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# Olfactory signaling components and olfactory receptors are expressed in tubule cells of the human kidney

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ABSTRACT

Cells of the renal tubule system are in direct contact with compounds dissolved in the urine, such as short chain fatty acids (SCFA). Murine OR78, a member of the olfactory receptor (OR) family, is involved in SCFA-related regulation of renal blood pressure in mice. It is still unclear whether OR signaling has an impact on human renal physiology. In our study, we showed that OR51E1 and OR11H7, both of which can be activated by the SCFA isovaleric acid, are expressed in the HK-2 human proximal tubule cell line. We observed a transient increase in intracellular $Ca^{2+}$ when isovaleric acid and 4-methylvaleric acid were added to HK-2 cells. The isovaleric acid-induced response was dependent on extracellular $Ca^{2+}$ and adenylyl cyclase (AC) activation. Furthermore, we demonstrated that the canonical olfactory signaling components $G\alpha_{olf}$ and ACIII are co-localized with OR51E1. The number of cells responding to isovaleric acid correlated with the presence of primary cilia on HK-2 cells. OR51E1 protein expression was confirmed in the tubule system of human kidney tissue. Our study is the first to show the expression of ORs and olfactory signaling components in human kidney cells. Additionally, we discuss ORs as potential modulators of the renal physiology.



## 1. Introduction

Olfactory receptors (ORs) are G protein-coupled receptors (GPCRs) that form the largest known gene superfamily in the human genome. Their expression was first described in the olfactory epithelium (OE) [1]. However, current studies have also verified the functional expression of ORs in non-olfactory human tissues, where they play essential roles in physiological processes. For example, ORs impact sperm motility [2], inhibition of proliferation of prostate and liver cancer cells, as well as myelogenous leukemia cells [3–5], wound healing of human skin [6], and serotonin release of enterochromaffin cells [7]. Therefore, ORs seem to have diverse physiological and pathophysiological functions and are interesting targets for basic and clinical research approaches. In olfactory sensory neurons, the binding of an odorant to an OR leads to the activation of a cAMP-mediated signaling pathway. The olfactory specific G protein $G\alpha_{olf}$ is activated after the conformational change of the receptor and in turn activates adenylyl cyclase III (ACIII), which converts ATP to cAMP [8–10]. The production of cAMP leads to the opening of cyclic nucleotide-gated (CNG) channels and an influx of $Ca^{2+}$ into the sensory neuron [11,12]. Interestingly, a previous study revealed the expression of olfactory signaling components $G\alpha_{olf}$ and ACIII in cells of the macula densa of rat and mice kidney [13]. ACIII-deficient mice showed a reduced glomerular filtration rate (GFR), and it is assumed that both $G\alpha_{olf}$ and ACIII play a role in the regulation of the GFR and blood pressure [14]. Further, OR78, a short chain fatty acid (SCFA)-activated receptor, is expressed in cells of the macula densa and has an impact on the modulation of blood pressure and renin secretion in the mouse kidney. ORs are therefore good candidates for the detection of fatty acids dissolved within the filtrate of the kidney glomeruli. The production of SCFA is associated with the fermentation of gut

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microbiota, but the accumulation of SCFA can also occur during pathological processes and change the odor of the urine. Patients with isovaleric aciduria, a genetic disorder of the leucine metabolism, exhibit an increased accumulation of isovaleric acid (IVA) that gives urine the odor of sweaty feet [15].

Until now, no studies have documented expression of ORs in cells of the human kidney. In this study, we demonstrated that some components of the olfactory signaling cascade and OR51E1 and OR11H7 are expressed in the human proximal tubule cell line HK-2. We showed that HK-2 cells can be activated by IVA, a ligand for OR51E1 and OR11H7. These findings could be useful for understanding the modulation of renal physiology by SCFA-sensing GPCRs.

## 2. Materials and methods

### 2.1. Cultivation of HK-2 cells

HK-2 cells were the kind gift of Dr. Ulla Ludwig (Department of Internal Medicine I, University of Ulm, Ulm, Germany). HK-2 cells were cultured in DMEM/F12 supplemented with 10% fetal bovine serum (FBS) and 100 U/ml of penicillin and streptomycin at 37 °C in a 5% $CO_2$ humidified atmosphere. Gibco™ supplements and media were purchased from Thermo Fisher Scientific (Waltham, USA) unless indicated otherwise.

### 2.2. Total RNA isolation and reverse transcriptase (RT)-PCR

Total RNA was extracted from HK-2 cells using an RNeasy® Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. RNA concentration and quality (A260/A280 ratio) were analyzed using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA). After DNase I treatment with a TURBO DNA-free™ Kit (Thermo Scientific, USA), complementary DNA (cDNA) was synthesized using an iScript™ cDNA Synthesis Kit (Bio-Rad, Berkeley, USA). For RT-PCR experiments, we used RNA controls (−RT) to exclude genomic DNA contamination. RT-PCR was performed using the GoTaq® qPCR Master Mix (Promega, USA) in a volume of 20 µl with 10 pmol of each primer. The following temperature cycle profile was used: 5 min at 95 °C followed by 40 cycles of 45 s at 95 °C, 45 s at 60 °C, 45 s at 72 °C and a final extension of 10 min at 72 °C. The following primers were used: β-actin (forward: 5′-GTACCCAGGCATTGCTGACA-3′, reverse: 5′-AGAAAGGGTGTAAAACGCAGC-3′), OR51E1 (forward: 5′-TTTGGCACTTGCGTCTCTCA-3′, reverse: 5′-GACACCTAGGGCTCTGAAGC-3′), OR11H7 (forward: 5′-TCCTCTGCCCCTACTCACAT-3′, reverse: 5′-GGCTGTAGATGAGGGGGTTT-3′), GNAL (forward: 5′-CAGACCAGGACCTCCTCAGA-3′, reverse: 5′-AGGGACTCTCTCAGCCTGTT-3′), and ADCY3 (forward: 5′-AAGGATTCAACCCTGGGCTC-3′, reverse: 5′-TCCAGCGTCGCATCTCATAG-3′).

### 2.3. Protein extraction and western blotting

Kidney tissues were obtained from patients undergoing surgical operations. Tissue collection was conducted according to the Declaration of Helsinki, and all patients gave their written consent. Whole protein lysate was extracted after sedimentation of cells or tissue in an appropriate volume of radioimmunoprecipitation assay (RIPA) buffer with protease inhibitors, followed by mechanical homogenization and a final centrifugation (1000 g for 10 min at 4 °C). For phosphorylation analysis, PhoshStop was added to RIPA buffer. A sample of the whole protein fraction was collected and prepared in Laemmli's buffer for Western blot analysis. For the membrane preparation, whole protein lysate was subjected to ultracentrifugation (35,000 g for 2 h). The precipitate and a sample of the supernatant were dissolved in Laemmli's buffer for further western blot analyses. Western blots were performed as described elsewhere (Neuhaus et al., 2009). After SDS-PAGE, proteins were transferred onto nitrocellulose membranes in a blotting chamber with 100 V at 4 °C for 30 min. After blocking at room temperature for 1 h in 50% casein (50% TBS buffer and 50% casein in TBS), membranes were incubated with primary antibodies against OR51E1 (custom designed, polyclonal, Eurogentec, Belgium) (Flegel et al., 2016), ACIII (Santa Cruz Biotechnology, USA), and $G\alpha_{olf}$ (Santa Cruz Biotechnology, USA) in a 1:250 dilution in 75% TBS buffer and 25% casein at 4 °C overnight. Nitrocellulose membranes were washed with TBS-T (3X, 10 min). For immunodetection, membranes were incubated with horseradish peroxidase (HRP)-coupled secondary antibodies (goat anti-rabbit, rabbit anti-goat, Bio-Rad, UK) diluted 1:10,000 in 75% TBS buffer with 25% casein at room temperature for 45 min. After additional TBS-T washes (3X, 10 min), chemiluminescence was imaged via Fusion-SL 3500-WL (Vilber Lourmat).

### 2.4. Immunocytochemical staining of HK-2 cells

HK-2 cells were cultured on 30 mm coverslips until 80% confluent. After washing in PBS, cells were fixed in ice-cold acetone for 5 min. To avoid nonspecific antibody binding, cells were blocked in 1% cold water fish gelatin (Sigma-Aldrich, USA) diluted in TBS with 0.05% Triton X-100 (Sigma-Aldrich, USA) before incubating with primary antibodies against OR51E1, ACIII, and $G\alpha_{olf}$ (dilution: 1:50). Cells were co-incubated with DAPI fluorescent dye to label the nucleus and washed 3 times in PBS for 10 min. Fluorophore-coupled secondary antibodies (Alexa Fluor 488 nm or 546 nm, Thermo Fisher Scientific, USA) were diluted 1:1000 in 1% fish gelatin/0.05% Triton X-100. After three PBS washes of 10 min each, cells were coated with Prolong Antifade Gold (Life Technologies, USA). Fluorescent signals were detected using a confocal microscope (Zeiss LSM 510 Meta, Germany) with a 40× oil immersion objective. The images were processed with the same settings using Corel Draw X5 (Corel, USA).

### 2.5. Immunohistochemical staining of kidney tissue

Paraffin-embedded human tissue was deparaffinized using Roti®-Histol (Roth, Karlsruhe, Germany) and dehydrated with isopropanol. The tissue was rehydrated with an ethanol-series and washed twice with 0.01 M PBS. Next, antigen retrieval and permeabilization were performed. To prevent nonspecific primary antibody binding, sections were blocked in 5% normal serum for 10 min. Sections were incubated with anti-OR51E1 antibodies (1:50 dilution) in 0.01 M PBS at 4 °C overnight. Sections were washed again with 0.01 M PBS and incubated with biotinylated secondary antibody (anti-rabbit) for 45 min (1:1000 dilution). A VECTASTAIN®Elite avidin/biotin (ABC)-based Kit (Vector laboratories, Burlingame, USA) was used according to the manufacturer's recommendations. Sections were incubated with 3,3'-diaminobenzidine until sufficiently stained, and the reaction was stopped with 0.1 M PBS at 4 °C. Immunohistochemical staining was detected with an Olympus BX 43 microscope (10× objective).

### 2.6. Calcium imaging

HK-2 cells cultured in 35-mm cell culture dishes (Sarstedt, Germany) were incubated with 3 µM fura-2-acetoxymethyl ester (Molecular Probes, Thermo Fisher Scientific, USA) for 30 min at 37 °C. The growth medium was exchanged with an extracellular solution and fluorometric imaging was performed as previously described [4]. Depending on the experimental approach, cells were