has long been clinically developed as an antiemetic and an antivertigo agent [14]. Incubation of HaCaT cells for 24 h with diphenidol dose-dependently led to an elevation of calcium-dependent fluorescence in fura-2-loaded HaCaT cells compared to the untreated control cells. Already with 30 μM could a significant calcium influx be detected (fig. 5a). However, diphenidol is a muscarinic receptor antagonist and therefore not specific for TAS2Rs. Diphenidol is also described as an inhibitor of voltage-gated K+ and Ca2+ channels without affecting store-operated Ca2+ channels, whereas the precise cellular and molecular action of diphenidol is still not fully clear [14]. Therefore, we performed additional activation experiments in HaCaT cells with amarogentin, the bitterest substance in nature that is present in higher amounts in G. lutea that can activate several TAS2Rs (TAS2R1, -4, -39, -43, -46, -47 and -50) to further prove the functionality of the TAS2Rs. A concentration of 100 μM amarogentin led to a significant calcium influx in HaCaT cells (fig. 5b). The required concentrations of diphenidol and amarogentin to induce calcium influx were not cytotoxic as demonstrated.
with the vialight assay in which the physiological activity of the cells after bitter compound treatment was measured (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000367631). Even 800 μM of the bitter compounds were not cytotoxic.

**Diphenidol and Amarogentin Induce Differentiation Markers in HaCaT Cells and Primary Keratinocytes**

High extracellular calcium concentrations (2 mM) trigger the differentiation process in keratinocytes. Therefore we tested whether diphenidol- or amarogentin-induced calcium influx could also increase the expression of differentiation markers in HaCaT keratinocytes and HPKs. The induction of differentiation markers caused by diphenidol or amarogentin was investigated by incubating subconfluent HaCaT cells or HPKs calcium-free with 100 μM of the respective bitter compounds for 72 h. Unstimulated HaCaT cells and HPKs expressed basal levels of keratin 10, involucrin and transglutaminase 1. This expression was upregulated during high extracellular calcium concentration (2 mM) or diphenidol or amarogentin treatment (fig. 6 and online suppl. fig. 2). Keratin 10 represents an early differentiation marker whereas involucrin and transglutaminase 1 are late markers of keratinocyte differentiation. The PCR results were densitometrically measured with the ImageJ program and shown in figure 6e and online suppl. fig. 2b.

**Discussion**

Previously, Clark et al. [15] have speculated that TAS2Rs might be expressed in other extraoral tissues apart from the respiratory and gastrointestinal endocrine cells. In the present study, TAS2R1 and TAS2R38 protein expression was determined for the first time in human keratinocytes, both in situ in the skin and in cultured cells.

Epidermal keratinocytes are highly specialized epithelial cells that separate the organism from its environment. One major factor that promotes keratinocyte differentiation is calcium, with the lowest concentration in the stratum basale and increasing concentrations up to the outer stratum granulosum, where it reaches its maximum concentration [16]. Activation of the keratinocyte cell line HaCaT with the synthetic bitter compound diphenidol and the bitterest substance in nature amarogentin (e.g. from *G. lutea*) leads to calcium influx and might influence the differentiation process. The required bitter compound concentrations were comparable to data from the literature [8]. The epidermis is a keratinized, multistratified epithelium that functions as a barrier to protect the organism from dehydration, mechanical trauma and microbial insults [17]. It seems that predominantly tissues that emerge from the ectoderm, the outer blastodermic

![Fig. 6. Diphenidol and amarogentin induce the expression of differentiation markers in HPKs. HPKs were incubated for 72 h in calcium-free medium (untreated control), 2 mM calcium (positive control), 100 μM diphenidol or 100 μM amarogentin. Total RNA of HaCaT cells was isolated, reverse transcribed and subjected to RT-PCR. a–d One of three independent experiments with similar results is shown.](image-url)
layer to protect the body, express TAS2Rs (e.g. the skin and mucous epithelial cells of the colon and stomach) in response to environmental factors, whereas mesodermal structures such as the lymphatic system are negative (data not shown).

In this context, it is interesting that many pharmaceuticals, including antibiotic (e.g. chloramphenicol), antimalarial (e.g. chloroquine and quinine) as well as immunosuppressive (e.g. azathioprine) or anti-inflammatory agents (hydrocortisone, flufenamic acid), taste bitter and therefore could mediate different new actions within the body in every bitter taste receptor-bearing cell [15].

Clark et al. [15] stated that drugs absorbed into the bloodstream could reach TAS2Rs in almost any tissue of the body; therefore it is necessary to know which cells express TAS2Rs and which application route should be preferred to avoid unwanted reactions. Here we provide evidence that also topically applied bitter compounds might influence cells, especially keratinocytes of the skin.

The signaling pathway of bitter compounds is also dependent on the particular cell type. Deshpande et al. [4] could show that activated TAS2Rs expressed on bronchial smooth muscle cells hyperpolarized these smooth muscle cells, eventually leading to relaxation. TAS2Rs were located in close proximity to calcium-activated big potassium channels that open after TAS2R activation. An increase in intracellular Ca\(^{2+}\) would otherwise lead to muscle contraction. In gustatory cells, intracellular Ca\(^{2+}\) triggers transduction channel transient receptor potential channel 5 to depolarize the cell and evoke transmitter release [18].

In summary we could show for the first time that TAS2Rs are expressed in keratinocytes, that ligand binding induces calcium influx and expression of differentiation markers. Future work will address the question if topical application of TAS2R ligands may improve the skin barrier in skin disorders with an impaired epidermal skin barrier.

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