



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Hydroxy- α -Sanshool and Capsaicin as Salt Taste Enhancers: Molecular Mechanisms of TRPV1/ENaC Activation and PKC α Signaling

Xingyu Wang^{† 1}, Yang Wang^{† 1,2,3}, Lei Zhao², Zhen Qin¹, Kui Zhong^{2*}, Haiyan Gao^{1*}

¹ School of Life Sciences, Shanghai University Shanghai 200444, China

² Food and Agriculture Standardization Institute, China National Institute of Standardization, Beijing, 100088, China

³ Periodical Agency of Nanchang University, Jiangxi, 330031, China

ABSTRACT: Excessive salt intake has emerged as a significant public health concern. Early-phase research suggests that pungent substances have the potential to reduce salt consumption, although the precise mechanism remains incompletely elucidated. This study systematically investigated the molecular basis of saltiness enhancement by má là compounds—hydroxy- α -sanshool (3 μ mol/L) and capsaicin (1 μ mol/L). We employed calcium imaging to monitor ionic flux in murine bud cells, Western blot and qPCR to quantify protein and gene expression in HEK293T cell models, and integrated molecular docking with dynamics simulations to characterize receptor-ligand interactions. Our findings demonstrate that both compounds significantly potentiated Ca²⁺ influx through TRPV1 and SCNN1A channels. Notably, the hydroxy- α -sanshool/capsaicin combination reverses the suppressive effect of capsaicin alone on SCNN1A expression and coordinately upregulates TRPV1 and SCNN1A expression, accompanied by enhanced PKC α phosphorylation. Structural simulations reveal that hydroxy- α -sanshool stabilizes TRPV1 conformational states that facilitate capsaicin binding. Collectively, these results establish a mechanistic foundation for má là-mediated saltiness enhancement and support their potential application in taste modulation strategies.

Keywords: má là compounds, Salt reduction, Hydroxy- α -sanshool, Capsaicin, TRPV1, SCNN1A

1. Introduction

Excessive sodium intake is a major public health concern, strongly associated with increased risks of cardiovascular diseases, stroke, cognitive impairment, diabetes mellitus, and kidney disease^[1-3]. The primary source of dietary sodium is salt (NaCl), and reducing sodium intake often equates to reducing salt consumption. However, global salt intake remains significantly higher than the recommended levels due to palatability^[4, 5], underscoring the urgent need for effective sodium reduction strategies.

Current salt reduction approaches include the use of salt substitutes, modifications in the size, shape, and distribution of salt crystals, novel food processing technologies, and leveraging multisensory interactions to enhance saltiness perception^[6]. However, maintaining food palatability while reducing sodium content remains a major challenge. Má describes the tingling and numbing sensations induced by compounds such as

[†] Xingyu Wang and Yang Wang contributed equally to this manuscript

*Corresponding author

zhongkui@cnis.ac.cn; hygao1111@126.com

Received 1 February 2026

Received in revised form 17 March 2026

Accepted 30 April 2026

those from Sichuan pepper (*Zanthoxylum spp.*), while là refers to the burning and pungent sensations primarily caused by chili compounds. Collectively known as má là, these flavors play crucial roles in many culinary traditions, particularly in Sichuan cuisine. The primary active compound in Sichuan pepper (*Zanthoxylum spp.*), hydroxy- α -sanshool (HS), and its counterpart in chili peppers (*Capsicum spp.*), capsaicin (Cap), provide má là taste in various dishes. Beyond their traditional culinary roles, both compounds are widely applied in the food industry as flavor enhancers in seasonings, meat products, and convenience foods, where they contribute to complex taste profiles and consumer appeal. Recent psychophysical studies have shown that HS and Cap significantly enhance human perception of saltiness^[7,8]. Sun et al. demonstrated that má là and umami enhanced saltiness perception in both NaCl aqueous solutions and oil-added systems^[9]. The ability of má là compounds to enhance saltiness perception offers a promising strategy for sodium reduction without compromising flavor. However, the physiological mechanisms underlying this enhancement remain poorly understood, limiting their application in salt reduction strategies.

Salty taste is primarily mediated by two receptor channels: the amiloride-sensitive epithelial sodium channel (ENaC) and amiloride-insensitive pathways, such as the transient receptor potential vanilloid 1 (TRPV1) channel^[6]. ENaC is composed of α , β , and γ subunits encoded by the SCNN1A, SCNN1B, and SCNN1G genes, respectively. It is mainly expressed in the fungiform papillae of the anterior tongue and is responsible for detecting low sodium concentrations (< 150 mmol/L NaCl)^[10,11]. In contrast, the transduction mechanism for high-salt stimuli (> 150 mmol/L NaCl) remains incompletely understood, though TRPV1 has been implicated in modulating high-salt taste transduction^[12]. TRPV1 is widely expressed in primary afferent neurons, oral epithelial cells, and taste bud cells of the fungiform, circumvallate, and foliate papillae, suggesting a potential role in saltiness modulation. As the primary ion in sodium chloride, sodium influx causes membrane depolarization, allowing calcium ions to enter taste receptor cells through voltage-gated channels. The subsequent rise in intracellular Ca^{2+} triggers neurotransmitter release at synaptic sites, enabling signal transmission to afferent fibers and completing taste sensation—a transduction pathway applicable to both channel types^[13]. HS is the most abundant alkylamide substance in Sichuan pepper, responsible for the characteristic má sensation. It activates multiple receptors, including TRPV1 and two-pore potassium channels (TASK-1, TASK-3, and TRESK), contributing to its tingling and numbing effects^[14-17]. Cap is the primary pungent compound in chili peppers, inducing a burning sensation through direct activation of TRPV1^[18]. Although evidence indicates that HS and Cap enhance salt perception, their specific molecular mechanisms in salt transduction remain unclear.

Therefore, this study aims to systematically elucidate the neurophysiological and molecular mechanisms by which má là compounds regulate salt perception. Using HS and Cap as representative compounds, we focus on two key ion channels involved in salt taste transduction—TRPV1 and α -ENaC—to investigate their individual and combined effects on salt perception. First, using a mouse papillary cell model, we evaluated the regulatory effects of HS and Cap on NaCl-induced Ca^{2+} signaling. Second, by constructing a salty taste receptor cell model expressing human TRPV1 and SCNN1A, we analyzed the regulation of salty taste receptor

function at the transcriptional, translational, and phosphorylation levels. Finally, combining molecular docking and molecular dynamics simulations, we revealed the binding patterns, dynamic mechanisms, and potential synergistic effects of HS and Cap on the TRPV1 receptor at the atomic level. This study provides new experimental evidence for elucidating the complex mechanisms by which má là compounds enhance salt perception and lays a theoretical foundation for their scientific application in salt reduction strategies.

2. Materials and methods

2.1 Main Reagents

Capsaicin (CAS: 404-86-4) was purchased from Sigma-Aldrich (Denmark). SB-366791 (CAS: 472981-92-3), amiloride (CAS: 2609-46-3), and hydroxy- α -sanshool (CAS: 83883-10-7) were obtained from MedChemExpress (USA). TRPV1 and SCNN1A primers were sourced from Shanghai Generay Bioengineering Co., Ltd (China). Collagenase A and Dispase® II were acquired from Roche (Switzerland). DMEM-H, Opti-MEM™ I reduced serum medium, fetal bovine serum (FBS), and trypsin-EDTA (0.25%) with phenol red were purchased from Gibco (USA). Fluo-4 AM, Hieff® qPCR SYBR Green Master Mix (No Rox), and Hifair® II 1st Strand cDNA Synthesis SuperMix for qPCR (gDNA digester plus) were provided by Yeasen Biotechnology Shanghai Co., Ltd (China). Lipofectamine 3000 was obtained from Invitrogen (USA). Phosphatase inhibitor mixture A (50×), PVDF membrane (0.45 μ m), transfer filter paper, horseradish peroxidase-labeled goat anti-rabbit IgG (H+L), and Pluronic F-127 were supplied by Shanghai Beyotime Biotechnology Co., Ltd (China). TRPV1 Rabbit pAb (A8564), SCNN1A Rabbit pAb (A5538), and PKC alpha Rabbit pAb (A0267) were purchased from Wuhan ABclona Biotechnology Co., Ltd (China).

2.2 Animals and Cells

Six-to-eight-week-old male and female C57BL/6 mice (specific pathogen-free grade) were purchased from Shanghai JieSiJie Laboratory Animal Co., Ltd. All experimental procedures were approved by the Ethics Committee of the School of Life Sciences at Shanghai University (Approval No. SCXK(HU)2018-0004). These mice were used for the isolation of tongue epithelial tissue. HEK293T cells were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and were used to establish a salt-taste perception cellular model.

2.3 Calcium Signaling in Taste Bud Cells of the Anterior Tongue Epithelium

2.3.1 Isolation of Fungiform Papillae from Mouse Tongue Epithelium

Euthanized mice were placed on their backs, and the upper and lower jaws were separated to open the mouth widely. The cheeks were cut open with tissue scissors to expose the entire tongue. The tongue was excised at the base inner to the circumvallate papillae and soaked in cold Tyrode's solution. Using forceps, a 1 mL syringe needle was inserted subepithelially from the lingual base toward the apex, taking care not to puncture the epithelial layer. While slowly withdrawing the needle, 1 mL of a freshly prepared enzyme

solution containing Dispase II (2 mg/mL) and Collagenase A (1 mg/mL) was injected. This injection caused the epithelium to swell and separate from the underlying muscle layer. The injection was performed slowly to ensure even distribution of the enzymes. The tongue was subsequently incubated in Tyrode's solution at 37 °C for 15 minutes. Under a stereomicroscope, the epithelium was carefully peeled from the base to the apex of the tongue using straight and fine-tipped forceps. The anterior tongue epithelium, which contains the fungiform papillae, was retained for subsequent experiments.

2.3.2 Determination of Calcium Signal in Tongue Epithelial Taste Bud Cells

To monitor intracellular calcium dynamics, freshly isolated murine fungiform papillae epithelial tissues were incubated for 1 hour at 37 °C under light-protected conditions in Tyrode's solution containing 4 μ mol/L Fluo-4 AM and 0.04% – 0.05% Pluronic F-127. Following incubation, tissues were rinsed and transferred to fresh Tyrode's solution to remove excess dye, then secured in an imaging chamber (Warner Instruments). Calcium imaging was performed using a Bergamo® II series multiphoton microscope (Thorlabs), with an excitation wavelength set at 920 nm and a 447/60 nm emission filter, operating in bidirectional raster scan mode at a resolution of 512×512 pixels. The focal plane was carefully adjusted along the Z-axis to ensure optimal signal acquisition from sufficient numbers of taste cells^[19]. During calcium imaging, data were continuously acquired in streaming mode for 300 seconds per sample, with 20 seconds of stimulus application followed by a 3-minute washout period with bath solution. Changes in intracellular calcium concentration were quantified as $\Delta F/F_0$, where ΔF represents the difference between the maximum fluorescence intensity (F_{max}) during stimulus application and the baseline fluorescence intensity (F_0) measured over 30 seconds in bath solution prior to stimulation. A response was considered positive only when the fluorescence change exceeded three standard deviations (SD) of F_0 .

2.4 Cell Culture, Transfection, and RNA Extraction

A HEK293T cellular model co-expressing human TRPV1 and SCNN1A (encoding the α -ENaC subunit) was established to investigate the regulatory effects of "má là" substances—specifically, HS and Cap—on these channels. Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO₂ incubator. At 70%–80% confluency, cells were co-transfected with equal amounts (1:1 ratio) of TRPV1 and SCNN1A plasmids using the Lipo3000 transfection reagent. Forty-eight hours post-transfection, cells were treated for 1 hour with various working solutions containing different concentrations of NaCl, compounds (C4, P4, CnPm), and/or pharmacological inhibitors (SB-366791, a TRPV1 channel antagonist; Amiloride, an ENaC blocker), as detailed in Table 1^[20-22]. Following treatment, cells were harvested for RNA or protein extraction.

RNA extraction was performed using the Trizol-chloroform method^[23]. The RNA was dissolved in DEPC-treated water, and concentration and purity were assessed using a spectrophotometer. Only samples with OD_{260/280} ratios between 1.9 and 2.1 and concentrations between 200 ng/ μ L–1,000 ng/ μ L were used for further analysis.

Table 1. Concentrations of "Má Là" Stimulants and Antagonists

Substance	Working Concentration ($\mu\text{mol/L}$)				
	1	2	3	4	5
Capsaicin (C)	0.10	0.29	0.50	1.00	6.00
Hydroxy- α -sanshool (P)	0.50	0.80	1.10	3.00	6.00
Amiloride (A)			100.00		
SB-366791 (SB)			10.00		

2.5 RT-qPCR for Gene Expression Analysis

An RNase-free centrifuge tube was loaded with $5 \times \text{g}$ DNA digester Mix ($3 \mu\text{L}$), mRNA, and RNase-free H_2O (to a total volume of $15 \mu\text{L}$). The contents were gently mixed with a pipette and the mixture was incubated at 42°C for 2 minutes to remove residual genomic DNA. Next, $4 \times$ Hifair® III SuperMix plus ($5 \mu\text{L}$) was added to the tube and was gently mixed again with a pipette. Reverse transcription was performed using the following program: 25°C for 5 minutes, 55°C for 15 minutes, and 85°C for 5 minutes. The resulting cDNA was diluted with ultrapure water for subsequent qPCR analysis.

Each sample and target gene was analyzed in three independent replicates, with GAPDH serving as the internal control. The qPCR reaction system ($20 \mu\text{L}$ total volume) consisted of $10 \mu\text{L}$ of Hieff® qPCR SYBR Green Master Mix (No Rox), $0.4 \mu\text{L}$ each of forward and reverse primers ($10 \mu\text{mol/L}$; Table 2), $1 \mu\text{L}$ of cDNA template, and ultrapure water to volume. The amplification protocol comprised an initial denaturation at 95°C for 3 min; 30–35 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 15 sec, and extension at 72°C for 60 sec/kb; and a final extension at 72°C for 5 min. Relative gene expression levels were calculated using the $2^{-\Delta\Delta\text{CT}}$ method, normalized to GAPDH expression, and are presented relative to the control group (set to 1)

Table 2. Primers used for RT-qPCR experiments

Gene	Sequence (5'-3')	Length (bp)
TRPV1	Forward: CCCTGTTATCACCATCCA	139
	Reverse: TCTGAGCAACGGCTTCA	
SCNN1A (α -ENaC)	Forward: ATGCGGTGAGGGAGTG	112
	Reverse: GCAGGCGAAGATGAAGT	
GAPDH	Forward: CAGGAGGCATTGCTGATGAT	138
	Reverse: GAAGGCTGGGGCTCATTT	

2.6 Protein Immunoblotting Assays

HEK293T cells were lysed using a lysis buffer consisting of RIPA buffer supplemented with PMSF (final concentration of 1 mmol/L) and phosphatase inhibitor mixture A ($1\times$). The lysate was centrifuged at $13,000 \text{ g}$ for 10 minutes at 4°C . A small portion of the supernatant was collected to determine protein concentration using the BCA assay, and all samples were adjusted to a uniform concentration of $1 \mu\text{g}/\mu\text{L}$ using lysis buffer. Protein samples ($10 \mu\text{g}$ – $20 \mu\text{g}$ per lane) were separated under reducing conditions via SDS-PAGE and transferred onto a polyvinylidene fluoride (PVDF) membrane. Following transfer, the membrane was briefly rinsed with TBST for 1 minutes–3 minutes and then blocked with 5% BSA for 1 hours–2 hours at room

temperature. The membrane was incubated overnight at 4 °C with the following primary antibodies: TRPV1 Rabbit pAb, SCNN1A Rabbit pAb, and PKC α Rabbit pAb. After primary antibody incubation, the membrane was washed with TBST and then probed with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (H+L) for 1 h at room temperature. Following secondary antibody incubation and subsequent washes, the membrane was treated with freshly prepared ECL luminescent solution (prepared by mixing solutions A and B at a 1:1 ratio) for 1 min–2 min. Chemiluminescence signals were detected and recorded using a chemiluminescence imaging system.

2.7 Molecular Docking and Molecular Dynamics

2.7.1 Molecular Docking

Molecular docking was performed according to a previously described protocol^[24] with slight modifications to assess the binding energies and interaction patterns between the small molecules and target proteins. The three-dimensional structures of hydroxy- α -sanshool (HS) and capsaicin (Cap) were retrieved from the PubChem Compound Database (<https://pubchem.ncbi.nlm.nih.gov/>). The crystal structure of the human TRPV1 protein (PDB ID: 7LPB, resolution: 3.54 Å) was obtained from the RCSB Protein Data Bank. Prior to docking, all water molecules were removed from the protein structure, and polar hydrogen atoms were added. Molecular docking was performed using AutoDock Vina 1.5.7. A cubic docking grid with dimensions of 100 Å $\times$ 100 Å $\times$ 100 Å and a grid spacing of 1.0 Å was defined to encompass the entire vanilloid binding pocket of TRPV1. The binding free energy (ΔG) was calculated using the AutoDock Vina scoring function, which approximates the sum of intermolecular interactions including hydrogen bonding, hydrophobic interactions, and van der Waals forces. The resulting docking poses were visualized and analyzed using Discovery Studio 2017 R2 and PyMOL.

2.7.2 Molecular Dynamic Simulation

Unconfined molecular dynamics simulations of molecular docking complexes of small molecules with proteins were performed using the molecular dynamics simulation software GROMACS 2023, the CHARMM36 force field, and the TIP3P water model. The MOL2 format file of the small molecule was uploaded to the CGenFF server (<https://cgenff.umaryland.edu/initguess/>) to generate the str file. The cgenff_charmm2gmx.py script (executed with Python 2.7) was then used to convert this file into a CHARMM36-compatible molecular topology. Each system was solvated in a cubic box with a 1 nm periodic boundary condition applied. Energy minimization was followed by two equilibration phases: NVT for 500 ps and NPT for 1000 ps, using the Nose-Hoover thermostat and Parrinello-Rahman barostat, respectively, to stabilize the system at 310 K and 1 bar. Production MD simulations were then run for 200 ns. The simulations used a 2 fs time step, saving coordinates every 10 ps. Non-bonded interactions were treated with a 1.2 nm cutoff, and long-range electrostatics were handled using the Particle Mesh Ewald (PME) method. Temperature

coupling was achieved using the modified Berendsen algorithm with a time constant of 0.1 ps, and pressure coupling was maintained with the Parrinello-Rahman algorithm at 1.0 bar with a 2.0 ps time constant.

2.8 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0, while data visualization and graphing were conducted with Excel 2016, OriginPro, and GraphPad Prism software. Each experiment was conducted in at least triplicate. Data were presented as the mean $\pm$ standard error of the mean (SEM), with $N \geq 3$. Based on the normality assessment of the data, either parametric or non-parametric tests were selected for differential analysis. The interaction effects between factors were compared using ANOVA or MANOVA, with a significance level set at $P < 0.05$.

3. Results and Discussion

3.1 Effect of Pungent Substances on Calcium Ion Signal Strength for NaCl Perception by Taste Bud Cells in the Tongue Epithelium

Calcium signals in NaCl solutions (100 or 250 mmol/L) containing pungent substances (C4, P4, and C4P4) were significantly higher than in NaCl solutions alone (Figure 1), indicating that these substances enhanced the cellular calcium response ($P < 0.05$). In the presence of the TRPV1 antagonist SB-366791, calcium signaling was significantly attenuated across all treatment groups compared to the corresponding conditions without the antagonist ($P < 0.05$). Notably, the calcium signal intensity in the NaCl+C4+A+SB group was comparable to that in the NaCl+A+SB group and both were significantly lower than in the NaCl-only group. This suggested that 10 $\mu\text{mol/L}$ TRPV1 antagonist SB largely abolished the C4-induced calcium elevation in both 100 mmol/L and 250 mmol/L NaCl solutions. The fact that calcium levels in the NaCl+C4 group exceeded those in the NaCl-only group further confirmed the enhancing role of C4. Similarly, calcium levels in both the NaCl+C4+SB and NaCl+SB groups were suppressed to levels below that of the NaCl-only treatment. This indicated that SB also essentially abolished the capsaicin-induced calcium rise at these NaCl concentrations. In contrast, SB did not fully inhibit the enhanced calcium signals induced by P4 or the C4P4 mixture at the same NaCl concentrations. These differential responses implied that under high-salinity conditions, C4 and P4/C4P4 activated TRPV1 via distinct mechanisms. A possible explanation was that, besides activating TRPV1-mediated signaling, high salt has also been reported to depolarize sensory neurons by inhibiting pH- and anesthetic-sensitive two-pore domain potassium channels (such as KCNK3, KCNK9, and KCNK18), leading to neuronal excitation and a subsequent rise in intracellular Ca^{2+} [25].

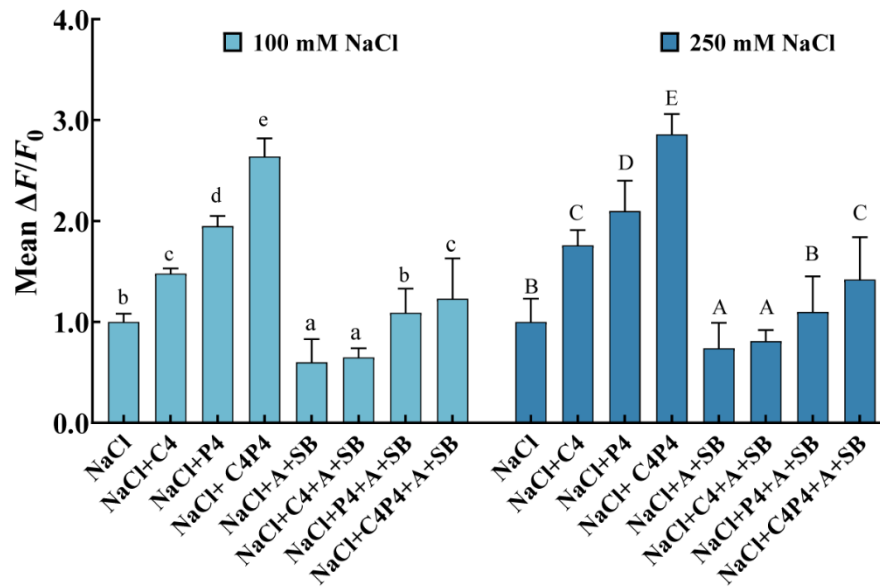
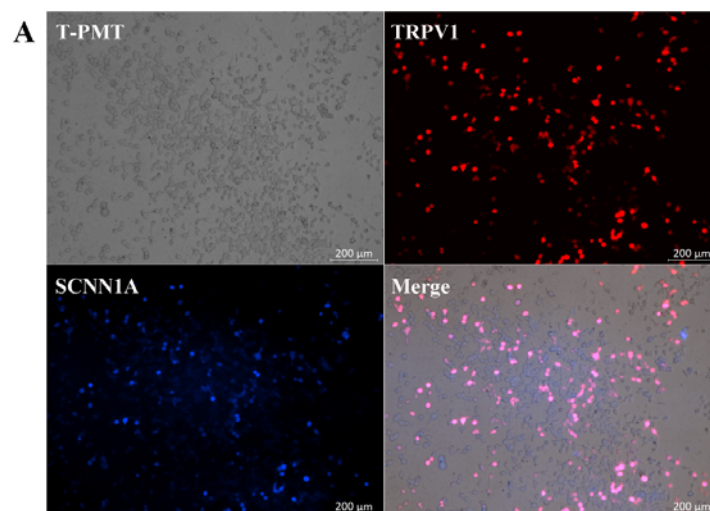


Fig.1 Má là compounds enhance the intensity of Ca^{2+} signals in fungiform taste bud cells for perceiving NaCl (C4, Cap 1.00 $\mu\text{mol/L}$; P4, HS, 3.00 $\mu\text{mol/L}$; A, Amiloride, 100.00 $\mu\text{mol/L}$; SB, SB-366791, 10.00 $\mu\text{mol/L}$). Lowercase letters a-e and uppercase letters A-E denote differences in $\Delta F/F_0$ between samples in the 100 mmol/L and 250 mmol/L NaCl groups, respectively. Means followed by different letters are significantly different ($P < 0.05$).

3.2 Effect on mRNA Expression of Human-derived TRPV1 and SCNN1A Genes in HEK293T Cell Models

3.2.1 Development of a HEK293T Cellular Model for Salty Taste Sensing Co-expressing Human TRPV1 and SCNN1A

Fluorescence microscopy confirmed the successful co-expression of TRPV1 fused to the red fluorescent protein mCherry and SCNN1A fused to the blue fluorescent protein mTagBFP2 in HEK293T cells (Figure 2A). Subsequent RT-qPCR and Western Blot analysis further confirmed the successful overexpression of both TRPV1 and SCNN1A at the mRNA and protein levels, respectively (Figure 2B, 2C). Collectively, these results indicated the successful establishment of a HEK293T salt-taste perception model engineered to express the human TRPV1 and SCNN1A genes.



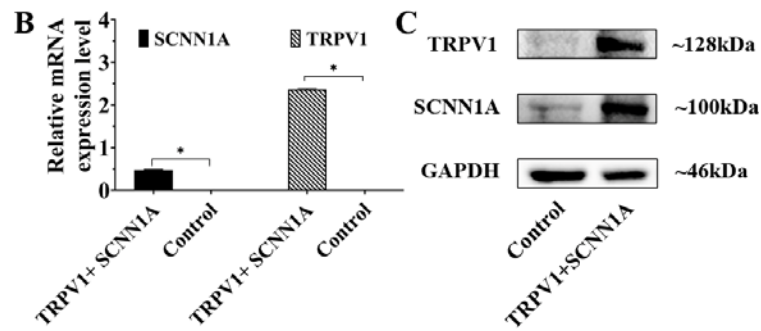
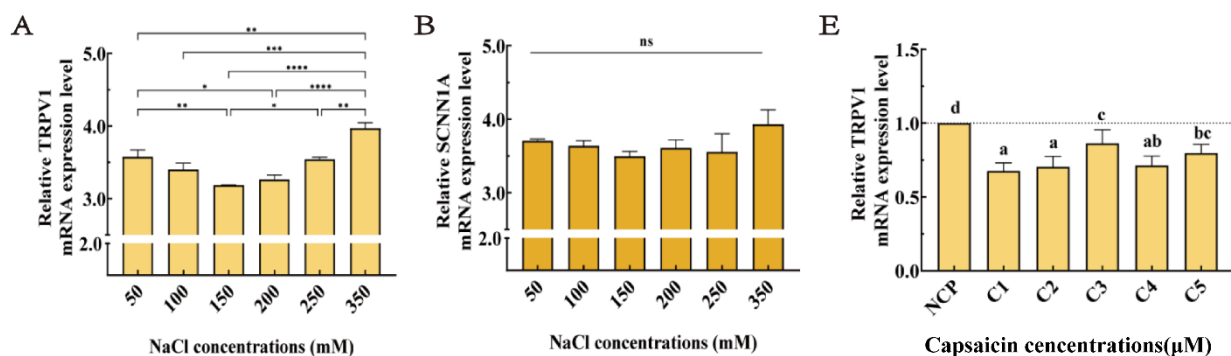


Fig.2 HEK293T cells successfully overexpressed TRPV1 and SCNN1A. (A) Representative images of HEK293T cells 24 h after transfection, captured under bright-field, 594 nm (mCherry-TRPV1), and 408 nm (mTagBFP2-SCNN1A) wavelengths. (B) RT-qPCR analysis of TRPV1 and SCNN1A mRNA expression in HEK293T cells. Relative mRNA levels were calculated using the $2^{-\Delta\Delta CT}$ method with GAPDH as the reference gene. (C) Western blot analysis of TRPV1 and SCNN1A protein expression. * $P < 0.05$.

3.2.2 Effects of Hydroxy- α -sanshool or Capsaicin on TRPV1 and SCNN1A mRNA Expressions Individually

The relative mRNA expression of TRPV1 and SCNN1A in HEK293T cells was significantly affected by NaCl, HS, and Cap (Figure 3, $P < 0.05$). When exposed to increasing NaCl concentrations (50–350 mmol/L), TRPV1 mRNA expression displayed a biphasic response. At NaCl concentrations below 150 mmol/L, TRPV1 mRNA expression significantly decreased, potentially due to the translation of intracellular TRPV1 mRNA reserves into proteins. Conversely, at concentrations exceeding 150 mmol/L, TRPV1 mRNA expression significantly increased, indicating TRPV1 involvement in high-salt sensing pathways (Figure 3A). In contrast, SCNN1A mRNA expression remained unchanged across all NaCl concentrations, suggesting that NaCl did not significantly impact SCNN1A at the mRNA level (Figure 3B). HS exerted a dose-dependent effect on TRPV1 and SCNN1A mRNA expression. At low concentrations (P1–P3), HS slightly inhibited expression of both mRNAs, whereas at higher concentrations (P4–P5), it significantly increased their expression (Figure 3C, 3D). This indicated that HS had a threshold concentration, below which it might suppress receptor expression. Similarly, Cap significantly altered the relative mRNA expression of both TRPV1 and SCNN1A in a dose-dependent manner (C1–C5) (Figure 3E, 3F). However, across all concentrations tested (C1–C5), the mRNA expression of both genes was lower than in the control group. This observed downregulation is consistent with previous findings that capsaicin can inhibit the expression of ENaC subunits at certain concentrations, suggesting a potential suppressive effect at the transcriptional or post-transcriptional level^[26].



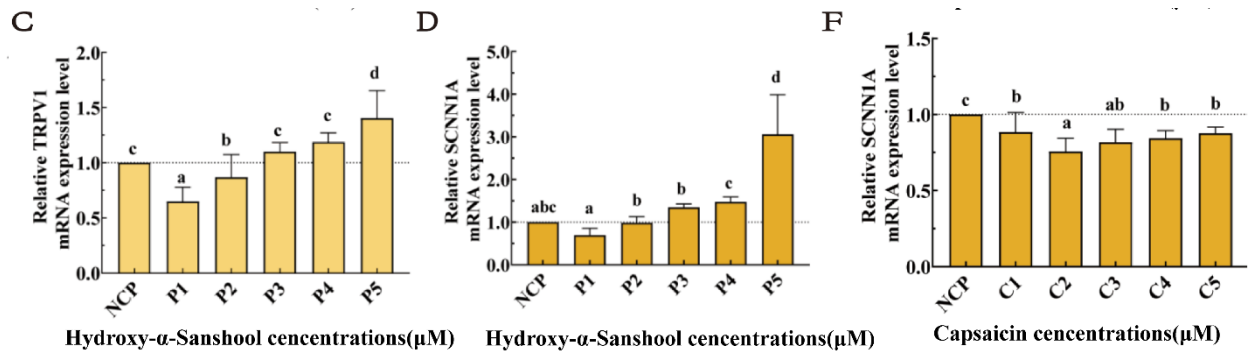
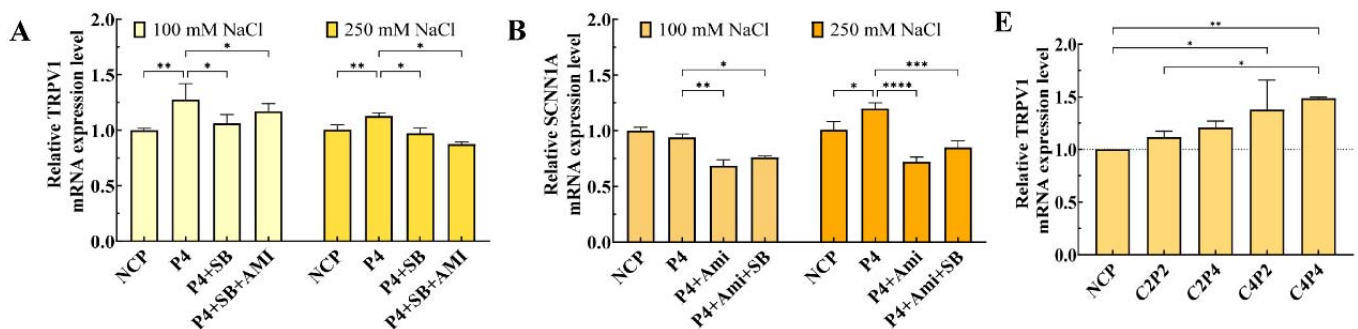


Fig.3 Relative mRNA expression of TRPV1 and SCNN1A in HEK293T cells after stimulation (P1-P5, HS 0.50, 0.80, 1.10, 3.00, 6.00 $\mu\text{mol/L}$; C1-C5, Cap 0.10, 0.29, 0.50, 1.00, 6.00 $\mu\text{mol/L}$). (A and B) The relative mRNA expression of TRPV1 and SCNN1A genes in HEK293T cells stimulated by different concentrations of NaCl. (C and D) The relative mRNA expression of TRPV1 and SCNN1A genes in HEK293T cells stimulated by different concentrations of HS. (E and F) The relative mRNA expression of TRPV1 and SCNN1A genes in HEK293T cells stimulated by different concentrations of Cap. The relative expression levels of the NCP group are standardized as 1. Lowercase letters indicate significant differences between groups ($P < 0.05$).

3.2.3 Effects of Hydroxy- α -Sanshool and Capsaicin Combinations on TRPV1 and SCNN1A mRNA Expression

C4P4 significantly increased the mRNA expression of TRPV1 and SCNN1A compared to individual treatments (Figure 4E, 4F). This synergistic effect was particularly pronounced at higher concentrations, where the combination of Cap and HS substances not only enhanced TRPV1 expression but also counteracted the inhibitory effect of Cap on SCNN1A. The combination of 100 or 250 mmol/L NaCl with P4 significantly upregulated TRPV1 mRNA expression relative to NaCl alone, and this effect was partially suppressed by the TRPV1 antagonist SB (Figure 4A). The combination of NaCl and Cap (100 or 250 mmol/L NaCl+C4) did not significantly alter TRPV1 mRNA expression compared to NaCl alone (Figure 4C). However, SCNN1A mRNA expression was significantly reduced in the presence of Cap, and this reduction was further potentiated by Amiloride (Figure 4D). The failure of NaCl + Cap to alter TRPV1 expression may be attributed to the classic desensitization of the TRPV1 receptor following prolonged capsaicin exposure^[27]. Additionally, Amiloride exacerbated the Cap-induced inhibition of SCNN1A. This strongly suggests that the inhibitory effect of Cap on the sodium channel (ENaC) may depend either on ENaC activity per se or on a downstream pathway that overlaps with the mechanism of action of Amiloride.



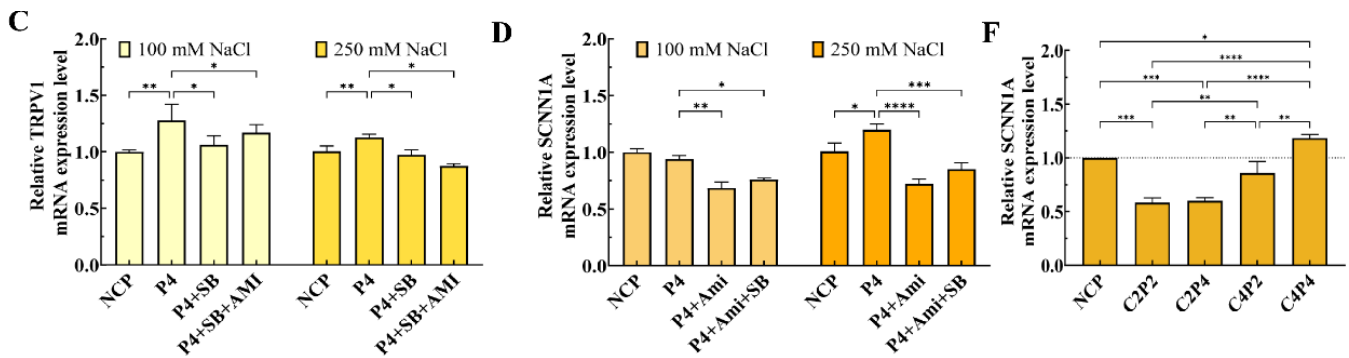


Fig.4 Relative mRNA expression of TRPV1 and SCNN1A in HEK293T cells after stimulation with different *má là* groups (C2 and C4, Cap 0.29, 1.00 $\mu\text{mol/L}$; P2 and P4, HS 0.80, 3.00 $\mu\text{mol/L}$; Ami, Amiloride 100.00 $\mu\text{mol/L}$; SB, SB-366791 10.00 $\mu\text{mol/L}$). (A and B) HS and NaCl modulated TRPV1 and SCNN1A mRNA expression in saltiness perception cells. (C and D) Cap and NaCl modulated TRPV1 and SCNN1A mRNA expression in saltiness perception cells. (E and F) HS and Cap modulated TRPV1 and SCNN1A mRNA expression in saltiness perception cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

3.2.4 Effect of *má là* Compounds and NaCl on TRPV1 and SCNN1A mRNA Expressions

The relative mRNA expression levels of TRPV1 and SCNN1A in cells stimulated with the combination of C4P4 and 100/250 mmol/L NaCl were significantly higher than those with C4 alone ($P < 0.05$), while no significant difference was observed compared with single P4 alone ($P > 0.05$). These findings indicated that, relative to individual P4 or C4 stimulation, the C4P4 combination upregulated TRPV1 and SCNN1A mRNA expression and counteracted the inhibitory effect of Cap on SCNN1A. Specifically, the C4P4 combination not only enhanced TRPV1 expression but also markedly reversed the suppressive effect of Cap alone on SCNN1A. This phenomenon was consistent with previous studies, indicating that Cap may shift the regulation of the epithelial sodium channel (ENaC) from inhibition to synergism under specific physicochemical conditions, such as high-salt environments^[28].

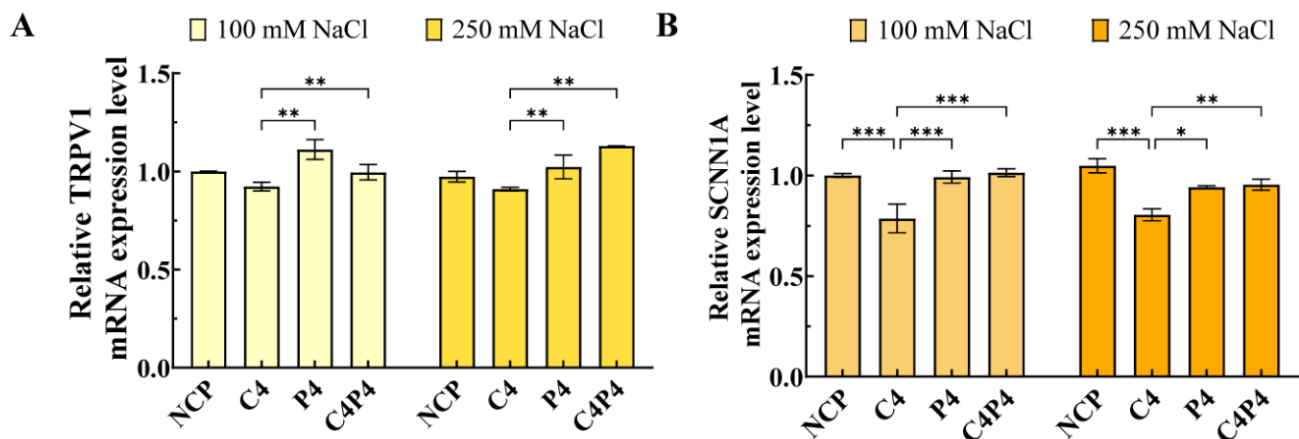


Fig.5 Relative mRNA expression of TRPV1 (A) and SCNN1A (B) in saltiness perception cells after stimulation with Cap, HS, and NaCl (C4, Cap 1.00 $\mu\text{mol/L}$; P4, HS 3.00 $\mu\text{mol/L}$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

3.3 Effect of *má là* Compounds and NaCl on the Expression of TRPV1 and SCNN1A Proteins

Western Blot analysis revealed that P4, C4 and CnPm increased the relative expression of human TRPV1 and SCNN1A proteins in 100 mmol/L and 250 mmol/L sodium solutions in the HEK293T saltiness perception cell model (Figure 6). In 100 mmol/L NaCl, P4, C4, and CnPm significantly increased TRPV1 expression (P

< 0.05), while significant upregulation of SCNN1A was induced by P4 and C4P4 ($P < 0.05$) (Figure 6A, 6D). Under the 250 mmol/L NaCl condition, TRPV1 expression was significantly enhanced by P4 and CnPm ($P < 0.05$; Figure 6B, 6E), whereas only C4P4 produced a significant increase in SCNN1A expression. ($P < 0.05$; Figure 6D, 6F).

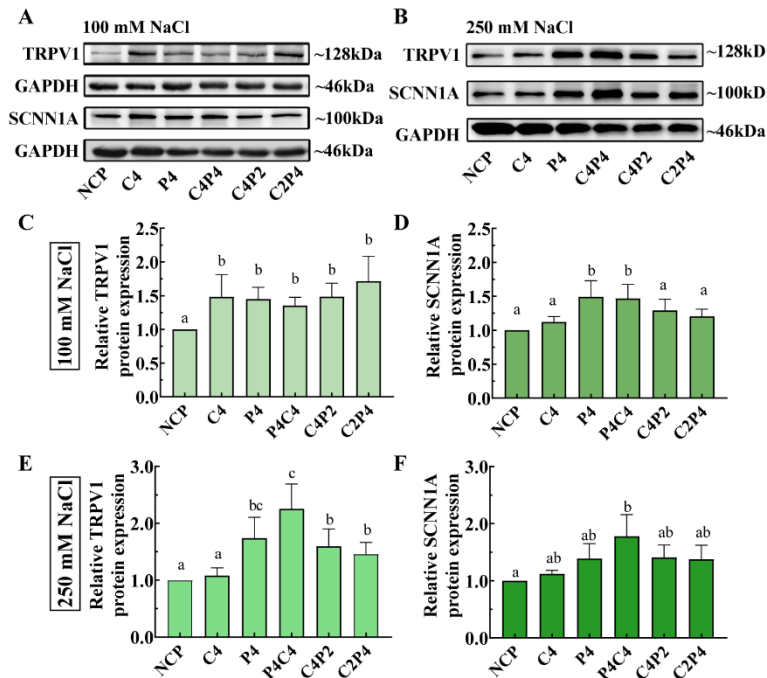


Fig.6 Relative protein expression of TRPV1 and SCNN1A in saltiness perception cells after stimulation with Cap, HS and NaCl (C2 and C4, Cap 0.29, 1.00 $\mu\text{mol/L}$; P2 and P4, HS 0.80, 3.00 $\mu\text{mol/L}$). (A and B) The results of Western blots of TRPV1 and SCNN1A proteins in saltiness perception cells after stimulation with 100 mmol/L and 250 mmol/L NaCl solutions containing different *má là* substances. (C and E) The relative expression of intracellular TRPV1 protein after stimulation with 100 mmol/L and 250 mmol/L NaCl solutions containing different *má là* substances. (D and F) The relative expression of intracellular SCNN1A protein after stimulation with 100 mmol/L and 250 mmol/L NaCl solutions containing different *má là* substances. Different lowercase letters indicate significant differences between groups ($P < 0.05$).

Li et al. investigated cultured renal cortical collecting duct (M1-CCD) cells and found that incubation with a 10 mmol/L salt solution for 24 h reduced the expression of TRPV1 in M1-CCD cells while increasing the expression of SCNN1A^[28]. Conversely, incubation with 1.0 $\mu\text{mol/L}$ Cap plus high-salt solution for 24 h up-regulated the expression of TRPV1 in M1-CCD cells and down-regulated the expression of SCNN1A^[28]. In this study, Cap activation of TRPV1 did not significantly inhibit SCNN1A protein expression ($P > 0.05$), but did significantly reduce SCNN1A mRNA expression ($P < 0.05$). This discrepancy might be because the present study monitored the effects of short-term stimulation, whereas Li et al. focused on the long-term effects of Cap.

3.4 Effects of *má là* Compounds on PKC α Protein Expression

Figure 7 illustrated the changes in intracellular PKC α protein relative expression following different compounds and NaCl. The PKC α relative expression of combination stimulation (100 mmol/L NaCl C4P2 and 250 mmol/L NaCl C4P4) was significantly higher than that of the control group in both 100 mmol/L and 250 mmol/L NaCl solutions ($P < 0.05$). Moreover, in the 100 mmol/L NaCl solution, CnPm exhibited higher PKC α relative expression than the individual Cap and HS group. Furthermore, in 250 mmol/L solution, the

interaction between HS and Cap significantly affected PKC α protein relative expression, and the effect was concentration-dependent.

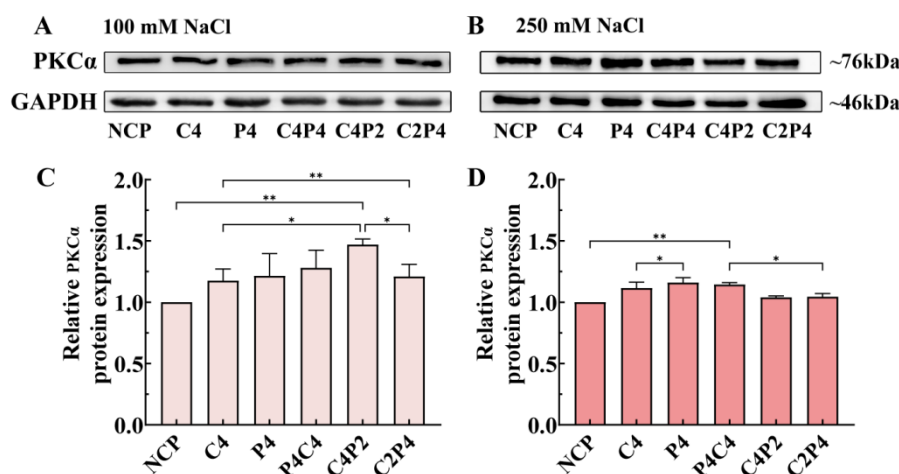


Fig.7 Relative protein expression of PKC α in saltiness perception cells after stimulation with Cap, HS and NaCl (C2 and C4, Cap 0.29, 1.00 μ mol/L; P2 and P4, HS 0.80, 3.00 μ mol/L). (A and B) The protein blotting results of intracellular PKC α protein after stimulation with 100 mmol/L and 250 mmol/L NaCl solutions containing different má là substances. (C and D) The relative expression of intracellular PKC α protein after stimulation with 100 mmol/L and 250 mmol/L NaCl solutions containing different má là substances. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Studies have shown that the activities of TRPV1 channels and SCNN1A channels are closely linked to their phosphorylation status. The activation of TRPV1 channels can subsequently activate kinases such as PKC, PLC, and CAMKK2. In turn, these kinases enhance TRPV1 channel function by increasing intracellular free calcium levels, thereby establishing a positive feedback loop between TRPV1 and these kinases. However, activation of PKC is found to decrease Na⁺ transport and ENaC expression in amiloride-sensitive epithelial cells in rat lung and alveolar cells^[29-31]. Further studies have also shown that PKC promotes degradation of β - and γ -ENaC subunits and loss of intracellular γ -ENaC^[32]. Collectively, this suggests that the altered activity of TRPV1 and ENaC might be closely related to the activation of the PKC signaling pathway.

3.5 Molecular Affinity of Hydroxy- α -sanshool and Capsaicin with TRPV1 Protein

The calculated binding energy of HS to TRPV1 was -7.5 kcal/mol (Figure 8A, 8B), indicating a favorable binding affinity. Within the ligand-binding pocket of TRPV1, HS formed multiple interactions with several residues. Conventional hydrogen bonds were established with residues SER512 (S512), GLN700 (Q700), ARG557 (R557), GLU570 (E570), and ASP509 (D509), with bond lengths of 4.04 Å, 4.89 Å, 5.80 Å, 4.88 Å, and 4.75 Å, respectively. Additionally, hydrophobic interactions occurred between HS and residues LEU515 (L515) and PHE516 (F516), involving alkyl and π -alkyl interactions with bond lengths of 4.30 Å and 4.43 Å, respectively. Van der Waals interactions were also detected with residues TYR511 (Y511) and TYR554 (Y554), further stabilizing the binding complex. These results suggested that hydroxy- α -sanshool could stably bind to the TRPV1 channel, potentially influencing its activity and modulation.

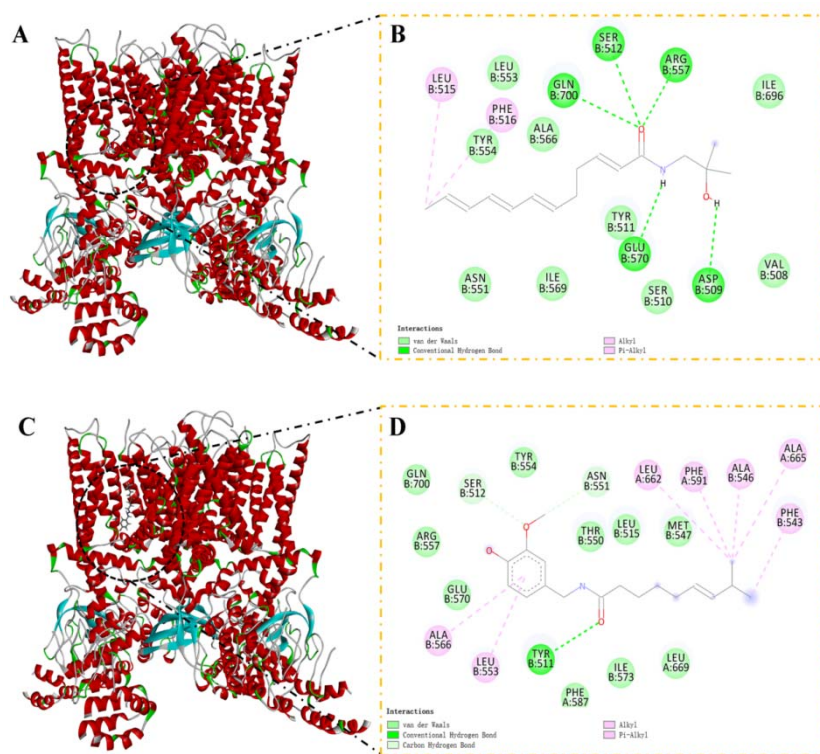


Fig. 8 Schematic diagram of molecular docking between Hydroxy- α -sanshool, Capsaicin, and TRPV1. (A and B) The docking poses and molecular forces with the full-length TRPV1 protein with HS. (C and D) The docking poses and molecular forces of the full-length TRPV1 protein with Cap.

The binding posture of Cap and the TRPV1 protein were shown in (Figure 8C and 8D), derived directly from the reported cryo-electron microscopy structure (PDB ID: 7LPB) of full-length TRPV1 at 25 °C^[33]. The TRPV1 protein binding site residue Y511 (TYR511) formed hydrogen bonds with Cap; residues F543 (PHE543), A546 (ALA546), L553 (LEU553), and others formed hydrophobic alkyl (Alkyl) and hydrophobic π -alkyl (Pi-Alkyl) interactions with Cap; residues L515 (LEU515), M547 (MET547), T550 (THR550), I573 (ILE573), and F587 (PHE587) formed van der Waals interactions with Cap. The hydrogen bond between Cap and Y511 is critical for anchoring Cap within the binding pocket, while the surrounding hydrophobic residues create a favorable non-polar environment that stabilizes the ligand-receptor complex. Furthermore, the extensive van der Waals network suggests that Cap maintains close contact with multiple residues distributed across the binding cavity, potentially restricting ligand mobility and prolonging its residence time on the channel. These findings indicated that capsaicin could stably bind to TRPV1. Comparative analysis revealed that while both HS and Cap engage the same general region of TRPV1, their interaction profiles differ substantially. HS relies more heavily on a diverse array of hydrogen bonds (involving five distinct residues) and hydrophobic contacts, whereas Cap exhibits a stronger dependence on aromatic and alkyl hydrophobic interactions centered around Y511 and F543. Although the binding region of hydroxy- α -sanshool to the TRPV1 protein was close to the active binding region of capsaicin, the binding sites were distinct. When these two ligands interact with the TRPV1 protein, the forces between the different ligands and the protein may influence each other, thereby affecting the binding affinity of the two ligands to the same protein molecule. This interaction may alter channel activity and jointly modulate subsequent signaling changes and other biological processes.

3.6 Validation of Molecular Dynamics Simulations of Hydroxy- α -sanshool with TRPV1 Protein

3.6.1 Stability of the Overall Structure

To explore the structural dynamics of the interaction between free hydroxy- α -sanshool and TRPV1, this study conducted a 180 ns all-atom molecular dynamics (MD) simulation of free hydroxy- α -sanshool and its TRPV1 complex, analyzing key conformational metrics: root mean square deviation (RMSD), radius of gyration (Rg). As shown in Figure 9B, the root mean square deviation (RMSD) of the complex plateaued after approximately 50 ns, with an average value of 0.613 nm and fluctuations confined within 0.365 nm over the final 120 ns of simulation, indicating that the system attained a stable, equilibrated state upon ligand binding. Conformational dynamics analysis indicated that the ligand hydroxy- α -sanshool exhibited relatively high flexibility during the 20 ns–50 ns interval, after which its structural deviations gradually stabilized, following a trend similar to that of the protein–ligand complex (Figure 9A). The radius of gyration (Rg) of the ligand initially increased and began to stabilize after 50 ns (Figure 9C), consistent with the RMSD trend. In contrast, the Rg of the complex gradually decreased and stabilized after 50 ns (Figure 9D), suggesting that the TRPV1 conformation became more compact and stable following hydroxy- α -sanshool binding. Overall, the RMSF and Rg results indicated that the structures of proteins and ligands changed. HS occupied the active pocket and interacted with TRPV1, resulting in traction movement and conformational changes of active residues.

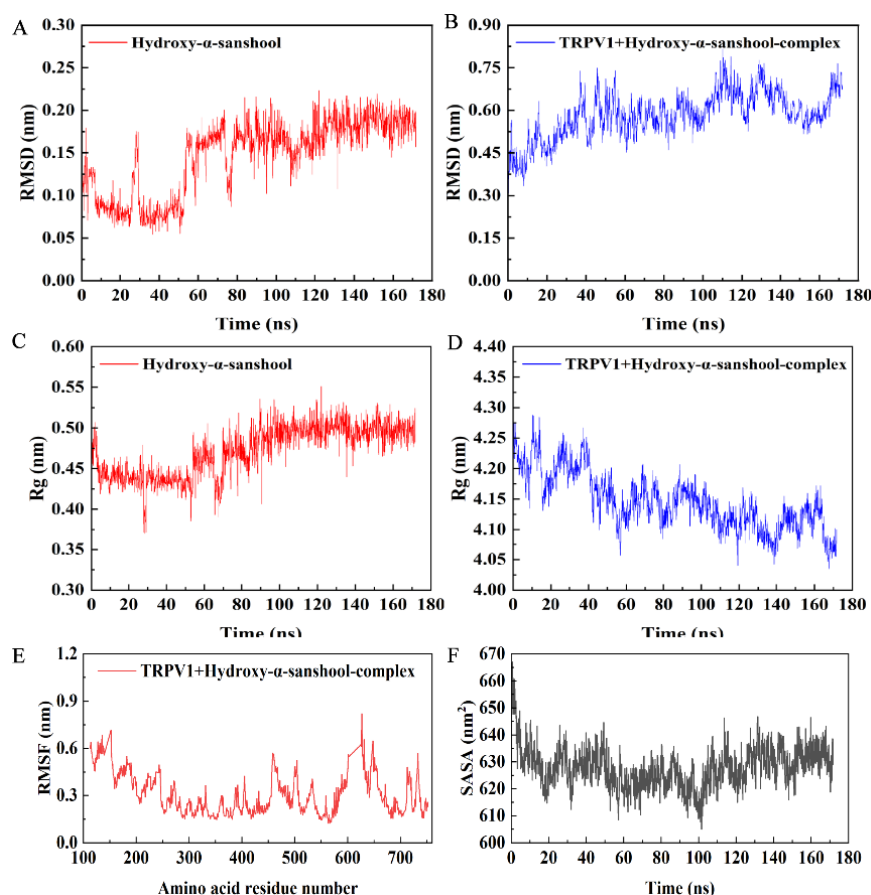


Fig.9 Molecular dynamics simulation analysis of the TRPV1-Hydroxy- α -sanshool complex stability. (A and C) RMSD and Rg of the HS ligand. (B and D) RMSD and Rg of the TRPV1-HS complex over simulation time. (E and F) Root-mean-square fluctuation (RMSF) per amino acid residue and Solvent-accessible surface area (SASA) of the TRPV1-HS complex.

3.6.2 Structural Flexibility

Figure 9E demonstrated that the majority of the higher fluctuations occurred in the C-terminal structural domain. Residues ILE446-PHE543, ASP601-TYR631, etc. exhibited significant fluctuations, indicating that HS binding to TRPV1 enhanced the flexibility of structural domains near these amino acid sites. Furthermore, the key residues involved in the binding of HS and Cap to TRPV1, as identified through molecular docking, were found within the structural domains responsible for the previously mentioned fluctuations. Figure 9F indicated that the average solvent-accessible surface area (SASA) value for the full simulation of the TRPV1-HS complex was $(627.95 \pm 7.58) \text{ nm}^2$. After 120 ns, this average SASA value increased to $(631.06 \pm 5.74) \text{ nm}^2$ and remained stable until the end of the MD trajectory. The results showed that the combination of hydroxy- α -sanshool and TRPV1 expanded the structure of TRPV1. Other ligand small molecules may be easier to enter the TRPV1 protein cavity and interact with its active site. Some small signaling molecules such as Ca^{2+} , Na^+ may enter the cell due to the opening of TRPV1 ion channel caused by the conformational change of the protein and have an impact on subsequent biological activities.

4. Conclusion

This study provides a comprehensive exploration of the physiological mechanisms by which hydroxy- α -sanshool and capsaicin, representative "má là" compounds enhance saltiness perception (Figure 10). We demonstrated that these compounds significantly augmented Ca^{2+} influx through TRPV1 and SCNN1A channels in mouse fungiform taste bud cells. Furthermore, the synergistic action of hydroxy- α -sanshool and capsaicin upregulates the mRNA and protein expression levels of TRPV1 and SCNN1A, concomitant with elevated intracellular PKC α levels in HEK293T cells. Molecular docking and dynamics simulations revealed that hydroxy- α -sanshool and capsaicin bound to similar regions of the TRPV1 protein, with hydroxy- α -sanshool inducing conformational changes that enhanced the flexibility and accessibility of the binding site. Collectively, these results suggest that co-activation of TRPV1 and ENaC channels by má là substances facilitates Ca^{2+} and Na^+ influx, thereby amplifying downstream neural signaling and enhancing saltiness perception. Through cytological and computational approaches, this work elucidates the molecular and cellular mechanisms for pungent substance-mediated salt taste enhancement and outlines potential strategies for reducing dietary sodium intake. However, fully clarifying the regulatory mechanisms underlying salty taste signal transduction requires further investigation. For instance, measuring real-time dynamics of Ca^{2+} and Na^+ in taste cell models could quantitatively assess the modulation of salt perception at the cellular level. Additionally, introducing genetic mutations into salty taste receptor protein molecules could identify key amino acid residues responsible for enhancing salt perception, facilitating the discovery of novel salty taste enhancers with similar properties. These findings have important implications for dietary sodium control and public health.

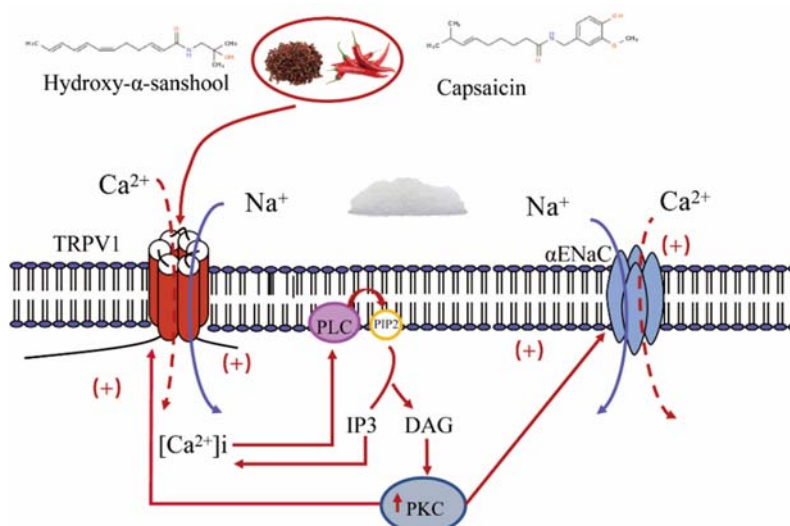


Fig.10 The underlying physiological mechanism of TRPV1-mediated salt taste enhancement by hydroxy- α -sanshool and capsaicin

Acknowledgements

This work was supported by the National Key Research and Development Plan of China (grant no. 2017YFD0400101).

Conflict of interest

The authors declare no competing financial interest.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] L.P. Cohen, J.D. Wessler, M.S. Maurer, S.L. Hummel, Salt Taste Sensitivity and Heart Failure Outcomes Following Heart Failure Hospitalization, *The American Journal of Cardiology*. 127 (2020) 58-63. <https://doi.org/https://doi.org/10.1016/j.amjcard.2020.04.008>.
- [2] G. Faraco, K. Hochrainer, S.G. Segarra, et al., Dietary Salt Promotes Cognitive Impairment through Tau Phosphorylation, *Nature*. 574 (2019) 686-690. <https://doi.org/10.1038/s41586-019-1688-z>.
- [3] F.J. He, M. Tan, Y. Ma, G.A. MacGregor, Salt Reduction to Prevent Hypertension and Cardiovascular Disease: JACC State-of-the-Art Review, *Journal of the American College of Cardiology*. 75 (2020) 632-647. <https://doi.org/https://doi.org/10.1016/j.jacc.2019.11.055>.
- [4] D.B. Hipgrave, S. Chang, X. Li, Y. Wu, Salt and Sodium Intake in China, *The Journal of the American Medical Association*. 315 (2016) 703-705. <https://doi.org/10.1001/jama.2015.15816>.
- [5] J. Powles, S. Fahimi, R. Micha, et al., Global, Regional and National Sodium Intakes in 1990 and 2010: a Systematic Analysis of 24 h Urinary Sodium Excretion and Dietary Surveys Worldwide, *BMJ Open*. 3 (2013) e003733. <https://doi.org/10.1136/bmjopen-2013-003733>.
- [6] J.K. Roebber, S.D. Roper, N. Chaudhari, The Role of the Anion in Salt (NaCl) Detection by Mouse Taste Buds, *The Journal of Neuroscience*. 39 (2019) 6224-6232. <https://doi.org/10.1523/jneurosci.2367-18.2019>.
- [7] Y. Wang, K. Zhong, B. Shi, et al., Cross-modal Effect of Capsaicin and Pepper Oleoresin on the Enhancement of Saltiness Perception in a NaCl Model Solution, *Food Quality and Preference*. 98 (2022) 104542.

- <https://doi.org/https://doi.org/10.1016/j.foodqual.2022.104542>.
- [8] L.L. Zhang, B.L. Shi, P. Sun, et al., The Correlation of Taste and Chemesthetic Sensation in Individuals with Different Suprathreshold Sensitivities, *LWT - Food Science and Technology*. 141 (2021) 111070. <https://doi.org/https://doi.org/10.1016/j.lwt.2021.111070>.
- [9] X.X. Sun, K. Zhong, D. Zhang, et al., Saltiness Enhancement by “má là” Umami Flavor in NaCl Model Aqueous and Oil-added Systems, *Food Research International*. 173 (2023) 113277. <https://doi.org/https://doi.org/10.1016/j.foodres.2023.113277>.
- [10] M.A. Kido, H. Muroya, T. Yamaza, et al., Vanilloid Receptor Expression In the Rat Tongue and Palate, *Journal of Dental Research*. 82 (2003) 393-397. <https://doi.org/10.1177/154405910308200513>.
- [11] N. Takahashi, T. Tsuzuno, S. Mineo, et al., Epithelial TRPV1 channels: Expression, Function, and Pathogenicity In the Oral Cavity, *Journal of Oral Biosciences*. 62 (2020) 235-241. <https://doi.org/https://doi.org/10.1016/j.job.2020.05.005>.
- [12] B. Wang, A. Danjo, H. Kajiya, et al., Oral Epithelial Cells are Activated via TRP Channels, *Journal of Dental Research*. 90 (2011) 163-167. <https://doi.org/doi:10.1177/0022034510385459>.
- [13] A. Bernal, M. Zafra, M. Simón, J. Mahía, Sodium Homeostasis, a Balance Necessary for Life, *Nutrients*. 15 (2023) 395. <https://doi.org/10.3390/nu15020395>.
- [14] C.E. Riera, C. Menozzi-Smarrito, M. Affolter, et al., Compounds from Sichuan and Melegueta Peppers Activate, Covalently and Non-covalently, TRPA1 and TRPV1 Channels, *British Journal of Pharmacology*. 157 (2009) 1398-1409. <https://doi.org/10.1111/j.1476-5381.2009.00307.x>.
- [15] J. Slack. Chapter 21 - Molecular Pharmacology of Chemesthesis. In: Zufall F., S.D. Munger, editors. *Chemosensory Transduction*. Academic Press; 2016, p. 375-391. <https://doi.org/https://doi.org/10.1016/B978-0-12-801694-7.00021-4>.
- [16] E. Sugai, Y. Morimitsu, Y. Iwasaki, et al., Pungent Qualities of Sanshool-Related Compounds Evaluated by a Sensory Test and Activation of Rat TRPV1, *Bioscience, Biotechnology, and Biochemistry*. 69 (2005) 1951-1957. <https://doi.org/10.1271/bbb.69.1951>.
- [17] M. Tsunozaki, R.C. Lennertz, D. Vilceanu, et al., A ‘toothache tree’ Alkylamide Inhibits Aδ Mechanonociceptors to Alleviate Mechanical Pain, *The Journal of Physiology*. 591 (2013) 3325-3340. <https://doi.org/https://doi.org/10.1113/jphysiol.2013.252106>.
- [18] S.T. McDonald, D.A. Bolliet, J.E. Hayes. *Chemesthesis: Chemical Touch in Food and Eating*. John Wiley & Sons; 2016. <https://doi.org/https://doi.org/10.1002/9781118951620.fmatter>.
- [19] J. Han, P. Choi, M. Choi, μ Tongue: A Microfluidics-Based Functional Imaging Platform for the Tongue In Vivo, *Journal of Visualized Experiments*. (2021) e62361. <https://doi.org/doi:10.3791/62361>.
- [20] M. Narukawa, S. Sasaki, T. Watanabe, Effect of Capsaicin on Salt Taste Sensitivity in Humans, *Food Science and Technology Research*. 17 (2011) 167-170. <https://doi.org/10.3136/fstr.17.167>.
- [21] Y. Wang, J. Zou, Y. Jia, et al., The Mechanism of Lavender Essential Oil in the Treatment of Acute Colitis Based on “Quantity–Effect” Weight Coefficient Network Pharmacology, *Frontiers in Pharmacology*. 12 (2021) 644140. <https://doi.org/10.3389/fphar.2021.644140>.
- [22] J. Xu, B.C. Lewandowski, T. Miyazawa, et al., Spilanthal Enhances Sensitivity to Sodium in Mouse Taste Bud Cells, *Chemical Senses*. 44 (2018) 91-103. <https://doi.org/10.1093/chemse/bjy069>.
- [23] L. Chong, *Molecular Cloning - A Laboratory Manual*, 3rd Edition, Science. 292 (2001) 446. <https://doi.org/10.1126/science.1060677>.
- [24] C.X. He, P.P. Wang, Y.Y. Zuo, et al., Constituents-composed Chinese Medicine of Dajianzhong Decoction Ameliorates Non-alcoholic Fatty Liver Disease Via AMPK/PPAR α /CPT1 Signaling, *Phytomedicine*. 149 (2025) 157515. <https://doi.org/10.1016/j.phymed.2025.157515>.
- [25] J.Y. Koo, Y. Jang, H. Cho, et al., Hydroxy- α -sanshool Activates TRPV1 and TRPA1 in Sensory Neurons, *European Journal of Neuroscience*. 26 (2007) 1139-1147. <https://doi.org/10.1111/j.1460-9568.2007.05743.x>.
- [26] A. Saffran. *Activation of TRPV1 by Capsaicin Regulates ENaC*. *Virginia Commonwealth University* Virginia. 2018.
- [27] L. Vyklicky, K. Nováková-Tousová, J. Benedikt, et al., Calcium-Dependent Desensitization of Vanilloid Receptor TRPV1: A Mechanism Possibly Involved in Analgesia Induced by Topical Application of Capsaicin, *Physiological Research*. 57 (2008) S59-S68. <https://doi.org/10.33549/physiolres.931478>.

- [28] L. Li, F. Wang, X. Wei, et al., Transient Receptor Potential Vanilloid 1 Activation by Dietary Capsaicin Promotes Urinary Sodium Excretion by Inhibiting Epithelial Sodium Channel α Subunit-Mediated Sodium Reabsorption, *Hypertension*. 64 (2014) 397. <https://doi.org/10.1161/hypertensionaha.114.03105>.
- [29] P.C. Rosa, V. Pierluigi, F.M. Antonio, et al. Complex Regulation of TRPV1 and Related Thermo-TRPs: Implications for Therapeutic Intervention. In: Islam M.S., editor *Transient Receptor Potential Channels*. Dordrecht: Springer Netherlands; 2011, p. 491-515. https://doi.org/10.1007/978-94-007-0265-3_27.
- [30] G. Bhawe, H.J. Hu, K.S. Glauner, et al., Protein Kinase C Phosphorylation Sensitizes But Does not Activate the Capsaicin Receptor Transient Receptor Potential Vanilloid 1 (TRPV1), *Proc Natl Acad Sci U S A*. 100 (2003) 12480-12485. <https://doi.org/10.1073/pnas.2032100100>.
- [31] *Frontiers in Neuroscience*. In: Liedtke W.B., S. Heller, editors. *TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades*. Boca Raton (FL): CRC Press/Taylor & Francis Copyright © 2007, Taylor & Francis Group, LLC.; 2007.
- [32] G.G. Robins, G.I. Sandle, Liddle-Mutation of the β -Subunit, but not the γ -Subunit, Attenuates Protein Kinase C-Mediated Inhibition of Human Epithelial Sodium Channels (hENaC), *The Journal of Membrane Biology*. 249 (2016) 271-279. <https://doi.org/10.1007/s00232-015-9866-x>.
- [33] D. Kwon, S.Y. Zhang F, Bouvette J, Borgnia MJ, Lee SY, Heat-dependent Opening of TRPV1 in the Presence of Capsaicin, *Nature Structural & Molecular Biology*. 28 (2021) 554-563. <https://doi.org/10.1038/s41594-021-00616-3>.