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Humulus lupulus L.: Evaluation of Phytochemical Profile and Activation of Bitter Taste Receptors to Regulate Appetite and Satiety in Intestinal Secretin Tumor Cell Line (STC-1 Cells)

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Scope: Inflorescences of the female hop plant (*Humulus lupulus* L.) contain biologically active compounds, most of which have a bitter taste. Given the rising global obesity rates, there is much increasing interest in bitter taste receptors (TAS2Rs). Intestinal TAS2Rs can have beneficial effects on obesity when activated by bitter agonists. This study aims to investigate the mechanism of action of a hydroalcoholic hop extract in promoting hormone secretion that reduces the sense of hunger at the intestinal level through the interaction with TAS2Rs.

Methods and results: The results demonstrate that the hop extract is a rich source of bitter compounds (mainly α-, β-acids) that stimulate the secretion of anorexigenic peptides (glucagon-like peptide 1 [GLP-1], cholecystokinin [CCK]) in a calcium-dependent manner while reducing levels of hunger-related hormones like ghrelin. This effect is mediated through interaction with TAS2Rs, particularly *Tas2r138* and *Tas2r120*, and through the activation of downstream signaling cascades. Knockdown of these receptors using siRNA transfection and inhibition of *Trpm5*, *Plcβ-2*, and other calcium channels significantly reduces the hop-induced calcium response as well as GLP-1 and CCK secretion.

Conclusions: This study provides a potential application of *H. lupulus* extract for the formulation of food supplements with satiating activity capable of preventing or combating obesity.

1. Introduction

Humulus lupulus L., commonly known as hop(s), has a very long history and ancient origins. Its flowers have been traditionally used to flavor food due to their bitter properties.[1] *H. lupulus* is also a well-known medicinal remedy, rich in bitter molecules primarily composed of α- and β-acids. Oxidized acids and other compounds present in hop inflorescence extracts or essential oils are reported to promote sleep, relieve mental stress, reduce body fat, and exhibit antimicrobial activity, largely through the secretion of intestinal hormones.[1–3]

Bitter substances are detected by a class of receptors called bitter taste receptors (TAS2Rs), first discovered in the tongue. These receptors are now known to be present in extra-oral tissues such as the gastrointestinal tract, lungs, and heart.[4] Currently, 26 bitter receptors have been identified in humans and 35 in mice. Mouse and human genomes contain orthologous pairs of bitter receptors genes,[5,6] primarily clustered in mouse chromosome 6, with their human counterparts in human chromosome 7 and 12.[7–9] Conditions like overweight and obesity are characterized by excessive fat accumulation due to an imbalance between calorie intake and expenditure. Reducing these conditions lowers the risk of developing diseases such as diabetes and cardiovascular issues. Appetite and food intake are regulated by a balance of anorexigenic and orexigenic signals, largely influenced by hormones, which are stimulated

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by the TAS2Rs. In vivo and some in vitro studies indicate that hop extracts and/or their compounds can suppress food intake by releasing intestinal hormones.[10–12] This study aims to identify novel bitter receptors and hop targets involved in calcium homeostasis and in downstream pathways related to the release of key satiety hormones like cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1). Mouse intestinal neuroendocrine cells (intestinal secretin tumor cell line [STC-1]), which mimic characteristics of L cells in the ileum and I cells in the duodenum, were used for this study.[13]

2. Experimental Section

2.1. Chemicals and Reagents

DMEM, trypsin-EDTA solution, feta bovine serum (FBS), glutamine, penicillin–streptomycin, and phosphate saline buffer (PBS) were purchased from Euroclone (Milan, Italy). Fluo 4-AM and Pluronic F-127 were purchased from ThermoFisher Scientific. Triphenylphosphine oxide (TPPO; Trpm5 inhibitor), U73122 (Plcβ-2 inhibitor), 2-aminoethyl diphenylborinate (2-APB; Ip3r inhibitor), nitrendipine (an L-type voltage-sensitive Ca^{2+} channel blocker), NPS2143 (CaSR antagonist), and A967079 (selective Trpa1 channel blocker) were purchased from Sigma–Aldrich.

2.2. Plant Material

H. lupulus dried female inflorescences were provided by EVRA s.r.L. (Galdo di Lauria, Italy), batch number 20E0076D3101 and extracted by maceration. A 55% hydroalcoholic mixture of ethanol/water (EtOH/H_2O) was used as the solvent and the extraction was conducted for 60 min using a 1:15 plant material:solvent ratio. These conditions were developed with industrial application and human consumption in mind using solvents that are cost-effective and environmentally safe such as water and ethanol. Ethanol, in particular, enhances the extraction of poorly soluble compounds like polyphenols.

2.3. Quantitative Phytochemical Analysis

The quantitative HPLC-DAD analysis of bitter compounds from *H. lupulus* was performed with an HPLC-DAD reversed-phase system (Shimadzu LC- 20AB, Prominence Diode Array Detector, Shimadzu Corporation, Japan), equipped with a binary pump. The column used was a Macherey-Nagel C18 column (4.6 mm × 250 mm × 5 µm). The wavelength was set at 325 nm to monitor α-acids, β-acids, and at 370 for xanthohumol. As mobile phases, solvent A (MeOH:H_2O:H_3PO_4 75:24:1) and solvent B (MeOH), were used with the following gradient: 0–8 min 0% B, 13–15 min 50%, 20–30 min 100% B, 32–42 min 100% B, and 42–50 min 0% B. The flow rate was 1.0 mL min^{-1}, the time analysis was 50 min, and the injected sample was 20 µL. The identification of hop active molecules compounds was achieved based on retention time and the UV-Visible spectra of the corresponding sample compounds. The amount of each molecule was determined considering the peak areas and the linear regression equation achieved from standard calibration curves ranging from 0.20 to 0.005 mg mL^{-1}; correlation coefficients were 0.993 and 0.997 for α-acids (cohumulone and adhumulone + humulone, respectively), 0.998 and 0.999 for β-acids (colupulone and adlupulone + lupulone, respectively), and 0.995 for xanthohumol. Experiments were performed in triplicates, and data were represented in mg g^{-1} dry weight as mean ± standard deviation (±SD).

2.4. LC-MS Detection of Phytochemicals in *Humulus lupulus*

For the phytochemical profile characterization of hop inflorescences, the chromatographic separation of the extract was achieved using a Shimadzu Nexera UPLC system equipped with an Inertsil Phenyl-3 column (150 × 4.6 mm, 5 µm). Mobile phases included water with 0.1 % formic acid (phase A), and methanol with 0.1% formic acid (phase B) and the injection volume was 10 µL. The gradient started with 5% B that was held for 1 min, followed by a 10-min linear gradient from 5% to 30% B. The gradient was then increased linearly from 30% to 100% B, reached at 35 min, and maintained for 15 min. Finally, stepped back to 5% B to equilibrate the column. The column temperature was maintained at 50 °C, the flow rate was 0.4 mL min^{-1}, and the auto-sampler was set at 10 °C. The column effluent was introduced into a QTOF mass spectrometer (AB Sciex Triple TOF 5600) equipped with a TurboSpray electrospray ionization source operated in the negative and positive ionization modes. The instrument was operated in the information-dependent acquisition (IDA) mode using a collision energy setting of 35 V. Compound identification was based on accurate mass, isotopic similarity, retention time, and MS/MS spectral comparison with authentic standards in OSU's library of >500 natural products (Enzo Life Sciences, Farmingdale, NY) and spectral databases (Human Metabolome Database, Massbank of North America, and the Global Natural Products Social Network database).

2.5. Cell Line and Culture Conditions

STC-1 cells were cultured in DMEM and supplemented with 10% FBS, streptomycin (100 µg mL^{-1}), penicillin (100 units mL^{-1}), and 2 mM glutamine and maintained in a humidified atmosphere with 5% CO_2 at 37 °C. *H. lupulus* extract was dissolved in DMSO. DMSO-treated cells (0.8% v/v) were used as control cells in all experiments. All experiments were conducted by treating the cells for 2 h with 100 and 300 µg mL^{-1} of hop extract. STC-1 cell viability was measured by MTT assay after treatment with the extract reporting no cytotoxic effect at the concentrations tested (data not shown).

2.6. GLP-1, CCK, PYY, and Ghrelin Secretion Assay

STC-1 cells (2×10^5 cells per well) were seeded into 12-well plates for 48 h. After treatment, the medium was collected and centrifuged at 12 000 × *g* for 5 min to remove cellular debris. The supernatant was stored at −80 °C until analyses. CCK was measured by CCK EIA kit (Sigma–Aldrich RAB0039),

peptide YY (PYY) was measured by PYY ELISA Kit (Sigma–Aldrich RAB1078), active GLP-1 by GLP-1 ELISA kit (Invitrogen BMS2194) while total ghrelin by Ghrelin EIA Kit (Sigma–Aldrich RAB0207) according to the manufacturer's instruction. For the evaluation of hormonal secretion following the use of blockers and inhibitors, STC-1 cells were seeded in 12-well plates and after 48 h they were pretreated for 30 min with TPPO (400 μM), U73122 (50 μM), 2-APB (50 μM), nitrendipine (1 μM), NPS2143 (30 μM), and A967079 (50 μM). At the end of the pretreatment, cells were treated with hop extract, and GLP-1 and CCK were evaluated as previously described.

2.7. Inhibitory Effect of *H. lupulus* Extract on DPP-IV Activity

STC-1 cells were seeded in black 96-well plates with clear bottoms (1.5×10^4 cells per well). Two days after seeding, cells were treated with 50, 100, and 300 μg mL^{-1} of hop extract or diprotin A (6.125–200 μM) used as a dipeptidyl peptidase-4 (DPP-IV) inhibitor, for 2 h. At the end of treatment, cells were washed with 100 μL of PBS before the addition of the substrate Gly-Pro-AMC (50 μM). Fluorescence ($\lambda_{ex}/\lambda_{em}$ 350/450 nm) was recorded using the GLOMAX Multidetection System (Promega, Madison, WI, USA) every 30 min for up to 90 min.[14]

2.8. cAMP Accumulation Assay

STC-1 cells (2×10^5 cells per well) were seeded into 12-well plates for 48 h. 3-Isobutyl-1-methylxanthine (IBMX, 1 mM) was added to the treatment to prevent cAMP degradation. Forskolin 10 μM was used as a positive control. The supernatant was discarded after 10 min incubation with 0.1 mL of 0.1 M hydrochloric acid at room temperature. Lysate was centrifuged (1200 × *g* for 5 min) and stored at −80 °C before cAMP analysis. A direct cAMP ELISA kit (CA200, Sigma–Aldrich) was used to measure intracellular cAMP levels. Absorbance was read at 405 nm using a microplate reader (Varioskan TM Flash Multimode Reader, ThermoScientific, Waltham, MA, USA).[15]

2.9. Western Blot Analysis

Total protein STC-1 cell extract was quantified by Bradford assay. For each sample, about 40 μg of protein was subjected to 8% SDS-PAGE w/v, and protein bands were transferred to nitrocellulose membranes. For the detection of target proteins, membranes were incubated overnight with specific mouse or rabbit antibodies at 4 °C: anti-*β*-ACTIN (1:5000) and anti-CASR (1:500) from Sigma–Aldrich, Merck, Milan, Italy; anti-ITP3R3 (1:1000), anti-TRPM4 (1:500), anti-Cav1.1 (1:250), anti-Cav1.2 (1:1000), anti-Cav1.3 (1:1000), anti-TAS2R10 (mTas2r105, 1:500), and anti-TAS2R1 (mTas2r119, 1:500) from ThermoFisher Scientific, Milan, Italy; anti-TAS2R38 (1:1000) from Invitrogen; and anti-Plc*β*-2 (1:500), anti-TRPA1 (1:500), anti-TRPM5 (1:500), and anti-TAS2R7 (mTas2r130, 1:500) from Antibodies. After incubation with suitable secondary antibodies, the bands were visualized with chemiluminescence reagents by Chemidoc TM XRS detection system. For image acquisition, Image Lab Software was used (Bio-Rad, Hercules, CA, USA). The protein levels were quantified using Image J software and normalized with *β*-actin content.

2.10. Quantitative RT-PCR

Considering that TAS2Rs were involved in the release of intestinal hormones with satiating activity, qRT-PCR was used to measure the expression profile of key genes involved in the mechanism of transduction of bitter taste in intestinal cells. The choice of the bitter receptor isoforms considered (*Tas2r105*, *Tas2r119*, *Tas2r130*, *Tas2r120*, and *Tas2r138*) was made based on the literature including also the previous study.[16–18]

After treatment, RNA was isolated by using a specific kit (Qiagen, Hilden, Germany) and stored at −80 °C until further analysis. The concentration and purity of the RNA was measured using a Nanodrop Lite Spectrophotometer (Thermo Scientific). All PCR primers were designed toward available *Mus musculus* sequences from the NCBI Genebank Sequences databases using the Primer-BLAST software and purchased from Integrated DNA Technologies (IDT). Primer sequences are listed in **Table 1**. For real-time quantitative polymerase chain reaction, RNA samples were reverse transcribed using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Waltham, MA, USA). The cDNA amplification was performed by real-time PCR using iTAQTM Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA) by the StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA). To ensure the quality of the amplification product, a melt curve analysis was performed as a final step. Samples were tested in triplicates and the gene expression was normalized to levels of polymerase-II. The comparative threshold cycle method (ΔΔCt) was used to quantify the relative amounts of product transcripts.

2.11. Intracellular Calcium Measurement

STC-1 cells (1.5×10^4 cells per well) were seeded in 96-well black plates, and after 48 h, cell permeant Ca^{2+} sensitive fluorescent dye Fluo-4-AM (5 μM) was loaded together with pluronic F-127 into the cells for 30 min at 37 °C using PBS. Fluorescence measurements were performed using a microplate reader (Varioskan TM Flash Multimode Reader, Thermoscientific, Waltham, MA, USA; $\lambda_{ex.}$ 490 nm, $\lambda_{em.}$ 516 nm, Invitrogen).[15] Ionomycin 1 μg mL^{-1} was used as a positive control. The baseline was recorded before the injection of the bitter extract. Fluorescence was recorded every 37 s for 5 min. Cells were treated for 30 min with specific substances (blockers and inhibitors), the fluorescent probe Fluo-4-AM was added, and data recording was carried out as described in section 2.11.

2.12. RNA Interference of Ta2r138 and Tas2r120

Mouse *Tas2r138* and *Tas2r120* were transiently knocked down in STC-1 cells by small interfering RNA (siRNA) (ThermoFisher Scientific). A nontargeting siRNA was used as a negative control (Silencer™ Select Negative siRNA 4390843, TermoFisher Scientific). The transfection of STC-1 cells was performed according to the manufacturer's instructions for the Lipofectamine RNAiMAX Transfection Reagent. The efficiency of the transfection was evaluated by qRT-PCR. Forty-eight hours after the transfection, cells were treated with hop extract, and the supernatant was collected for the GLP-1 and CCK analysis.

Table 1. Primer sequences for real-time PCR.

Gene	Accession number	Primer sequence
Pol II	NM_027231	F: 5′-GAC AAC GAG GAC AAT TTC GAC G-3′ R: 5′-GGA GAA TCT CGA CAT TTT CCT GG-3′
Trpm4	NM_175130	F: 5′-CCT TCT GTG GCT GTG GAG GAT G-3′ R: 5′-CAA GAG CGG GTA ACG AGA CTG TA-3′
Trpm5	NM_020277	F: 5′-GTC ACC TGT AGA ATG GTG CCC T-3′ R: 5′-ATGTGGATGAGCCGA AGT GTG A-3′
Trpa1	NM_177781	F: 5′-GCA AAG AAG TGA TCC AAA TTT TCC A-3′ R: 5′-GCT CCA CAT TGC CAC TGC AT-3′
Plcβ-2	NM_177568	F: 5′-TCA AAA TCA GAG CCA GAA ACT CCG-3′ R: 5′-AAG GTG AGG TCC ACC ATG TCA G-3′
Ip3r3	NM_080553.3	F: 5′-GAG CAC ACT TTG AAG AGG CAA T-3′ R: 5′-GGG CAC ACT TTG AAG AGG CAA T-3′
Tas2r105	NM_020501	F: 5′-AGG ATT TTT ATT GTG TGG C-3′ R: 5′-AGA AGA TGC TTA GGC TGG T-3′
Tas2r119	NM_020503	F: 5′-ATG AGG ACT ATG GTG GGA-3′ R: 5′-CGA GTG TAA GGA GGG GTA-3′
Tas2r130	NM_199156	F: 5′-TCC TGG CCC TGT TTG TTG TCT-3′ R: 5′-GCA CCC TCA CAG ATG CTT GTT-3′
Tas2r138	NM_001001451	F: 5′-AAC GCC TTC ATT GTC TTG-3′ R: 5′-CTC AGT GGG TCT TTC ATC-3′
Tas2r120	NM_207023.1	F: 5′-AACCAACCATTTTACCAC-3′ R: 5′-AAACAGAGACGATCCCAG-3′
Casr	NM_013803.3	F: 5′-TGG CAC AGT TGC CTT GTG AT-3′ R: 5′-CAT CCC AGG CTT CCG ATC CT-3′
Cav1.1	NM_014193.4	F: 5′-GCT GGA GCT CTT CAA AGG CAA G-3′ R: 5′-AGC ACA GGG TGA TGG TTT CTC A-3′
Cav1.2	NM_009781.4	F: 5′-AGT GAC AGG GCC ACA TGA GA-3′ R: 5′-GGG GTG TGG ATG GCT GAA CA-3′
Cav1.3	NM_028981.3	F: 5′-GCC CGA TAT AAA GGC AGA GTT GT-3′ R: 5′-CCT TGG CCT GTC TAG CAG CA-3′
Cav1.4	NM_019582.2	F: 5′-ACA TGG TCT TCA CTG GCC TCT T-3′ R: 5′-CGC CAA GAT GGC CTC CGT T-3′
Pyy	NM_145435.1	F: 5′-TGCCTCTCCCTGTT TCTCGTATCC-3′ R: 5′-AAGTCCACCTGTGT TCTCCTCCTC-3′
Pde1A	NM_001009979.2	F: 5′-CACTGGCTCACTGA ACTGGA-3′ R: 5′-ATTCTCAAGCACTGA GCGGT-3′

2.13. Statistical Analysis

Data were expressed as mean ± SD. Statistical analysis was performed using GraphPad Prism 8 Software. Inc. (San Diego. CA. USA) and p values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Phytochemical Analysis

HPLC-DAD allowed identifying α-acids (cohumulone, adhumulone, and humulone), β-acids (colupulone, adlupulone, and lupulone), and xanthohumol at 325 nm and quantifying them based on the respective standard (**Figure 1**). The quantitative analysis reported xanthohumol (peak **1**) 0.46 ± 0.07 mg g^{-1}, total α-acid content (peaks **2**, **3**) 8.18 ± 0.30 mg g^{-1}, and total β-acid (peaks **4**, **5**) 9.14 ± 0.56 mg g^{-1}. Phytochemicals of hops were detected by LC-MS through PeakView software, XIC manager, using a list of 164 compounds created based on comparison with literature reports[19–24] and the KNApSAcK database. More precisely, the methods of identification of the compounds fall within level 1 by using a reference standard or level 2 by using a comparison with data present in the literature or specific databases.[25] The peak peaking parameters were $S/N \geq 10$ and intensity ≥ 100 count. Hop phytochemicals include a wide and heterogeneous range of specialized metabolites with different structures and bioactivities. In addition to the bitter acids mentioned above, posthumulone, postlupulone, cohumulinone, hulupinic acid, and cohulupone were also identified. Different polyphenols were also found in the extract, including the flavonols kaempferol, quercetin, and their conjugates; among the prenylflavonoids 8-prenylnaringenin, 6-prenylnaringenin, isoxanthohumol, and xanthohumol were identified (**Table 2**, **Figure 2**).

3.2. Secretion of Gut Hormones

Carbohydrate, fat, and protein in the lumen of the gut stimulate the secretion of the incretin hormone GLP-1. GLP-1 secretion was significantly increased after treatment with hop extract compared to untreated cells (**Figure 3a**). Both concentrations increased GLP-1 secretion compared to control cells (300, 100 µg mL^{-1}: 16.77 ± 0.76, 15.03 ± 1.37 pg mL^{-1}, respectively, versus CTRL: 8.66 ± 0.55 pg mL^{-1}). Oleic acid, used as a positive standard, was able to stimulate the secretion of GLP-1 from enteroendocrine L cells. CCK levels were also quantified; CCK secretion was significantly increased after treatment with hop extract compared to untreated cells (Figure 3b). Both tested concentrations (100 and 300 µg mL^{-1}) resulted in doubling the secretion of CCK (8.92 ± 0.85 and 9.33 ± 0.90 pg mL^{-1} respectively) compared to the control (4.64 ± 0.40 pg mL^{-1}). PYY was not measured by ELISA probably because of its low levels in STC-1 cells[26]; therefore, PYY gene expression was evaluated by qRT-PCR. The extract favored the expression of PYY gene (Figure 3c). Furthermore, hop extract reduced the hunger hormone, ghrelin, in a dose-dependent manner: the highest concentration halved the amount of ghrelin compared to control cells (3.65 ± 0.30 ng mL^{-1} versus 6.61 ± 0.53 ng mL^{-1} of control cells).

3.3. Hop Extract Inhibition of DPP-IV Activity in Intestinal STC-1 Cells

After its release, GLP-1 is rapidly degraded by a specific enzyme, DPP-IV, and therefore its use for therapeutic purposes is not practicable. The inhibition of DPP-IV enzyme was investigated in STC-1 cells by a fluorescent assay. Diprotin, a DPP-IV inhibitor, was used as a positive control because inhibited the activity of DPP-IV on the Gly-Pro-AMC substrate in a time- and dose-dependent manner (**Figure 4a**). The increase of the fluorescence corresponding to DPP-IV activity was reduced by 20% in the presence of hop extract (300 µg mL^{-1}) compared to the control (CTRL) demonstrating the best activity after 60 and 90 min (Figure 4b).

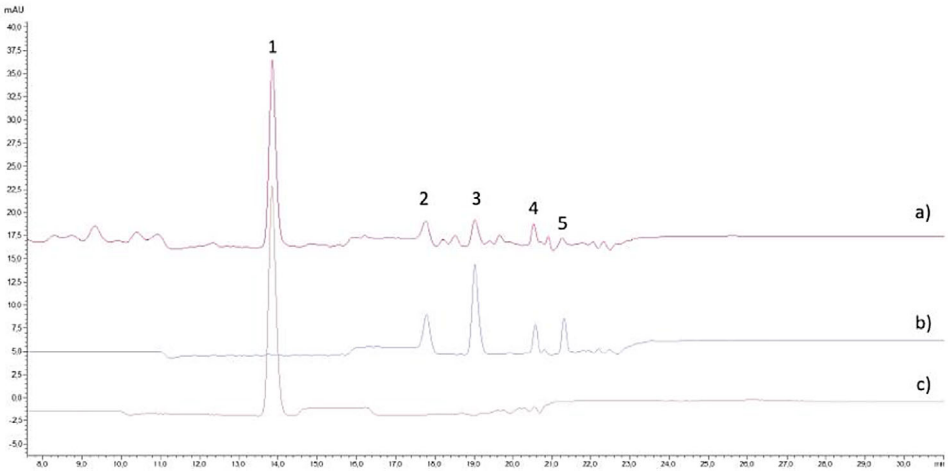

Figure 1. Representation of a) *H. lupulus* inflorescence, b) ICE-4, and c) xanthohumol chromatogram at 325 nm. 1) Xanthohumol (*Rt*: 13.78 min), 2) cohumulone (*Rt*: 17.74 min), 3) adhumulone + humulone (*Rt*: 18.99 min), 4) colupulone (*Rt*: 20.53 min), and 5) adlupulone+lupulone (*Rt*: 21.25 min).

3.4. Bitter Receptor Gene Expression Profiling

Since both concentrations of hop produced similar effects on hormone secretion, gene expression was evaluated using 100 µg mL^{-1}. Bitter receptors *Tas2r105, Tas2r119, Tas2r120, Tas2r130,* and *Tas2r138* exhibited significant changes in expression after stimulation with hop extract, indicating potential involvement of the β and γ subunits of TAS2Rs. Additionally, the enzyme *Plcβ2* and the potential transient cation channel *Trpm5* are key effectors involved in taste signaling transduction. Both exhibited increased expression levels together with *Ip3r3, Trpm4,* and *Trpa1* (**Figure 5**). Hop treatment also increased the expression of phosphodiesterase 1A (*Pde1A*) (Figure 5), possibly due to the rise in the intracellular levels of the G protein α subunit, α-gustducin.[27] Hop extracts resulted in increased expression levels of all tested receptors and ion channels. For all upregulated targets, protein levels were evaluated by western blot that confirmed the qRT-PCR data (**Figure 6**).

3.5. Hop Extract Effects in cAMP Levels and $[Ca^{2+}]_i$ in STC-1 Cells

Based on the previous results, it is possible that hop bitter extract increased gut hormones secretion through the activation of TAS2Rs. This effect involves activation of the α-subunit through the increase of the phosphodiesterase-mediated cAMP levels. Hop extract dose-dependently reduced cAMP levels in comparison with untreated cells (CTRL). In contrast, forskolin, a known positive control that activates adenyl cyclase and increases intracellular levels of cAMP, resulted in increasing cAMP levels (**Figure 7**).

In addition, hormone secretion involves the important second messenger calcium. To investigate whether bitter hops activate Tas2rs and induce a robust Ca^{2+} increase in enteroendocrine cells, the cell permeant Ca^{2+} sensitive dye Fluo-4-AM was used. Intracellular calcium concentrations ($[Ca^{2+}]_i$) rapidly increased in the STC-1 cells in response to different concentrations of hop extract (**Figure 8**). The fluorescence intensity of the highest concentration increased by 2.3-fold compared to the baseline.

3.6. Hop Extract Elicits Rapid Increase in $[Ca^{2+}]_i$ Through Ca^{2+} Influx

To determine whether the increase in the intracellular calcium is mediated by extracellular sources, additional targets involved in permeability pathways were identified by qRT-PCR. Treatment with hops (100 µg mL^{-1}) significantly increased the expression levels of three isoforms of the L-type voltage-gated calcium channels subunits α-1: *Cav1.1*, *Cav1.2*, and *Cav1.4*, which are activated by membrane depolarization. Additionally, expression levels of the calcium-sensing receptor (*Casr*), which is directly activated by elevated extracellular Ca^{2+}, were also increased (**Figure 9**). Protein content levels also increased following hop extract treatment, especially at 300 µg mL^{-1} (**Figure 10**).

3.7. Hops-Induced ($[Ca^{2+}]_i$) Increase Is Mediated by the GPCR CaSR, Trpa1, L-Type-Voltage Sensitive Calcium Channels and Bitter Transduction Mechanism

The results obtained suggest involvement of the G protein-coupled receptor CaSR and L-type VSSCs. Specific inhibitors were used to better understand the mechanism. Pretreatment of STC-1 cells for 30' with NPS214, a CaSR antagonist or nitrendipine, an L-type VSCCs antagonist, prevented $([Ca^{2+}])_i$ increase induced by hop extract indicating their importance in a hop-induced increase in intracellular calcium. Hop extract mediated $([Ca^{2+}])_i$ increase was also suppressed by A967079, a Trpa1 antagonist (**Figure 11a**). These results show that CaSR, L-type VSSCs, and Trpa1 act as mediators of hop-activated intracellular calcium increase. Activation of bitter receptors stimulates Plcβ2, which in turn stimulates the production of the second messenger IP_3 which results in increased levels of intracellular Ca^{2+} and consequently of Trpm5. The results obtained by RT-qPCR and western blot showed an increased expression of these markers. To evaluate their actual involvement in the hop-mediated increase in intracellular calcium, selective antagonists were used. Cells were pretreated with TPPO, U73122, 2-APB (Trpm5, Plcβ-2, and Ip3r3 antagonists, respectively). The increase of calcium levels by hop

Table 2. Phytochemicals detected in *Humulus lupulus* L. inflorescences extract.

Name	Molecular formula	MW	Adduct	Exact mass (*m/z*)	Retention time (min)
3-*p*-Coumaroylquinic acid [1]	$C_{16}H_{18}O_8$	338.10017	$[M-H]^-$	337.09289	17.42
Kaempferol 3,7,4′-O-triglucoside[2]	$C_{33}H_{40}O_{21}$	772.20621	$[M-H]^-$	771.19893	17.7
Dihydroferulic acid 4-O-glucuronide[3]	$C_{16}H_{20}O_{10}$	372.10565	$[M-H]^-$	371.09837	17.79
Myricetin 3-O-glucoside [4]	$C_{21}H_{20}O_{13}$	480.09039	$[M-H]^-$	479.08311	18.54
Manghaslin[5]	$C_{33}H_{40}O_{20}$	756.21129	$[M-H]^-$	755.20402	18.55
3-Feruloylquinic acid[6]	$C_{17}H_{20}O_9$	368.11073	$[M-H]^-$	367.10346	18.71
Kaempferol 3-O-(2,6-di-O-alpha-L-rhamnopyranosyl) –*b*-D-galactopyranoside)[7]	$C_{33}H_{40}O_{19}$	740.21638	$[M-H]^-$	739.2091	19.21
Rutin [8]	$C_{27}H_{30}O_{16}$	610.15339	$[M-H]^-$	609.14611	19.36
Quercetin-3-O-glucoside [9]	$C_{21}H_{20}O_{12}$	464.09548	$[M-H]^-$	463.0882	19.64
Quercetin 3-O-(6″-malonyl-glucoside)[10]	$C_{24}H_{22}O_{15}$	550.09587	$[M-H]^-$	549.08859	19.96
Kaempferol-3-O-rutinoside [11]	$C_{27}H_{30}O_{15}$	594.15847	$[M-H]^-$	593.15119	20.18
Kaempferol-3-O-glucoside[12]	$C_{21}H_{20}O_{11}$	448.10056	$[M-H]^-$	447.09329	20.42
Cohumulinone[13]	$C_{20}H_{28}O_6$	364.18859	$[M-H]^-$	363.18131	21.4
Quercetin[14]	$C_{15}H_{10}O_7$	302.04265	$[M-H]^-$	301.03538	21.51
Posthumulone[15]	$C_{19}H_{26}O_5$	334.17802	$[M-H]^-$	333.17075	22.04
Kaempferol[16]	$C_{15}H_{10}O_6$	286.04774	$[M-H]^-$	285.04046	22.38
Dehydrocycloxanthohumol hydrate (Xanthohumol B)[17]	$C_{21}H_{22}O_6$	370.14164	$[M-H]^-$	369.13436	22.85
Hulupinic acid[18]	$C_{15}H_{20}O_4$	264.13616	$[M-H]^-$	263.12888	23.41
Cohulupone[19]	$C_{19}H_{26}O_4$	318.18311	$[M-H]^-$	317.17583	23.9
Isoxanthohumol[20]	$C_{21}H_{22}O_5$	354.14672	$[M-H]^-$	353.13945	24.31
8-Prenylnaringenin[21]	$C_{20}H_{20}O_5$	340.13107	$[M-H]^-$	339.1238	24.76
6-Prenylnaringenin[22]	$C_{20}H_{20}O_5$	340.13107	$[M-H]^-$	339.1238	24.98
Xanthohumol D[23]	$C_{21}H_{22}O_6$	370.14164	$[M-H]^-$	369.13436	25.13
Xanthohumol[24]	$C_{21}H_{22}O_5$	354.14672	$[M-H]^-$	353.13945	25.27
Isocohumulone [25]	$C_{20}H_{28}O_5$	348.19367	$[M-H]^-$	347.1864	25.29
Adhumulone[a] [26]	$C_{21}H_{30}O_5$	362.20932	$[M-H]^-$	361.20205	25.53
Cohumulone[a] [27]	$C_{20}H_{28}O_5$	348.1937	$[M-H]^-$	347.1864	25.79
Palmitic acid[28]	$C_{16}H_{32}O_2$	256.24023	$[M-H]^-$	255.23295	25.95
Humulone[a] [29]	$C_{21}H_{30}O_5$	362.20932	$[M-H]^-$	361.20205	26.03
Postlupulone[30]	$C_{24}H_{34}O_4$	386.2457	$[M-H]^-$	385.23843	26.38
Colupulone[a] [31]	$C_{25}H_{36}O_4$	400.2614	$[M-H]^-$	399.25408	26.46
Lupulone[a] [32]	$C_{26}H_{38}O_4$	414.277	$[M-H]^-$	413.26973	26.72
Adlupulone[a] [33]	$C_{26}H_{38}O_4$	414.277	$[M-H]^-$	413.26973	26.72
Adprehumulone[34]	$C_{27}H_{40}O_4$	428.2927	$[M-H]^-$	427.28538	26.94

[a] Phytochemicals detected by using specific standards.

extract (L300 µg mL^{-1}) was inhibited or reduced in the case of pre-treatments. Particularly, 2-APB abolished the effect suggesting a major role of Ip3r3 in hop-induced intracellular calcium increase (Figure 11b).

3.8. Hop-Induced GLP-1 and CCK Production in STC-1 Cells Is Dependent on Bitter Transduction Mechanism and Intracellular Calcium Increase

Furthermore, we evaluated the contribution of the bitter taste signaling components described above on hormone secretion induced by hop extract using specific inhibitors and antagonists of the main downstream component of hop bitter transduction mechanism and of calcium channels. U73122, TPPO, 2-APB, A967079, nitrendipine, and NPS2143 were used to block *Plcβ-2*, *Trpm5*, and *Ip3r3*, *Trpa1*, L-type calcium channels, and *Casr*, respectively. Pretreatment for 30 min of STC-1 cells with the inhibitors significantly reduced hop-mediated GLP-1 and CCK production (**Figure** 12a). To further confirm that *Tas2r138* and *Tas2r120* were involved in hop-induced GLP-1 and CCK secretion, gene silencing was performed, and hormone levels were measured by ELISA. Hop treatment of STC-1 cells transfected with non-targeting siRNA dose-dependently induced GLP-1 and CCK secretion; this effect was significantly attenuated in cells transfected with *Tas2r138* and *Tas2r120* siRNA (Figure 12b). These data demonstrated the strong involvement of these two receptors in the mechanism of action of hops. Intracellular

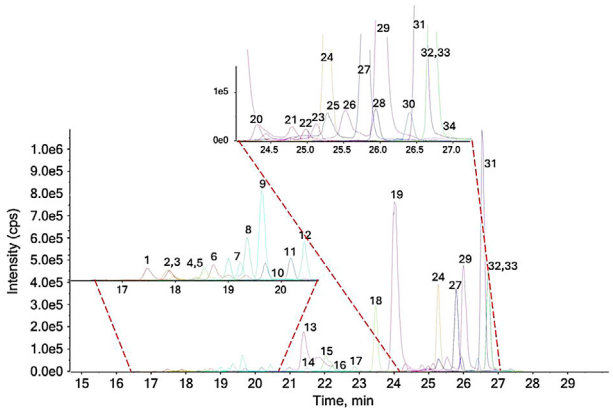

Figure 2. Extracted ion chromatogram of *H. lupulus* extract (negative ion mode). For key to peaks, see Table 2.

calcium concentration was also reduced in cells knockdown for the *Tas2r138* and *Tas2r120* genes compared to those transfected with nontargeting siRNA further confirming these observations (Figure 12c).

4. Discussion

Bitterness, one of the five tastes, has always been associated with toxic substances and therefore with danger; however, the activation of bitter receptors provokes several protective physiological responses.[28] Taste receptors have also been found in the gastrointestinal tract where upon stimulation with bitter agonists, they elicit the secretion of gut hormones such as PYY, CCK, or GLP-1 which provoke an aversive response to food, decrease in food intake, and delay in gastric emptying speed.[29] Hops are very well known for their use in the brewing process due to their bitter taste, antimicrobial properties, and bioactivity due to strobiles, the cone-shaped female structures.[29] Hop resins consist of bitter acids including α-acids, which are responsible for the foam stability and bitterness of the beer while β-acids are less stable and can be destroyed during beer brewing processes.[30,31] Despite their properties as flavoring agents and food additives, these compounds are involved in the maintenance of glucose homeostasis, reduction of body weight and fat mass in both rodent and human studies.[32,33] In this study, the hydroalcoholic extract of hop inflorescences was analyzed for its phytochemical profile and, in addition to the bitter ones, numerous other compounds belonging to different classes were also identified. Most of them possess important bioactivities and all together contribute to the health benefit effects of hops. The differences in the profile found in our previous study demonstrate how the use of different extraction methods and conditions influences the phytochemical composition of the extract.[34]

The murine enteroendocrine STC-1 cells were used as a cell model to better understand the regulation of gut hormones release. The evaluation of gene expression by qRT-PCR highlighted the activation of bitter receptors *Tas2r105, Tas2r119,* and *Tas2r130* by hops confirming what was previously reported.[12] The database BitterDB, which predicts bitterness of unknown compounds or ligands for bitter receptors, reported *Tas2r105* as a target of humulone (https://bitterdb.agri.huji.ac.il/dbbitter.php#main). However, for the first time was also established the involvement of *Tas2r120* and *Tas2r138* in the bitter taste transduction mechanism of hops. Among the compounds potentially involved in these effects are xanthohumol or hop bitter β-acids (lupulone, adlupulone, and colupulone) as we have previ-

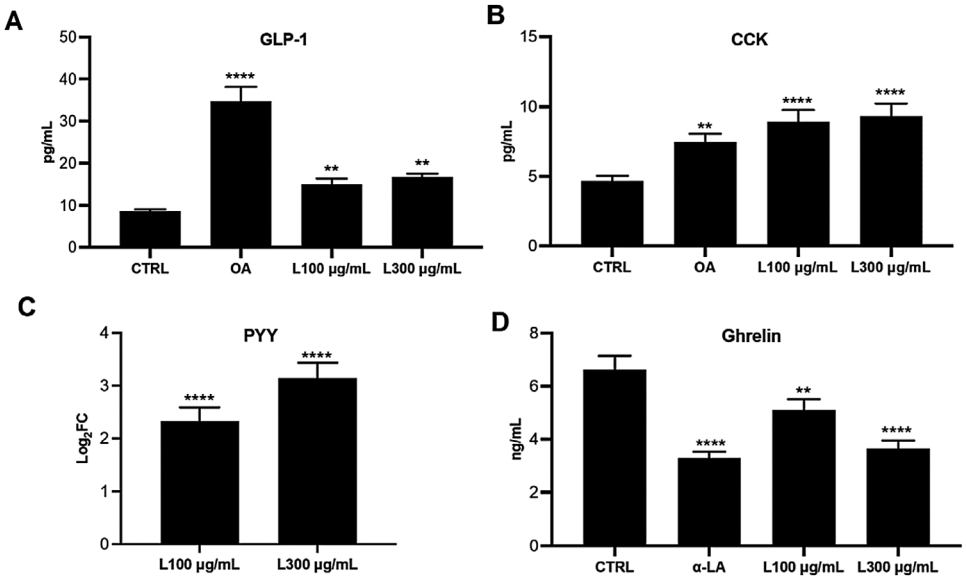

Figure 3. *H. lupulus* inflorescences extract effect on GLP-1 A), CCK B), PYY C), and ghrelin D) secretion or gene expression. Hop extract and the positive control oleic acid (OA; 0.3 mM) and α-linoleic acid (α-LA; 10 µM), significantly increased the secretion of gut hormones after 120' in STC-1 cell medium. Hop extract statistically increased PYY gene expression too. Data are displayed as the mean ± standard deviation (SD) of three independent experiments ($n = 3$) and were analyzed by one-way ANOVA followed by Tukey's post-hoc test; ****$p < 0.0001$, ***$p < 0.001$ **$p<0.01$ versus untreated cells (CTRL). PYY results are normalized with the housekeeping gene polymerase II (Pol II) and are shown as Log_2 fold change treated/control ± SD. ANOVA, analysis of variance; CCK, cholecystokinin; GLP-1, glucagon-like peptide 1; PYY, peptide YY, STC-1, intestinal secretin tsumor cell line.

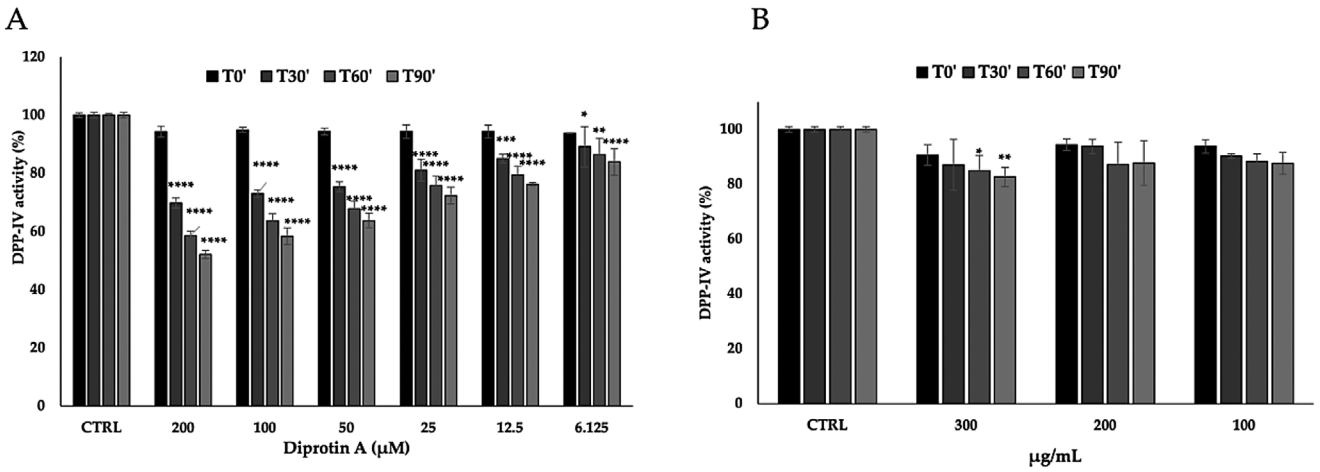

Figure 4. Inhibitory activity on DPP-IV enzyme measured in STC-1 cells: diprotin A inhibited DPP-IV activity dose-and time-dependently A); hop extract inhibited DPP-IV activity dose-and time-dependently B). Data are reported as the mean ± standard deviation (SD) of three independent experiments ($n = 3$) and were analyzed by one-way ANOVA followed by Tukey's post-hoc test; ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$ versus control cells (CTRL). ANOVA, analysis of variance; DPP-IV, dipeptidyl peptidase-4; STC-1, intestinal secretin tumor cell line.

ously shown.[18] After the link of specific agonists, bitter receptors interact with α-gustducin. In taste cells, this mechanism stimulates phosphodiesterase, which subsequently reduces intracellular cAMP levels. The released Gβ-3 and Gγ-13 subunits activate phospholipase-β2, a component of the signaling cascade of the sweet and bitter taste, which in turn catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol-1,4,5-trisphosphate (IP_3). Binding of the generated IP_3 to its receptor induces calcium release from the endoplasmic vesicles which acts on *Trpm5* and induces Na^+ influx and membrane depolarization.[35,36] Less known is the involvement of *Trpm4* in the transduction of taste. Loss of *Trpm4* in mice causes a reduction in taste transduction, and loss of both *Trpm4* and *Trpm5* suppresses the stimuli of taste sensation.[37]

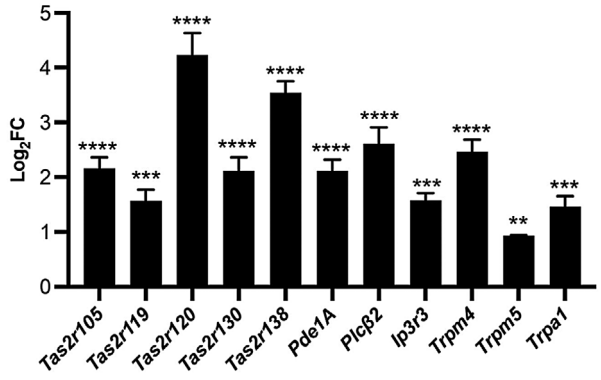

Figure 5. *H. lupulus* inflorescences extract effect on gene expression. Hop extract significantly induces the expression of the bitter receptors *Tas2r105*, *Tas2r119*, *Tas2r120*, *Tas2r130*, and *Tas2r138* and the transient receptor potential cation channel subfamily A (Trpa1) or M (Trpm4 and Trpm5), in STC-1 cells treated for 120 min. Expression of phospholipase C (*Plcβ2*), inositol 1,4,5-trisphosphate receptor, type 3 (*Ip3r3*), and phosphodiesterase 1A (*Pde1A*) was also increased. Data are normalized with the housekeeping gene polymerase II (*Pol II*) and are shown as Log_2 fold change treated/control ± standard deviation (SD), of three independent experiments ($n = 3$) and were analyzed by one-way ANOVA followed by Tukey's post-hoc test; **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$ versus control cells. ANOVA, analysis of variance; STC-1, intestinal secretin tumor cell line.

The transient receptor potential ankyrin1 (*Trpa1*) encodes for a nonselective cation channel highly permeable to sodium, potassium, and calcium.[38] *Trpa1* is expressed in neuronal cells where it responds to cold, mechanical, and chemical stressors. It is also expressed in nonneuronal cell types like gastrointestinal mucosa of colon and small intestine. Its activation is related to mediate pain and inflammatory response[39,40] and it triggers the release of Ca^{2+} from the endoplasmic reticulum.[41] Human TRPA1 is activated by eudesmol, an oxygenated-sesquiterpenoid of hops.[42] The downstream product of the bitter cascade is represented by gut anorexigenic hormones secreted from the vesicles.

GLP-1 not only significantly improves insulin secretion and glucose control but also decreases gastric emptying and increases satiety by acting on the brain with consequently benefits in terms of weight loss.[43] To support our findings, a previous randomized, crossover clinical trial conducted on healthy humans, observed an increased secretion of GLP-1 after gastric and duodenal treatment with 250 mg of supercritical CO_2 hop extract.[44] In addition, a pure analogue of hop-derived isohumulones, KDT501, was found to increase GLP-1 secretion in diet induced obese mice and in STC-1 cells through the TAS2Rs Tas2r108.[29] However, GLP-1 has a short half-life as it is rapidly degraded in the blood plasma by the enzyme DPP-IV. DPP-IV (also called cluster of differentiation 26 [CD26]) is a transmembrane glycoprotein that cleaves X-proline dipeptides from polypeptides including hormones at their N-termini.[45] DPP-IV inhibitors have been recently classified as antidiabetic agents because of their effect in reducing plasma glucose by increasing GLP-1 effects. Many natural substances have inhibitory effects against DDP-IV such as polyphenols and particularly rutin. They act by potentially binding to the active site of the enzyme and preventing the interaction with its substrate.[46] Furthermore, a derivative of hop tetrahydro iso-α-acids in combination with a DPP-IV inhibitor was

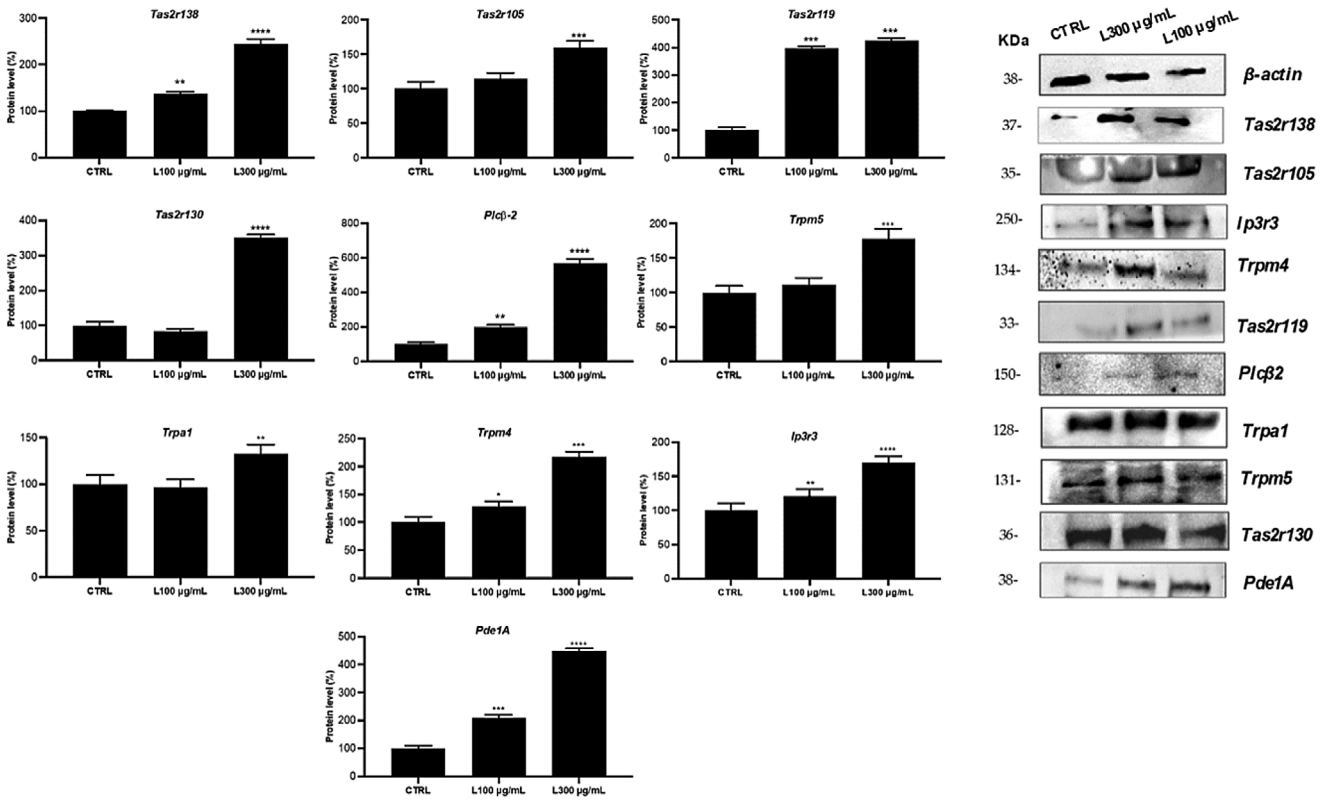

Figure 6. Effect of hop treatment (L300 and L100 µg mL^{-1}) on protein expression. Cell lysate was subjected to western blot analysis and protein content was normalized against β-actin. Immunoreactive bands are from a single experiment representative of multiple experiments and results from densitometric analysis are expressed as the mean ± standard deviation (SD) of three independent experiments ($n = 3$) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test; ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$ versus control cells (CTRL).

previously shown to amplify incretin secretion, suggesting a possible involvement of bitter compounds in this mechanism exerted by the extract of hop.[47]

Two previous studies have reported a strong correlation between the human TAS2R38, which shares 65.3% of identity with the mouse *Tas2r138* and GLP-1 secretion.[48,49] In fact, the bitter compound phenylthiocarbamide (PTC), by acting on that receptor, can stimulate the secretion of GLP-1.[50] Moreover, siRNA knockdown of TAS2R38 reduced GLP-1 secretion by another natural compound called berberine.[42] The most

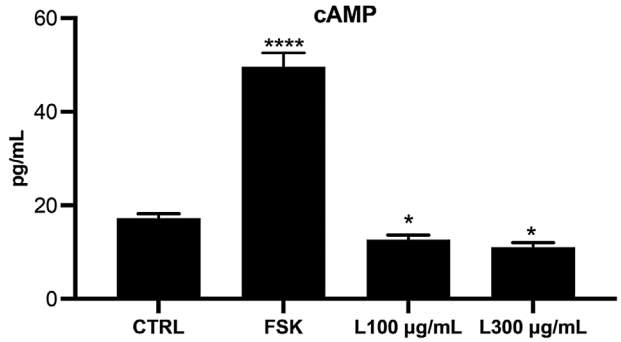

Figure 7. Intracellular cyclic adenosine monophosphate (cAMP) levels in STC-1 cells after 2 h exposure to hop extract and positive control forskolin (FSK) (10 µM). Test samples and controls were supplemented with 1 mM 3-isobutyl-1-methylxanthine (IBMX). Data are expressed as mean ± standard deviation (SD) of three independent experiments ($n = 3$) and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test; ****$p < 0.0001$, *$p < 0.05$ versus control cells (CTRL).

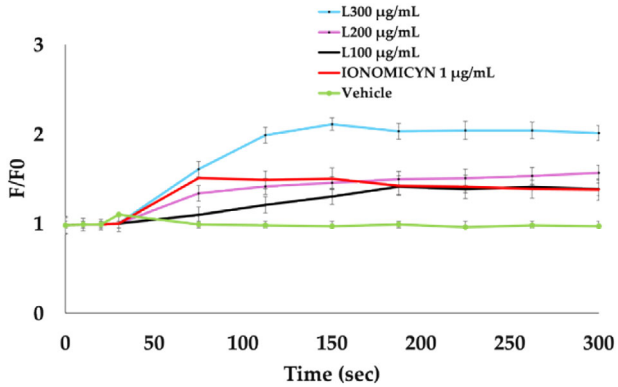

Figure 8. Hop bitter stimuli rapidly increased intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) dose-dependently in intestinal secretin tumor cell line (STC-1) cells. Cells were loaded with 5 µM of calcium indicator dye Fluo 4-AM for 30' at 37 °C in the dark and fluorescence was recorded every 37 s. Ionomycin was used as a positive control. Results are expressed as mean ± standard deviation (SD) of three independent experiments ($n = 3$) and reported as F/F0 where F0 is the baseline fluorescence.

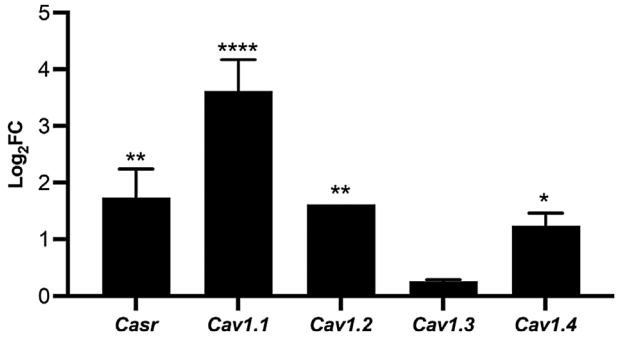

Figure 9. *H. lupulus* inflorescences extract effect on calcium gene expression. Hop extract significantly induces the expression of calcium-sensing receptor (*Casr*), and the L-type voltage-gated calcium channels (*Cav1.1*, *Cav1.2*, and *Cav1.4*) in STC-1 cells treated for 120 min with 100 µg mL^{-1}. Data are normalized with the housekeeping gene polymerase II (*Pol II*) and are shown as Log_2 fold change treated/control ± standard deviation (SD), of three independent experiments ($n = 3$) and were analyzed by one-way ANOVA followed by Tukey's post-hoc test; ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$ versus control cells. ANOVA, analysis of variance; STC-1, intestinal secretin tumor cell line.

common variant alleles of human TAS2R38, which are associated with taste sensitivity to PTC and 6-*n*-propylthiouracil (PROP), occur at positions encoding amino acids 49, 262, and 296, and comprise the "taster" PAV (proline, alanine, valine) and "non-taster" AVI (alanine, valine, isoleucine) haplotypes.[51] An extremely rare (frequency <1%) haplotype has also been identified: Pro, Ala, Ile (PAI), that is conserved among primates and rodents (*M. musculus*).[52] Together with PAV, PAI is the most sensitive to PTC and PROP, and this knowledge is interesting for this study as STC-1 cells are of murine origin.[9,53] With these results, we demonstrated that hops act through bitter receptors (*Tas2r120* and *Tas2r138* as siRNA knockdown confirmed) to secrete peptides responsible for the feeling of satiety and involved in the processes of regulating glucose homeostasis. A key mediator of the taste transduction mechanism is represented by intracellular calcium. The increase obtained in STC-1 cells by hop extract agrees with that previously reported.[4] This effect is mediated by bitter transducers such as *Trpm5*, *Ip3r3*, or *Plcβ2*; in fact, when they were antagonized, the intracellular calcium increase was attenuated or inhibited. It was already demonstrated the expression of L-type voltage-gated calcium channels in STC-1 cells, but their involvement in hop bitter mechanism was unclear.[54] Normally, these channels promote extracellular calcium influx after membrane depolarization into neuroendocrine cells. After showing the increased mRNA and protein expression, it was determined whether the hop-induced increase in intracellular calcium concentration was mediated by L-type voltage-gated calcium channel. Pretreating STC-1 cells with the specific blocker nitrendipine, $[Ca^{2+}]_i$, and CCK and GLP-1 secretion increase was inhibited. In addition, it was considered another potential target which is the CaSR, a G-protein coupled receptor protein that control and maintain calcium homeostasis. CaSR is activated not only by extracellular calcium but also by other ligands including peptides and amino acids.[55,56] CaSR activation increases CCK production in STC-1 cells but it is not clear if CaSR is involved in the secretion of appetite-suppressant hormones mediated by bitter compounds or extract.[57] In this context, cells pretreatment with a CaSR antagonist reduced $[Ca^{2+}]_i$ and consequently the secretion of GLP-1 and CCK compared to cells treated with only extract. However, this effect was not completely inhibited suggesting that other mediators are involved in hop-induced peptide secretion. This increased calcium concentration could also explain the increased *Trpa1* expression mediated by hop extract which could further amplify calcium entry. A graphical representation of the mechanism of action of hops on the main channel receptors involved in calcium regulation is reported in **Figure 13**.

5. Concluding Remarks

These results demonstrate, for the first time, that hop extract specifically targets mouse *Tas2r120* and *Tas2r138*, leading to the secretion of satiating hormones through calcium and *Plcβ*-2 dependent pathways in STC-1 cells, which have properties similar to native intestinal hormone-secreting cells. These findings align with previous research and provide a foundation for future studies exploring plant-based extracts, including common ones like

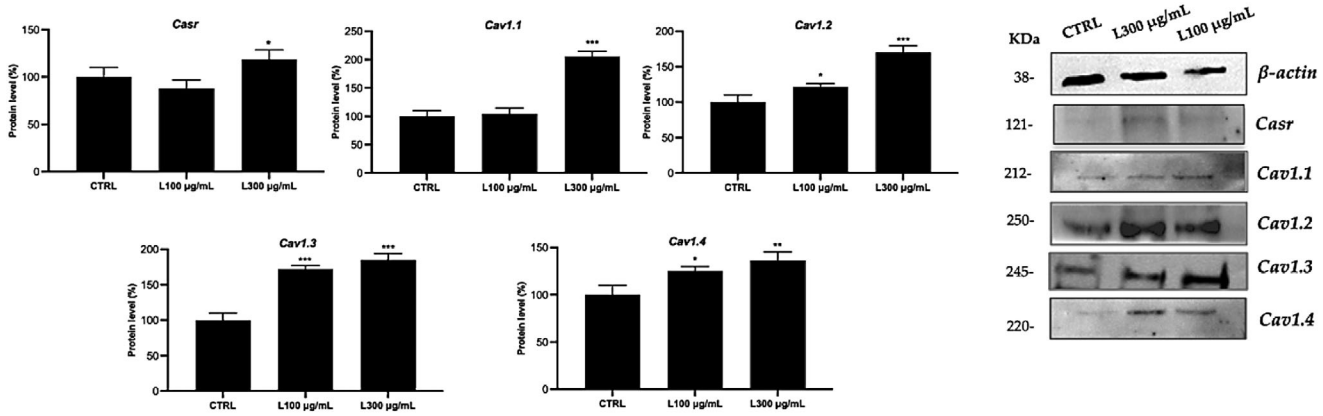

Figure 10. Effect of hop treatment on protein expression. Cell lysate was subjected to western blot analysis and protein content was normalized against *β*-actin. Immunoreactive bands are from a single experiment representative of multiple experiments and results from densitometric analysis are expressed as the mean ± standard deviation (SD). Data are expressed as the mean ± SD of three independent experiments ($n = 3$) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test; ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$ versus control cells (CTRL).

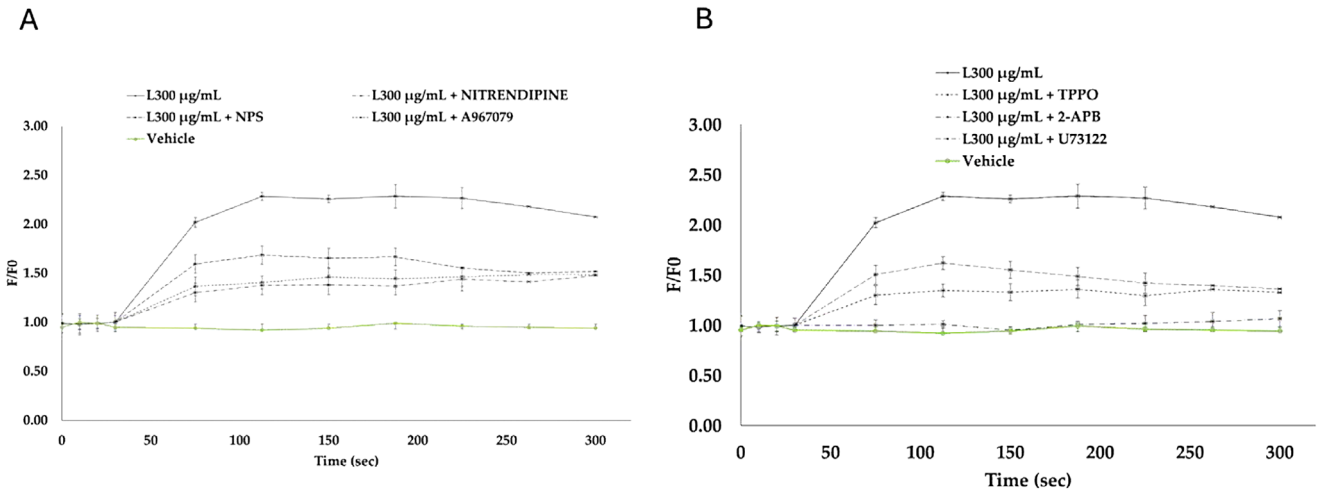

Figure 11. Hop-induced $[Ca^{2+}]_i$ increase is *Trpa1*, *Casr* and L-type calcium channels-dependent A) and bitter taste transduction mechanism dependent B). Induction of $[Ca^{2+}]_i$ by hop extract (L300 µg mL^{-1}) was reduced or inhibited by 30' pretreatment of STC-1 cells by the *Trpa1* antagonist A967079, the *Casr* antagonist NPS2143, the L-type voltage-gated calcium channel blocker nitrendipine, the *Trpm5* antagonist TPPO, the *Ip3r3* antagonist 2-APB, and the *Plcβ-2* inhibitor U73122. Results are expressed as mean ± standard deviation (SD) of three independent experiments and reported as F/F0 where F0 is the baseline fluorescence. 2-APB, 2-aminoethyl diphenylborinate; STC-1, intestinal secretin tumor cell line; TPPO, triphenylphosphine oxide.

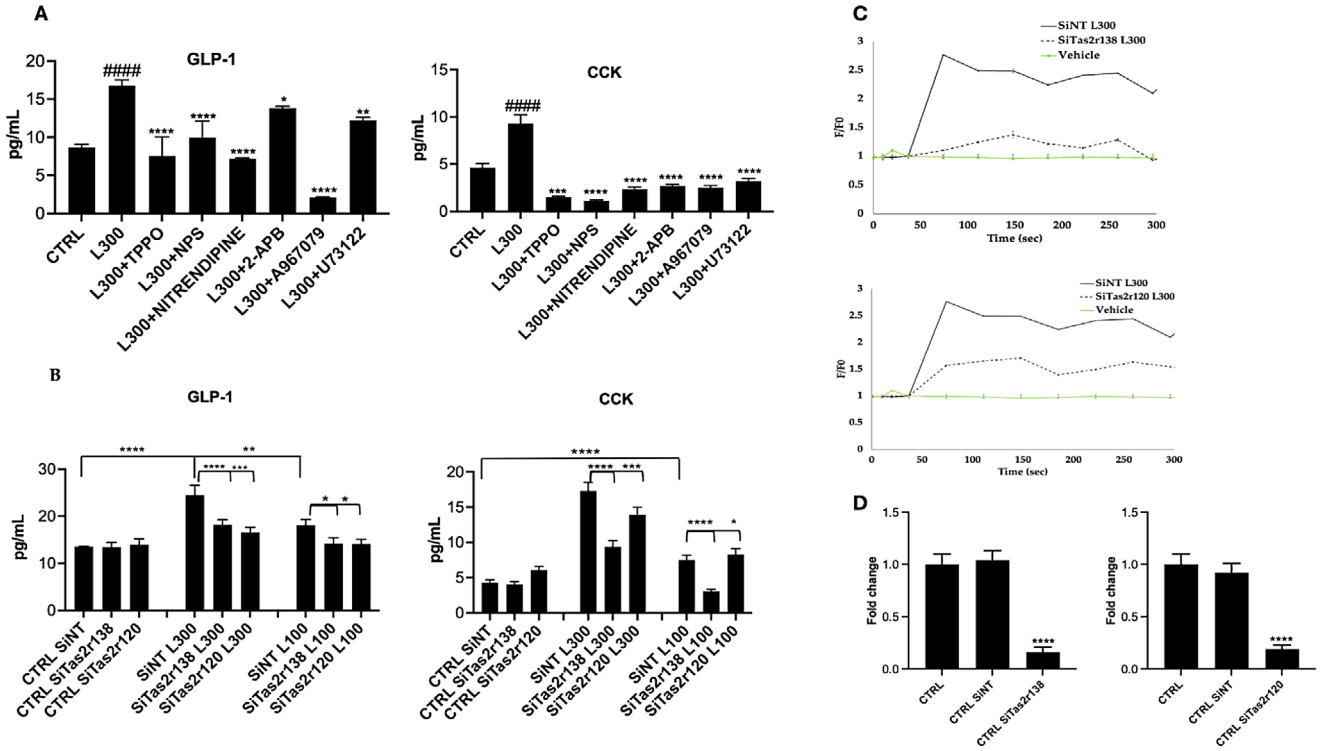

Figure 12. A) CCK and GLP-1 release from STC-1 cells in response to hop extract is mediated by L-type calcium channels and bitter taste key mediators. Exposure of STC-1 cells to *Trpm5*, *Trpa1*, *Casr*, *Plcβ-2*, *Ip3r*, and L-type calcium channels inhibitors for 30 min prior to hop treatment, reduced hormones secretion in comparison to cells only treated with hop extract. Data are expressed as mean ± standard deviation (SD), of three independent experiments ($n = 3$) and were analyzed by one-way ANOVA followed by Tukey's post-hoc test; ####$p < 0.0001$ versus control cells (CTRL), ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$ versus hop-treated cells (L300 µg mL^{-1}). B) *Tas2r138* and *Tas2r120* knockdown (SiTas2r138, SiTas2r120) attenuates the effects of hop extract (100 and 300 µg mL^{-1}) on the secreted levels of GLP-1 and CCK and intracellular calcium concentration (c) in comparison to non-targeting SiRNA (SiNT, negative control). Data are expressed as mean ± SD of three independent experiments ($n = 3$) and were analyzed by one-way ANOVA followed by Tukey's post-hoc test; ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. C) *Tas2r138* and *Tas2r120* knockdown (SiTas2r138, SiTas2r120) attenuates the effects of hop extract (300 µg mL^{-1}) on intracellular calcium concentration in comparison to nontargeting SiRNA (SiNT, negative control). D) Effect of *Tas2r138* and *Tas2r120* knockdown (Si*Tas2r138*, Si*Tas2r120*) on gene expression. Data are expressed as mean ± SD of three independent experiments ($n = 3$). ANOVA, analysis of variance; CCK, cholecystokinin; GLP-1, glucagon-like peptide 1; STC-1, intestinal secretin tumor cell line.

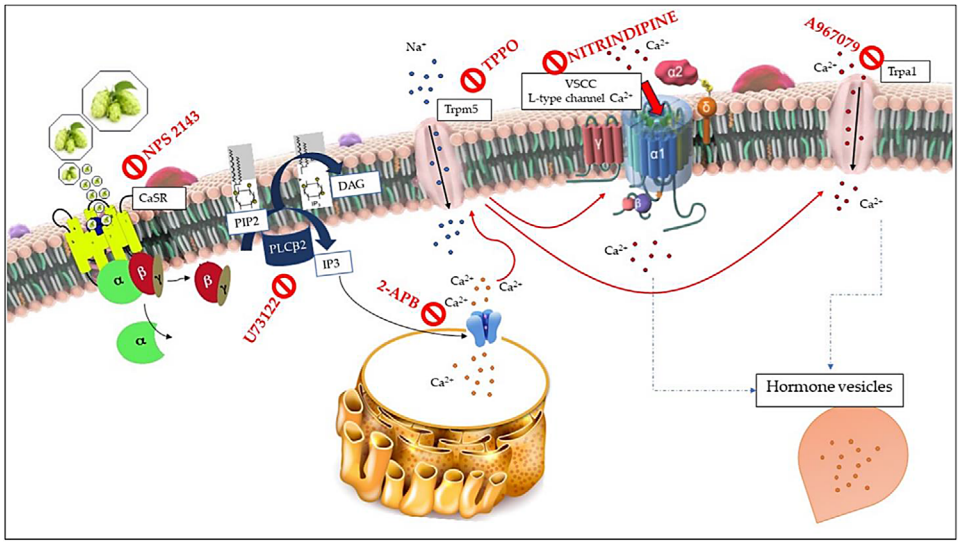

Figure 13. Representation of the mechanism of action of hops on the main channel receptors involved in calcium regulation.

hops, processed with no-toxic solvents. Such extracts could be useful to address metabolic-related diseases such as obesity by inhibiting appetite and food intake.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

L.M., C.K., and J.F.S.: Conceptualization, supervision, writing–review, and editing; L.L., D.R., V.C., and J.C.: Investigation, methodology, validation, and formal analysis; L.L. and L.M.: Writing original draft; All authors: Writing–review, and editing; L.M.: Funding acquisition.

Data Availability Statement

Data is available on request from the authors.

Keywords

bitter acids, calcium, gut hormones, hops, Tas2rs

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