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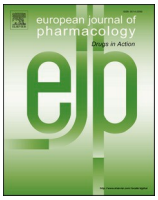

Expression and activity of the umami taste receptor (TAS1R1/TAS1R3) in rat corpus cavernosum

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ABSTRACT

The activation of umami taste receptors (TAS1R1/TAS1R3) promotes smooth muscle (SM) relaxation in the mouse stomach. The nitric oxide (NO)/cGMP signaling pathway is crucial for penile erection. Phosphodiesterase type 5 (PDE5) inhibitors, which specifically target cGMP, are the primary treatment for erectile dysfunction (ED). However, these drugs are ineffective in a significant number of patients, highlighting the need for alternative pharmacological targets for ED. Since umami taste receptors regulate SM contractility, this study investigates the role of TAS1R1/TAS1R3 in rat erectile tissue. We performed immunohistochemistry on the corpus cavernosum (CC) and dorsal penile artery (DPA) to detect TAS1R1/TAS1R3 expression. Isometric force recordings for the TAS1R1/TAS1R3 agonist monosodium glutamate (MSG), the NO donor SNAP, the hydrogen sulfide (H_2S) donor GYY 4137, and electrical field stimulation (EFS) and measured endogenous H_2S production. Immunohistochemistry revealed strong TAS1R1/TAS1R3 expression in nerve fibers of the CC and in the endothelium of the DPA, with limited expression in SM. In the CC, MSG enhanced relaxations induced by EFS, SNAP, and GYY 4137, and increased H_2S production, which was sensitive to NO and H_2S synthase inhibitors. MSG-induced relaxation was reduced by inhibition of neuronal voltage-gated calcium channels. In the DPA, MSG induced relaxation which was reduced by mechanical removal of the endothelium. These findings indicate significant neuronal and endothelial expression of TAS1R1/TAS1R3 in the CC and DPA, where MSG promotes SM relaxation. In the CC, MSG enhances nerve-mediated relaxation induced by NO and H_2S and stimulates H_2S production, suggesting TAS1R1/TAS1R3 as a potential therapeutic target for ED.

1. Introduction

Umami flavour is detected via activation of G protein-coupled receptors (GPCRs). Umami agonists activate the heterodimer TAS1R1/TAS1R3 of the taste receptor type 1 family. TAS1R1 confers selectivity for umami tastants, while TAS1R3 is co-expressed with receptor subunits for sweet and umami tastes (Xu et al., 2004). TAS1R1/TAS1R3 responds to L-amino acids, especially monosodium glutamate (MSG) (Xu et al., 2004). TAS1R1/TAS1R3 are found in enteroendocrine cells, where activation triggers peptide release such as cholecystokinin (Daly et al., 2013; Shirazi-Beechey et al., 2014) and calcitonin gene-related peptide (Kendig et al., 2014). However, the expression and function of these receptors in smooth muscle (SM) contractility regulation remains poorly understood. mRNA for L-amino acid sensing receptors, including TAS1R1, is detected in human and mouse stomach SM, whereas TAS1R3 is only expressed in mouse fundic SM (Vancleef et al., 2018). TAS1R1/TAS1R3 activation by MSG in mouse gastric SM reduces acetylcholine-induced contraction through $G\alpha_{i2}$ protein activation and intracellular Ca^{2+} decrease (Crowe et al., 2020).

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Abbreviations:

ACh	Acetylcholine
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CC	corpus cavernosum
CSE	cystathionine γ-lyase
DPA	dorsal penile artery
EFS	electrical field stimulation
ED	erectile dysfunction
GYY 4137	morpholin-4-ium 4-methoxyphenyl(morpholino) phosphinodithioate
H_2S	hydrogen sulfide
IR	immunoreactivity
L-NOARG	N^G-nitro-L-arginine
MSG	monosodium glutamate
NANC	non-adrenergic non-cholinergic
NO	nitric oxide
NOS	nitric oxide synthase
ODQ	1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one
PDE4	phosphodiesterase type 4
PDE5	phosphodiesterase type 5
PhE	phenylephrine
PPG	DL-propargylglycine
SM	smooth muscle
SNAP	(S)-nitroso-N-acetylpenicillamine
TAS1R1/TAS1R3	umami taste receptors

In the corpus cavernosum (CC), non-adrenergic, non-cholinergic (NANC) nerve-mediated relaxation involves the gasotransmitter nitric oxide (NO) (Ignarro et al., 1990; Holmquist et al., 1991). NO also mediates endothelium-dependent relaxation in human CC and penile arteries (Angulo et al., 2003). NO induces SM relaxation by stimulating soluble guanylate cyclase (sGC), increasing intracellular cyclic guanosine monophosphate (cGMP), which lowers cytosolic Ca^{2+} and alters protein phosphorylation (Andersson and Wagner, 1995). Phosphodiesterase type 5 (PDE5), expressed in CC, hydrolyzes cGMP. PDE5 inhibitors such as sildenafil, vardenafil, and tadalafil elevate cGMP in CC tissue, promoting penile erection. Thus, these inhibitors are commonly used to treat erectile dysfunction (ED) (Qiu et al., 2000; Francis and Corbin, 2003). Hydrogen sulfide (H_2S), another gaseous transmitter, contributes to nerve-mediated relaxation in human CC (Srilatha et al., 2007; d'Emmanuele di Villa Bianca et al., 2009). In diabetic rat models with ED, reduced activity of cystathionine γ-lyase (CSE) and cystathionine β-synthase decreases endogenous H_2S production (Zhang et al., 2016). H_2S promotes vasodilatory and pro-erectile effects via cAMP-dependent pathways (Srilatha et al., 2007). The interaction between endogenous H_2S and NO is crucial for maintaining erectile function (Coletta et al., 2012; Meng et al., 2013; Mostafa et al., 2019; Yilmaz-Oral et al., 2020). H_2S enhances NO signaling, correlating with NO enzyme activity and cGMP levels in CC tissue, highlighting their synergistic role in erectile regulation (Coletta et al., 2012; Meng et al., 2013; Mostafa et al., 2019; Yilmaz-Oral et al., 2020). Recent studies identify perivascular fibroblasts in the CC as key promoters of penile vasodilation by reducing norepinephrine availability (Guimaraes et al., 2024). Additionally, 6-nitrodopamine, a dopamine D2 receptor antagonist, is the most potent endogenous relaxant in rabbit CC, indicating that dopamine and 6-nitrodopamine balance regulates CC SM tone in vivo (Lima et al., 2024).

Although PDE5 inhibitors treat ED, about 30 % of patients, especially diabetics, respond poorly to them (Rendell et al., 1999; Sáenz de Tejada et al., 2002; Goldstein et al., 2003; Reffelmann and Kloner, 2005), highlighting the need for new therapies. Given that umami taste receptors (TAS1R1/TAS1R3) have been proposed as targets for disorders with impaired SM contractility (Kendig et al., 2014; Crowe et al., 2020), this study aims to investigate their expression and function in rat erectile tissue.

2. Materials and methods

2.1. Animals

Male Wistar rats, aged 17–18 weeks and with body weights ranging from 265 to 370g, were maintained on a 12 h light/dark cycle with unrestricted access to food and water at the Animal Facility of the Department of Physiology, Complutense University of Madrid. All animal care and experimental procedures were conducted in accordance with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes. The protocols were approved by the Institutional Animal Care and Use Committee of the Complutense University of Madrid and the Comunidad de Madrid (PROEX109/16).

2.2. Corpus cavernosum and dorsal penile artery harvesting

Rats were euthanized following isoflurane anesthesia (~5 %) and cervical dislocation. The study received approval from the Institutional Animal Care and Use Committee and adhered to the EU Directive 2010/63/EU. Once the rats were euthanized, the crura of the CC were isolated at their attachment sites to the pubic ramus. Additionally, rings of the dorsal penile artery (DPA) were obtained. After careful removal, the penis was placed in a physiological saline solution (PSS) at 4 °C, containing the following components (in mM): 119 NaCl, 24.9 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 0.027 EDTA, 11 glucose, and 1.5 $CaCl_2$. The PSS was continuously aerated with 5 % CO_2 and 95 % O_2 to keep the pH at 7.4. Next, the CC strips were isolated by removing the tunica albuginea and adjacent connective tissues. The CC samples and DPA rings were kept in PSS at 4 °C to maintain their physiological conditions.

2.3. Western blot

Proteins from rat CC were extracted and quantified using the Lowry method with the DC Protein Assay Kit (Bio-Rad, Madrid, Spain). A 30 μg amount was taken and separated via SDS-PAGE on a 10 % polyacrylamide gel. Following electrophoresis, the proteins were transferred to nitrocellulose membranes and allowed to incubate overnight at 4 °C with 1:500 dilutions of anti-TAS1R1 (OSR00182W, Invitrogen, Madrid, Spain) and anti-TAS1R3 (PA5-102260, Invitrogen, Madrid, Spain) antibodies (Reed et al., 2004; Lu et al., 2005; Oya et al., 2011; Muroi and Ishii, 2012). Mouse anti-β-actin antibodies (sc-47778, Santa Cruz Biotechnology Inc., Heidelberg, Germany) were utilized as a control for protein loading. Following the washing steps, the membranes were exposed to a secondary antibody (m-IgG2a BP-HRP, sc-542731, Santa Cruz Biotechnology Inc., Heidelberg, Germany) diluted at 1:5000 and incubated for 60 min at room temperature (RT). Detection of the blots was carried out using an ECL reagent (ECL Select-kit, GE Healthcare, Madrid, Spain) and visualized through chemiluminescence using an Image Quant LAS 500 system (GE Healthcare, Madrid, Spain).

2.4. Double-labeling immunofluorescence assays

Samples of rat erectile tissue were fixed in 4 % paraformaldehyde for 4 h and then washed with 1X PBS. Dehydration was performed using a 30 % sucrose solution. The tissues were then embedded in OCT and stored frozen until sectioning. Tissue sections, 5 μm thick, were treated

with a blocking solution containing 1X PBS, 5 % BSA, 0.3 % Triton X-100, and 10 % normal goat serum for 2–3 h. Then, sections were incubated for 48h at 4 °C, using the following primary antibodies: rabbit anti-TAS1R1 (1:100, OSR00182W, Invitrogen, Madrid, Spain), anti-TAS1R3 (1:100, PA5-102260, Invitrogen, Madrid, Spain) (Reed et al., 2004; Lu et al., 2005), mouse anti-protein gene product 9.5 (1:400, ab8189, Abcam, Cambridge, UK). After washing with 1X PBS, the sections were exposed to fluorochrome-conjugated secondary antibodies for 3 h at RT (Alexa Fluor 594 goat anti-rabbit, A11037, and Alexa Fluor 488 goat anti-mouse, A11029; 1:200, Invitrogen, Madrid, Spain). The nuclei were counterstained with DAPI using ProLong Gold antifade reagent with DAPI (P36935, Molecular Probes, Oregon, USA). Sections then were mounted for microscopic examination.

2.5. Endogenous H_2S measurement

Endogenous H_2S measurements were performed as previously (Li et al., 2005). To evaluate the effects of CSE, nitric oxide synthase (NOS), and TAS1R1/TAS1R3, CC samples were incubated for 1 h with DL-propargylglycine (PPG, 1 mM) and N^G-nitro-L-arginine (L-NOARG, 100 μM), inhibitors of CSE and NOS, respectively, or monosodium glutamate (MSG) at concentrations of 30, 50, and 100 mM. Post-incubation, the samples were homogenized (1:10 w/v) in a cold 50 mM potassium phosphate buffer, pH 6.8. Each assay chamber was prepared with 50 mg of homogenate mixed with 1 ml of incubation buffer (100 mM potassium phosphate buffer, 2 mM pyridoxal 5′-phosphate, and 10 mM L-cysteine, pH 7.4). Reactions were initiated by transferring the chambers from ice to a pre-warmed shaking water bath set at 37 °C. After 30 min, zinc acetate (1 %) was added to capture the produced H_2S, and the reaction was stopped by adding trichloroacetic acid (500 % w/v) to denature the proteins. Subsequently, the solution was treated with 0.5 ml of N, N-dimethyl-p-phenylenediamine sulfate (20 mM in 7.2 M HCl) and 0.4 ml of $FeCl_3$ (30 mM in 1.2 M HCl). After a 20-min incubation, absorbance was measured at 670 nm using a spectrophotometer. The concentration of H_2S was calculated from a NaHS standard curve and reported as nanomoles of H_2S per milligram of soluble protein per 20 min (nmol/mg.min^{-1}). Protein quantification was performed using the Lowry method (DC Protein Assay Kit, Bio-Rad, Madrid, Spain).

2.6. Isometric force recordings

From each penis, 2 CC samples, measuring 3 mm long and 2 mm wide, along with two DPA rings, each 2 mm in length, were harvested and placed horizontally in a myograph system (DMT, Muscle Strip System 820MS, Danish Myotechnology, Denmark). The myographs were filled with 5 ml of PSS and kept at a constant temperature of 37 °C. The pH level was stabilized at 7.4 by continuously bubbling the solution with a gas mixture of 95 % O_2 and 5 % CO_2. Each tissue sample was fastened at one end to a micrometer screw, and the opposite end was connected to an isometric force transducer for measuring force generation. Data were collected using the PowerLab v7.0 system (ADInstruments Inc, Oxford, UK). After the samples were mounted, they were allowed to equilibrate for 60 min under a passive tension of 4.0 mN for CC strips and the PSS was changed every 20 min. For each DPA, the resting wall tension and internal circumference were determined. From this, the internal circumference L100, corresponding to a transmural pressure of 100 mm Hg for a relaxed vessel in situ, was calculated. The DPA were then adjusted to an internal circumference of L1, where $L1 = 0.9 \times L100$, as force generation approaches its maximum at this circumference. Contractile responses were assessed using a potassium-rich solution (K-PSS, 124 mM).

The role of endothelial cells in MSG-induced relaxation was assessed using penile arteries from which the endothelium was mechanically removed. The endothelium was gently removed by rubbing the intimal surface with a stainless-steel wire. The artery was then challenged with KPSS to assess its viability, and the absence of endothelium was confirmed by the lack of relaxation to 10 μM acetylcholine. The tissues were stimulated with phenylephrine (PhE, 3 μM) to induce contraction, and subsequent relaxation responses were evaluated with varying concentrations of monosodium glutamate (MSG), a TAS1R1/TAS1R3 agonist. For electrical field stimulation (EFS) experiments, CC strips were pretreated for 1 h with guanethidine (10 μM) and atropine (1 μM) to inhibit, respectively, noradrenergic neurotransmission and muscarinic receptors. These samples were then precontracted with 3 μM PhE, and frequency-response curves were recorded in the presence or absence of MSG at 30 mM. EFS was performed using rectangular pulses with frequencies ranging from 0.5 to 16 Hz, pulse duration of 1 ms, 20 s stimulation trains, and a current output of 75 mA, applied every 4 min via two platinum electrodes positioned along the longitudinal axis of the samples (CS20 stimulator, Cibertec, Barcelona, Spain).

2.7. Compounds and solutions

Compounds used in the study: Acetylcholine (ACh), atropine, DL-propargylglycine (PPG), guanethidine, isoproterenol, monosodium glutamate (MSG), N^G-nitro-L-arginine (L-NOARG), and phenylephrine (PhE), all sourced from Sigma (St. Louis, MO, USA). Morpholin-4-ium4-methoxyphenyl(morpholino) phosphinodithioate (GYY 4137), 1H-[1,2,4]oxadiazolo [4,3-a] quinoxalin-1-one (ODQ), omega-conotoxin GVIA and (S)-nitroso-N-acetylpenicillamine (SNAP) were obtained from Tocris (Bristol, UK). GYY 4137, ODQ, PPG and SNAP were dissolved in dimethyl sulfoxide, while the remaining compounds were dissolved in purified water. These solvents did not influence the contractility of CC strips. PSS was freshly prepared with the following concentrations (in mM): NaCl 119, KCl 4.7, $NaHCO_3$ 24.9, $MgSO_4$ 1.2, KH_2PO_4 1.2, ethylenediamine tetraacetic acid (EDTA) 0.027, glucose 11, and $CaCl_2$ 1.5. To ensure a pH of 7.4, the PSS in the organ bath was continuously aerated with a 95 % O_2 and 5 % CO_2 gas mixture. The K-PSS was also freshly prepared by substituting NaCl with an equivalent amount of KCl.

2.8. Data analysis

The sensitivity to MSG is expressed as EC_{50}. The EC_{50} denotes the MSG concentration needed to achieve 50 % of the maximum effect. The extent of relaxation was measured as the percentage inhibition of the contraction induced by 3 μM PhE. Results are expressed as the mean ± SD for *n* (the number of rats). Statistical analyses were performed using either the paired or unpaired Student's *t*-test, or one-way analysis of variance (ANOVA), as appropriate. A *P*-value of less than 0.05 was considered statistically significant. Data were analyzed using GraphPad Prism version 8.3.0 (GraphPad Software, San Diego, CA).

3. Results

3.1. Expression of TAS1R1 and TAS1R3

The expressions of TAS1R1 and TAS1R3 in rat CC and DPA samples (n = 5) were investigated using double immunofluorescence with selective antibodies for TAS1R1 and TAS1R3, alongside the neuron-specific marker, protein gene product 9.5 (PGP 9.5). TAS1R1 (Fig. 1A–D) and TAS1R3 (Fig. 2A–D) were consistently found to co-express with PGP 9.5 in the nerve fibers of the CC. Additionally, strong TAS1R1 (Fig. 1E–H) and TAS1R3 (Fig. 2E–H) staining was detected in the endothelium of the DPA. Both receptors showed limited presence in SM (Fig. 1A–H and 2A-H). In the absence of primary antibodies, no immunoreactivity (IR) was observed in the samples (Fig. 1I and J and 2I-J). Furthermore, TAS1R1 and TAS1R3 expressions in the whole sample were confirmed by Western blot analysis (n = 7–9), which detected bands at 95 kDa and 110 kDa, representing the molecular weights for TAS1R1 and TAS1R3, respectively (Fig. 1K and 2K).

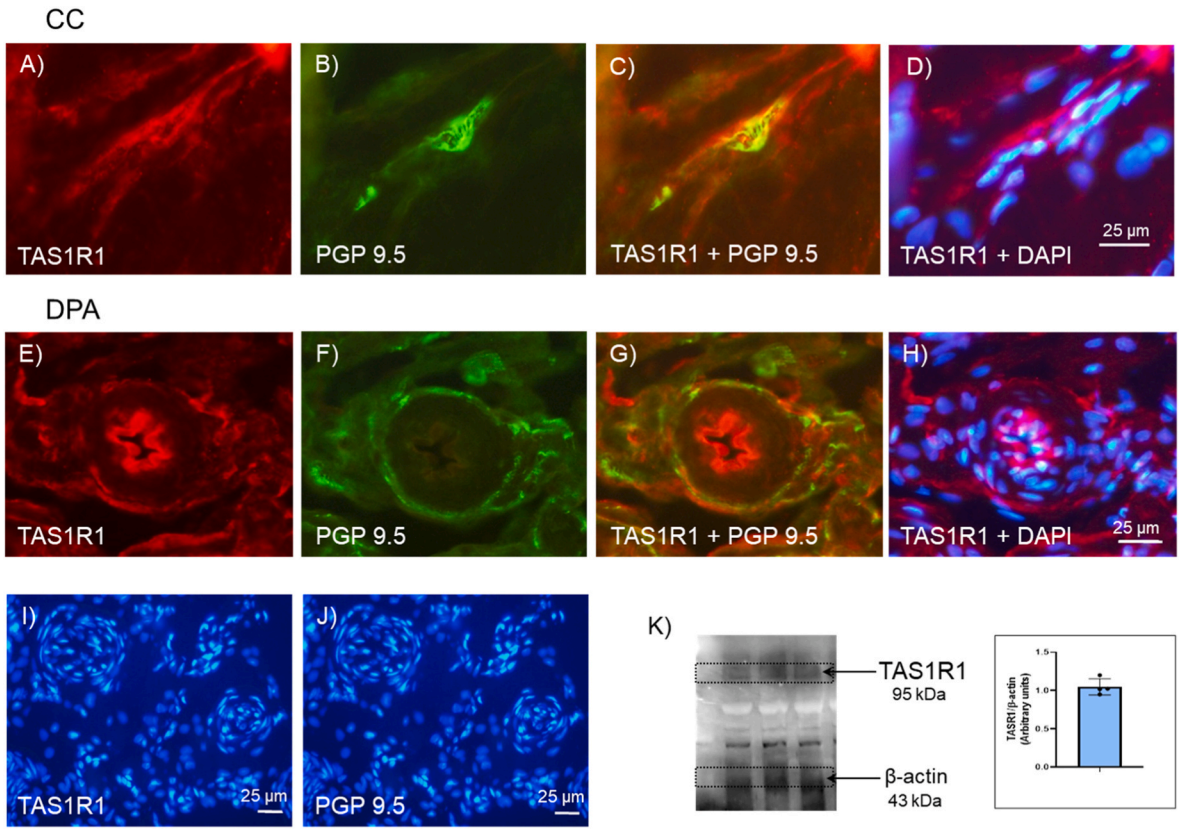

Fig. 1. TAS1R1 expression in nerve fibers and endothelium of rat erectile tissue. Immunofluorescence staining for TAS1R1 was performed in nerve fibers and endothelial cells within trabecular and arterial erectile tissues from rats. Panels (A–D) show the corpus cavernosum (CC), while panels (E–H) display the dorsal penile artery (DPA). TAS1R1 protein is marked in red in CC (A) and DPA (E). Protein gene product 9.5 (PGP 9.5), indicating nerve fibers, is labeled in green in CC (B) and DPA (F). The merged images (yellow) reveal TAS1R1 expression within nerve fibers running parallel to smooth muscle fibers (C and G). Cell nuclei are stained with DAPI (blue) (D and H). Scale bars indicate 25 µm. Negative controls, where the primary antibody was omitted, showed no immunoreactivity (I and J) (n = 5). Western blot analysis of homogenized cavernous tissue revealed a band at 95 kDa, matching the molecular weight for of TAS1R1 (K) (n = 7). Lanes represent different technical replicates (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Functional studies

Crura CC strips and DPA rings were normalized to a passive tension of 1.7 ± 0.43 mN and 0.46 ± 0.12 mN respectively. PhE (3 µM) elicited a sustained SM contraction of 4.2 ± 1.7 mN (n = 21) and 15.3 ± 5.6 mN (n = 6), respectively.

3.2.1. Effect of blockade of sGC and neuronal voltage-gated Ca^{2+} channels on relaxations to MSG in CC

MSG (1–200 mM) causes concentration-dependent relaxations, MSG induced slow-developing SM relaxations in the CC strips, with Emax value of 79.8 ± 9.3 %, respectively (n = 7) (Fig. 3A and B). The sGC inhibitor ODQ (5 µM) did not change the responses to MSG, resulting in Emax values of 71.5 ± 8.2 % and 75.3 ± 10.4 % in the absence and presence of ODQ, respectively, n = 6. However, the neuronal N-type voltage sensitive Ca^{2+} channel inhibitor, omega-conotoxin GVIA (1 µM), consistently reduced the relaxations to MSG, yielding Emax values of 79.8 ± 9.3 % and 58.2 ± 12.8 %** in the absence and presence of omega-conotoxin GVIA, respectively (n = 6) (**$P < 0.01$ vs control, paired Student's *t*-test) (Fig. 3B).

3.2.2. Role of endothelium in relaxations to MSG in DPA

MSG (1–200 mM) induced slow-developing SM relaxations in endothelium-intact DPA rings, with an Emax of 59.6 ± 18.2 % (n = 6) (Fig. 3C and D). In mechanically endothelium-denuded rings, MSG-induced relaxation was significantly reduced, with Emax of 33.9 ± 7.6 %** in the presence and absence of endothelium (n = 6) (**$P < 0.01$ vs control, unpaired Student's *t*-test).

3.2.3. Effects of threshold MSG concentrations on EFS-, SNAP-, GYY 4137-induced relaxations in CC

Maintaining NANC conditions, EFS (0.5–16 Hz) elicited frequency-dependent relaxation of rat CC strips contracted with PhE. The EFS-induced response was enhanced by threshold concentrations (30 mM) of MSG (Table 1) (Fig. 4A and B).

SNAP (1 µM) (Fig. 4C) and GYY 4137 (1 µM) (Fig. 4D), NO- and slow-release H_2S-donors, respectively, produced relaxations of CC samples, which were also increased in the presence of 30 mM MSG (SNAP- and GYY 4137-induced relaxations of 16.3 ± 7.6 % and 15.3 ± 9.6 % and 30.2 ± 13.2 %* and 49.3 ± 18.1 %***, in the absence and presence of MSG, respectively, n = 6 (*$P < 0.05$ and ***$P < 0.001$ vs control, paired Student's *t*-test).

3.3. Effect of NOS and CSE blockade and MSG on H_2S production

H_2S production in rat CC samples was measured at 8.8 ± 2.1 nmol/mg.min^{-1}. Following the inhibition of CSE and NOS with PPG (1 mM) and L-NOARG (100 µM), respectively, there was a significant reduction in the endogenous production of H_2S. However, increasing concentrations of the TAS1R1/TAS1R3 agonist MSG (30–100 mM) led to a dose-dependent restoration and significant elevation of basal H_2S levels (Table 2) (Fig. 5).

4. Discussion

This study suggests that umami taste receptors play a crucial role in controlling the tone of rat cavernous tissue. The TAS1R1/TAS1R3

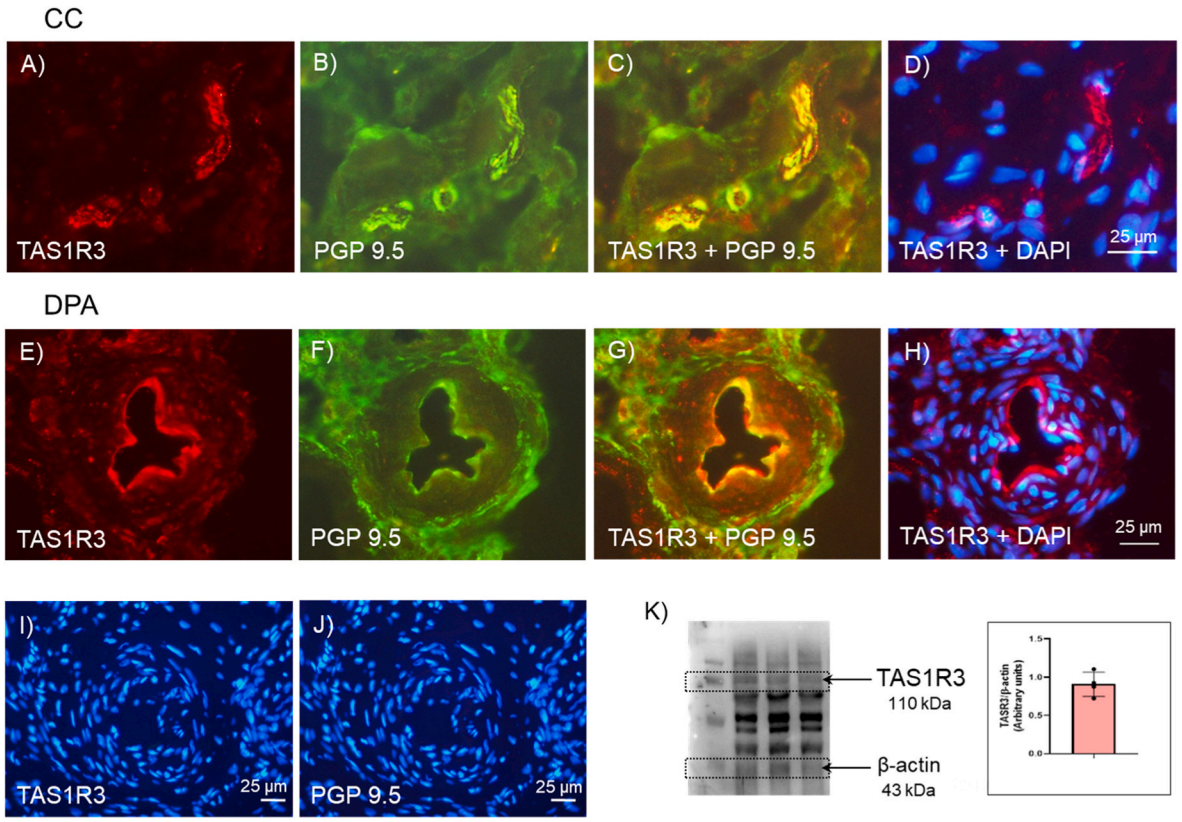

Fig. 2. TAS1R3 expression in nerve fibers and endothelium of rat erectile tissue. Immunofluorescence staining for TAS1R3 was performed in nerve fibers and endothelial cells within trabecular and arterial erectile tissues from rats. Panels (A–D) depict the corpus cavernosum (CC) and (E–H) the dorsal penile artery (DPA). TAS1R3 protein is marked in red in CC (A) and DPA (E). Protein gene product 9.5 (PGP 9.5), representing nerve fibers, is shown in green in CC (B) and DPA (F). The merged images (yellow) highlight TAS1R3 expression within nerve fibers running parallel to smooth muscle fibers (C and G). Cell nuclei are stained with DAPI (blue) (D and H). Scale bars: 25 µm. Negative controls, where the primary antibody was omitted, showed no immunoreactivity (I and J) (n = 5). Western blot analysis of homogenized cavernous tissue revealed a band at 110 kDa, matching the molecular weight for TAS1R3 (K) (n = 9). Lanes represent different technical replicates (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

heterodimer is widely expressed in erectile tissue, where its activation induces SM relaxation and promotes inhibitory neurotransmission mediated by gaseous molecules. This conclusion is suggested by the subsequent findings (i) High expression of TAS1R1/TAS1R3 in CC nerve fiber cells and endothelial cells of the DPA, with moderate presence in SM; (ii) The TAS1R1/TAS1R3 agonist MSG induces a slow onset and long-lasting concentration-dependent relaxation of trabecular and arterial SM; (iii) Threshold concentrations of MSG enhance nerve-mediated relaxation induced by NO and H_2S; and (iv) MSG also increases endogenous H_2S production.

We have demonstrated that bitter taste receptors regulate SM tone in rat cavernous tissue. Specifically, bitter taste receptor TAS2R10 is present in nerve fiber cells and SM, where its agonist denatonium induces significant SM relaxation and enhances inhibitory neurotransmission mediated by NO and H_2S (Navarro-Dorado et al., 2023). In the current study, Western blot analysis confirmed the presence of TAS1R1/TAS1R3 proteins throughout the erectile tissue. Double immunofluorescence labeling revealed intense TAS1R1/TAS1R3 IR primarily localized in cavernous nerve fiber cells and the DPA endothelium, suggesting their role in regulating SM tone. The pronounced expression of TAS1R1/-TAS1R3 in these tissues aligns with the functional effects of the TAS1R1/TAS1R3 agonist MSG, which causes a slow-developing relaxation of SM.

The endothelium plays a crucial role in penile arterial and trabecular SM relaxation, which is essential for penile erection. Diabetes is linked with endothelial dysfunction (McVeigh et al., 1992; Johnstone et al., 1993) and a high incidence of ED (Zietman et al., 1994). Endothelial dysfunction, commonly seen in aging and vascular conditions like hypercholesterolemia and hypertension, is strongly linked to an increased occurrence of ED (Martín-Morales et al., 2001). ED frequently serves as an early warning sign for underlying systemic vascular disorders, which can lead to significant cardiac events. The pathophysiology of ED is strongly related to cardiovascular disease, with endothelial dysfunction occurring early in both conditions (Blick et al., 2016). NO is a pivotal mediator of endothelium-dependent relaxation, facilitating penile vasodilation during erection by activating sGC in SM cells. This activation increases cGMP levels, which reduces intracellular Ca^{2+} and desensitizes the contractile apparatus to Ca^{2+}. Nevertheless, an unknown endothelial factor has been found to induce SM hyperpolarization and relaxation, independently of NOS and cyclooxygenase inhibition (Busse et al., 2002; Simonsen et al., 2002). This factor has functional significance in human resistance arteries (Coats et al., 2001). In human cavernous tissue, endothelium-dependent-relaxation is primarily mediated by NO. Conversely, in the small penile arteries, both NO and the endothelium-derived hyperpolarizing factor (EDHF) are key contributors to this relaxation (Angulo et al., 2003). Under physiological conditions, the regulation of penile vascular tone involves both NO-dependent and NO-independent pathways, ensuring equilibrium between vasoconstriction and vasodilation. Proper endothelial function is crucial for the action of pro-erectile agents and for maintaining normal erectile function. Dysfunction of the endothelium disrupts these regulatory mechanisms, adversely affecting SM contraction and the control of penile vascular tone. This dysfunction results in decreased NO bioavailability due to endothelial damage. Additionally, endothelium-derived mediators independent of NO also contribute to ED because of endothelial damage (Blick et al., 2016). In the current

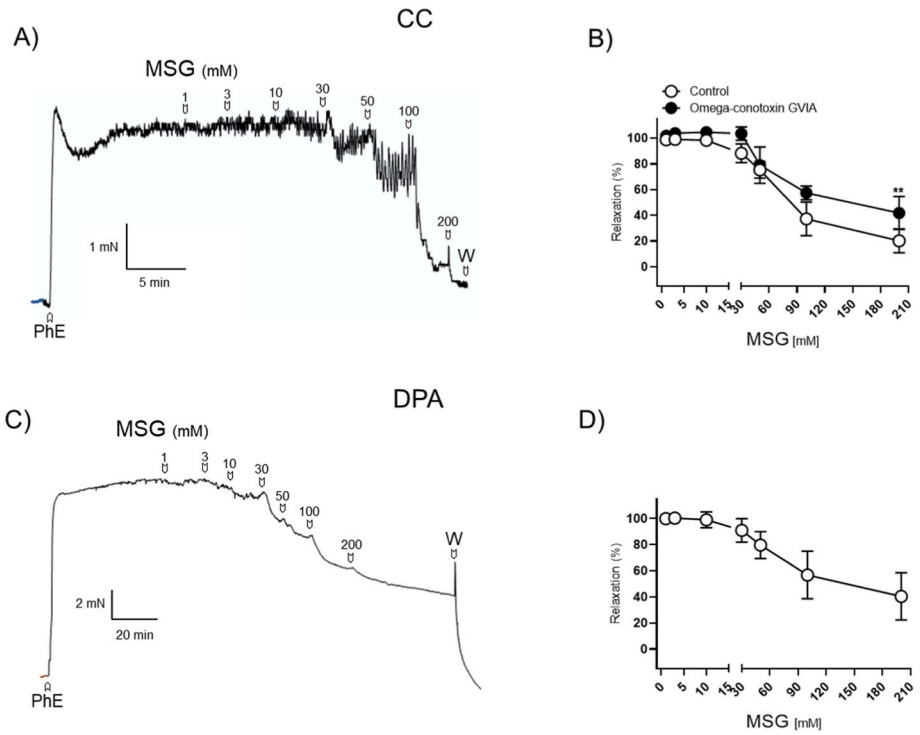

Fig. 3. Relaxation induced by MSG in smooth muscle of rat erectile tissue. Isometric tension recordings demonstrate the relaxant effects of MSG (1–200 mM) on rat corpus cavernosum (CC) strips pre-contracted with 3 μM phenylephrine (PhE) (A), and on dorsal penile artery (DPA) rings (C). The vertical axis represents tension (mN) while the horizontal axis denotes time (min). W: wash. Concentration-response relaxation curves to MSG (1–200 mM) are shown for CC strips in the absence and presence of omega-conotoxin GVIA (1 μM), respectively (n = 6) (**$P < 0.01$ vs control, paired Student's *t*-test) (B). Corresponding curves for DPA samples under control conditions are shown in panel D. Data are presented as mean ± SD from 6 to 7 rats.

Table 1
Effect of 30 mM MSG on relaxations to EFS (1ms duration, 0.5–16 Hz, 20 s trains, with constant current output adjusted to 75 mA) in rat corpus cavernosum strips.

	EFS (Hz)					
	0.5	*1*	*2*	*4*	*8*	*16*
Control	3 ± 1	11 ± 6	32 ± 8	64 ± 6	77 ± 7	80 ± 8
MSG	8 ± 2*	29 ± 14*	56 ± 14*	83 ± 6**	88 ± 3*	90 ± 3*

Results are expressed as inhibition percentage of 3 μM phenylephrine-induced precontraction and represent the mean ± SD of 6 experiments (*$P < 0.05$ and **$P < 0.01$ versus control value, paired Student's *t*-test).

study, double immunofluorescence labeling revealed pronounced TAS1R1/TAS1R3 IR primarily in the endothelial cells of the DPA, with minimal expression in SM cells. This finding contrasts with the localization of the bitter taste receptor TAS2R10 in rat CC, which was predominantly observed in SM cells (Navarro-Dorado et al., 2023). The slow relaxation of rat arterial and trabecular SM and the reduced response to MSG obtained in mechanically endothelium-denuded DPA rings may be attributed to the activation of the endothelial TAS1R1/-TAS1R3 heterodimer, which triggers the release of NO and EDHF. Although TAS1R1/TAS1R3 receptors are much less numerous in SM cells, their activation could still contribute marginally to penile vasodilation. Additionally, immunofluorescence studies demonstrated increased IR for the TAS1R1/TAS1R3 subunits co-localized with the neuronal marker PGP 9.5 in the nerve fibers of cavernous tissue samples, reinforcing the involvement of these receptors in modulating SM contractility. These findings are somewhat consistent with observations for the bitter taste receptor TAS2R10, which has also been detected in rat cavernous nerve fibers, albeit to a lesser extent (Navarro-Dorado et al., 2023).

In the present study, the slow-onset and sustained relaxations induced by high concentrations of MSG in the CC are likely mediated by an indirect mechanism, potentially involving the release of gasotransmitters such as NO or H_2S from nerve cells. This hypothesis is supported by the finding that omega-conotoxin GVIA, a blocker of neuronal N-type voltage-gated Ca^{2+} channels, consistently inhibits MSG-induced relaxations, thereby indicating a neurogenic component to this response. The activation of umami TAS1R1/TAS1R3 receptors in response to elevated MSG concentrations has been also previously reported in the mouse gastrointestinal tract, where increased peristalsis was observed following TAS1R1/TAS1R3 activation by 100 mM MSG in the mouse colon (Kendig et al., 2014). Furthermore, activation of TAS1R1/TAS1R3 by high MSG concentrations induces SM relaxation in the mouse stomach (Crowe et al., 2020).

In erectile tissue, PDE5 expression is notably high, playing a crucial role in modulating endogenous cGMP levels necessary for penile erection (Qiu et al., 2000; Corbin et al., 2002; Waldkirch et al., 2005). cGMP, in turn, enhances NO release from nitrergic neurons in rabbit cavernous tissue, underscoring the critical involvement of the NO/cGMP signaling pathway in nerve-mediated penile vasorelaxation (Hallen et al., 2005). In addition to PDE5, PDE4, a cAMP-specific phosphodiesterase, is present in the vascular endothelium and SM of human penile arteries and rat cavernous tissue. The presence of PDE4 suggests a functional interplay between cAMP- and cGMP-dependent pathways in regulating penile SM tone (Uckert et al., 2004; Waldkirch et al., 2005, 2010; Fernandes et al., 2022). Both NO (Burnett et al., 1996; Jung et al., 1997; González-Cadavid et al., 2000) and H_2S (d'Emmanuele di Villa Bianca et al., 2009; Zhang et al., 2016) are crucial for nerve-mediated relaxation of human cavernous SM, with their interplay being critical for erection (Meng et al., 2013; Mostafa et al., 2019; Yilmaz-Oral et al., 2020). In this study, inhibition of both H_2S and NO synthesis led to a decrease in endogenous H_2S production. Although the CSE inhibitor PPG has limited specificity and membrane permeability and may be subject to interference from other reactive sulfur species during H_2S

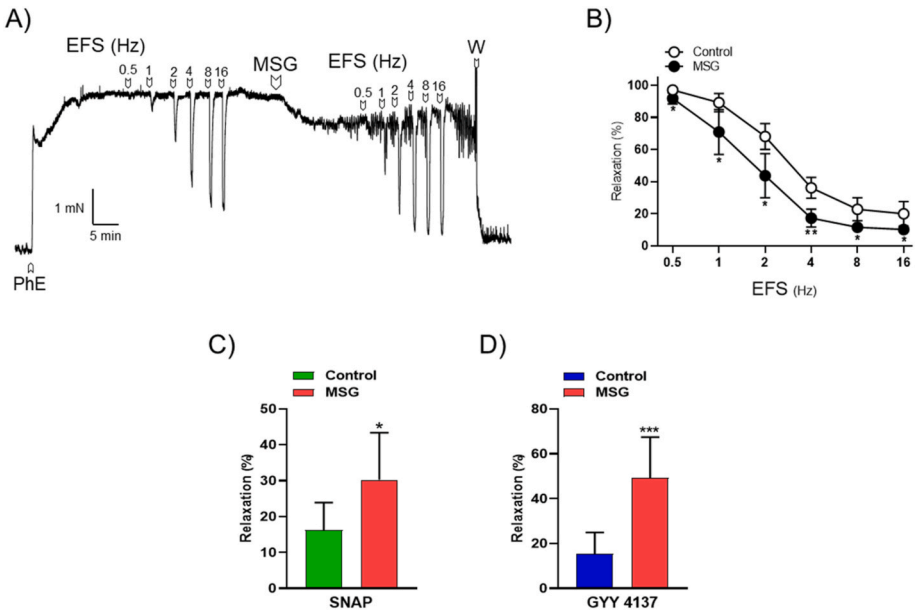

Fig. 4. MSG enhances relaxation induced by gaseous neurotransmitters in the rat corpus cavernosum. Original isometric force recordings demonstrate frequency-dependent relaxation in response to electrical field stimulation (EFS, 0.5–16 Hz, 1 ms pulse duration, and 20-s trains) both in the absence and presence of a threshold concentration of MSG (30 mM). EFS was applied to rat corpus cavernosum (CC) strips pre-contracted with 3 μM phenylephrine (PhE) and pretreated with guanethidine (10 μM) and atropine (1 μM). The vertical axis indicates tension (mN), while the horizontal axis represents time (min). W: wash (A). The graphs show relaxation to EFS (B), SNAP (1 μM) (C), and GYY 4137 (1 μM) (D) in rat corpus cavernosum preparations, either untreated (control; open circles, green and blue bars) or treated with MSG (30 mM; closed circles, orange bar). Data are expressed as mean ± SD from 6 rats. Statistical significance was set as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control (paired Student's t-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Effect of CSE and NO synthase blockade and MSG on endogenous H_2S production in rat corpus cavernosum samples.

	n	*H_2S level (nmol/mg.min− 1)*
Control	9	8.8 ± 2.1
PPG (1 mM)	11	4.4 ± 1.7***
L-NOARG (100 μM)	9	6.0 ± 1.3*
MSG (30 mM)	7	7.7 ± 1.9
MSG (50 mM)	5	13.2 ± 3.7**
MSG (100 mM)	6	15.5 ± 3.3****
PPG + L-NOARG + MSG (50 mM)	5	9.0 ± 0.6

Results are expressed as mean ± SD of *n* experiments ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ versus control value, analysis of variance followed by Bonferroni method).

quantification by spectrophotometry, the findings indicate a marked increase in CSE-dependent H_2S production triggered by TAS1R1/-TAS1R3 activation. Additionally, the synthesis of H_2S by CSE is also associated with NO synthesis pathway, highlighting the collaborative interaction between these two gaseous neurotransmitters in cavernous tissue (Meng et al., 2013; Mostafa et al., 2019; Yilmaz-Oral et al., 2020). H_2S production in cavernous tissue samples was restored and augmented in a concentration-dependent manner with levels surpassing those of the controls at higher MSG concentrations. This suggests that the stimulation of neuronal TAS1R1/TAS1R3 enhances the production and release of inhibitory gaseous neurotransmitters. Additionally, the enhanced responses to GYY 4137, SNAP, and EFS at threshold MSG concentrations suggest that neuronal TAS1R1/TAS1R3 activation potentiates nerve-mediated relaxation, primarily via H_2S and, to a lesser extent, NO. The absence of ODQ's effect on MSG responses further indicates that MSG-induced relaxation in the CC occurs independently of the sGC–cGMP pathway. These results indicate that a significant population of prejunctional TAS1R1/TAS1R3 receptors plays a crucial role in regulating cavernous SM contractility. Moreover, the production of endogenous H_2S in cavernous tissue, which is dependent on CSE, involves a NO synthetic pathway, highlighting the synergistic interaction between these two gasotransmitters (Navarro-Dorado et al., 2023). Neuronal TAS1R1/TAS1R3 activation likely enhances neuronal Ca^{2+} currents via plasma membrane voltage sensitive Ca^{2+} channels, leading to Ca^{2+} influx and membrane depolarization in response to action potentials at the motor nerve endings. The increase in neuronal cytosolic Ca^{2+}, through its interaction with calmodulin, leads to the activation of CSE and neuronal NOS. This activation facilitates the synthesis of H_2S and NO from L-cysteine and L-arginine, respectively, and promotes their release, leading to SM relaxation (Navarro-Dorado et al., 2023). Considering that ED, overactive bladder, and bladder outlet obstruction can coexist, drugs targeting these organs simultaneously might provide synergistic and beneficial pharmacological effects for patients with these conditions (Hedlund, 2005; McVary et al., 2005; Irwin et al., 2008; Kohler and McVary, 2009; Gacci et al., 2011). Given that the TAS1R1/TAS1R3 heterodimer has been proposed as a target for pathologies associated with impaired SM contractility (Kendig et al., 2014; Crowe et al., 2020), the significant presence of neuronal and endothelial umami receptors in rat erectile tissue, and their activation producing SM relaxation, suggests a potential role for TAS1R1/TAS1R3 activation in alleviating ED. However, the high concentrations of MSG required to induce SM relaxation may limit its therapeutic application.

Metabolic syndrome (MS) is a cluster of metabolic disorders and cardiovascular risk factors, including central obesity, type 2 diabetes mellitus, dyslipidemia, and hypertension, that share a common pathophysiological basis characterized by insulin resistance, compensatory hyperinsulinemia, and glucose intolerance (Ford et al., 2002). Given the well-established association between MS and ED (Esposito et al., 2005; Corona et al., 2006; Demir et al., 2006), further studies will be conducted to investigate the function and expression of neuronal TAS1R1/TAS1R3 receptors in the CC of the obese Zucker rat, a well-established genetic model of MS (Guerre-Millo, 1997).

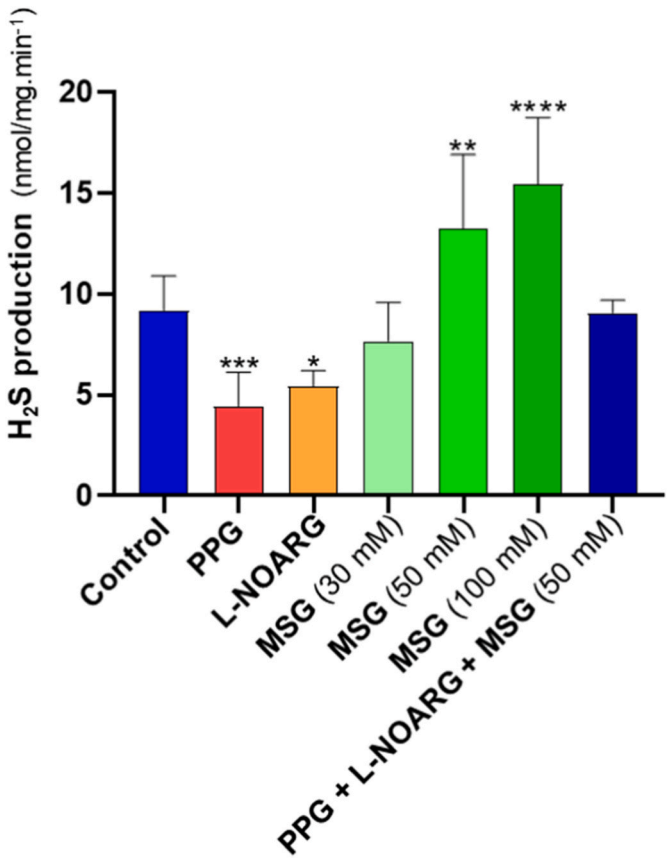

Fig. 5. MSG increases endogenous H2S production in the rat corpus cavernosum. H_2S levels were quantified under control conditions and in the presence of DL-propargylglycine (PPG, 1 mM) and N^G-nitro-L-arginine (L-NOARG, 100 μM), inhibitors of CSE and NO synthase, respectively. The effects of MSG were assessed at concentrations of 30, 50, and 100 mM. Data are presented as mean ± SD from 5 to 11 rats. Statistical significance was set at $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ compared to control (one-way ANOVA with Bonferroni post hoc test).

5. Conclusions

These results demonstrate a strong expression of neuronal and endothelial TAS1R1/TAS1R3 receptors in rat erectile tissue. Activation of these receptors by MSG induces SM relaxation and enhances inhibitory neurotransmission mediated by NO and H_2S. Therefore, TAS1R1/TAS1R3 receptors could be a valuable therapeutic target for managing ED. Nevertheless, the high concentration needed to produce SM relaxation may limit its therapeutic potential.

CRediT authorship contribution statement

Jorge Navarro-Dorado: Writing – original draft, Investigation, Formal analysis, Data curation. **Laura Juan-José Gibello:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **María Elvira López-Oliva:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **María Pilar Martínez:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Marina Hernández-Martín:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Belén Climent:** Writing – original draft, Supervision, Data curation, Conceptualization. **Ángel Agis-Torres:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Paz Recio:** Validation, Methodology, Investigation, Formal analysis, Data curation. **María Victoria Barahona:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara Benedito:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Vítor S. Fernandes:** Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization. **Medardo Hernández:** Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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.

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Data availability

Data will be made available on request.

References

Andersson, K.E., Wagner, G., 1995. Physiology of penile erection. Physiol. Rev. 75, 191–236. https://doi.org/10.1152/physrev.1995.75.1.191.

Angulo, J., Cuevas, P., Fernández, A., Gabancho, S., Videla, S., Sáenz de Tejada, I., 2003. Calcium dobesilate potentiates endothelium-derived hyperpolarizing factor-mediated relaxation of human penile resistance arteries. Br. J. Pharmacol. 139, 854–862. https://doi.org/10.1038/sj.bjp.0705293.

Blick, C., Ritchie, R.W., Sullivan, M.E., 2016. Is erectile dysfunction an example of abnormal endothelial function? Curr. Vasc. Pharmacol. 14, 163–167. https://doi.org/10.2174/1570161114666151202205950.

Burnett, A.L., Nelson, R.J., Calvin, D.C., Liu, J.X., Demas, G.E., Klein, S.L., Kriegsfeld, L. J., Dawson, V.L., Dawson, T.M., Snyder, S.H., 1996. Nitric oxide-dependent penile erection in mice lacking neuronal nitric oxide synthase. Mol. Med. 2, 288–296.

Busse, R., Edwards, G., Feletou, M., Fleming, I., Vanhoutte, P.M., Weston, A.H., 2002. EDHF: bringing the concepts together. Trends Pharmacol. Sci. 23, 374–380. https://doi.org/10.1016/s0165-6147(02)02050-3.

Coats, P., Johnston, F., MacDonald, J., McMurray, J.J., Hillier, C., 2001. Endothelium-derived hyperpolarizing factor : identification and mechanisms of action in human subcutaneous resistance arteries. Circulation 103, 1702–1708. https://doi.org/10.1161/01.cir.103.12.1702.

Coletta, C., Papapetropoulos, A., Erdelyi, K., Olah, G., Modis, K., Panopoulos, P., Asimakopoulou, A., Gero, D., Sharina, I., Martin, E., Szabo, C., 2012. Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. Proc. Natl. Acad. Sci. U. S. A. 109, 9161–9166. https://doi.org/10.1073/pnas.1202916109.

Corbin, J.D., Francis, S.H., Webb, D.J., 2002. Phosphodiesterase type 5 as a pharmacologic target in erectile dysfunction. Urology 60, 4–11. https://doi.org/10.1016/s0090-4295(02)01686-2.

Corona, G., Mannucci, E., Schulman, C., Petrone, L., Mansani, R., Cilotti, A., Balercia, G., Chiarini, V., Forti, G., Maggi, M., 2006. Psychobiologic correlates of the metabolic syndrome and associated sexual dysfunction. Eur. Urol. 50, 595–604. https://doi.org/10.1016/j.eururo.2006.02.053.

Crowe, M.S., Wang, H., Blakeney, B.A., Mahavadi, S., Singh, K., Murthy, K.S., Grider, J. R., 2020. Expression and function of umami receptors T1R1/T1R3 in gastric smooth muscle. Neuro Gastroenterol. Motil. 32, e13737. https://doi.org/10.1111/nmo.13737.

d'Emmanuele di Villa Bianca, R., Sorrentino, R., Maffia, P., Mirone, V., Imbimbo, C., Fusco, F., De Palma, R., Ignarro, L.J., Cirino, G., 2009. Hydrogen sulfide as a mediator of human corpus cavernosum smooth-muscle relaxation. Proc. Natl. Acad. Sci. U. S. A. 106, 4513–4518. https://doi.org/10.1073/pnas.0807974105.

Daly, K., Al-Rammahi, M., Moran, A., Marcello, M., Ninomiya, Y., Shirazi-Beechey, S.P., 2013. Sensing of amino acids by the gut-expressed taste receptor T1R1-T1R3 stimulates CCK secretion. Am. J. Physiol. Gastrointest. Liver Physiol. 304, G271–G282. https://doi.org/10.1152/ajpgi.00074.2012.

Demir, T., Demir, O., Kefi, A., Comlekci, A., Yesil, S., Esen, A., 2006. Prevalence of erectile dysfunction in patients with metabolic syndrome. Int. J. Urol. 13, 385–388. https://doi.org/10.1111/j.1442-2042.2006.01310.x.

Esposito, K., Giugliano, F., Martedì, E., Feola, G., Marfella, R., D'Armiento, M., Giugliano, D., 2005. High proportions of erectile dysfunction in men with the metabolic syndrome. Diabetes Care 28, 1201–1203. https://doi.org/10.2337/diacare.28.5.1201.

Fernandes, V.S., López-Oliva, M.E., Martínez, M.P., Agis-Torres, A., Recio, P., Navarro-Dorado, J., Barahona, M.V., Benedito, S., Prieto, D., Climent, B., Hernández, M., 2022. In vitro inhibition of phosphodiesterase type 4 enhances rat corpus cavernosum nerve-mediated relaxation induced by gasotransmitters. Life Sci. 296, 120432. https://doi.org/10.1016/j.lfs.2022.120432.

Ford, E.S., Giles, W.H., Dietz, W.H., 2002. Prevalence of the metabolic syndrome among US adults: findings from the third national health and nutrition examination survey. JAMA 287, 356–359. https://doi.org/10.1001/jama.287.3.356.

Francis, S.H., Corbin, J.D., 2003. Molecular mechanisms and pharmacokinetics of phosphodiesterase-5 antagonists. Curr. Urol. Rep. 4, 457–465. https://doi.org/10.1007/s11934-003-0027-x.

Gacci, M., Eardley, I., Giuliano, F., Hatzichristou, D., Kaplan, S.A., Maggi, M., McVary, K. T., Mirone, V., Porst, H., Roehrborn, C.G., 2011. Critical analysis of the relationship between sexual dysfunctions and lower urinary tract symptoms due to benign prostatic hyperplasia. Eur. Urol. 60, 809–825. https://doi.org/10.1016/j.eururo.2011.06.037.

Goldstein, I., Young, J.M., Fischer, J., Bangerter, K., Segerson, T., Taylor, T., Vardenafil Diabetes Study, G., 2003. Vardenafil, a new phosphodiesterase type 5 inhibitor, in the treatment of erectile dysfunction in men with diabetes: a multicenter double-blind placebo-controlled fixed-dose study. Diabetes Care 26, 777–783. https://doi.org/10.2337/diacare.26.3.777.

González-Cadavid, N.F., Burnett, A.L., Magee, T.R., Zeller, C.B., Vernet, D., Smith, N., Gitter, J., Rajfer, J., 2000. Expression of penile neuronal nitric oxide synthase variants in the rat and mouse penile nerves. Biol. Reprod. 63, 704–714. https://doi.org/10.1095/biolreprod63.3.704.

Guerre-Millo, M., 1997. Regulation of ob gene and overexpression in obesity. Biomed. Pharmacother. 51, 318–323. https://doi.org/10.1016/S0753-3322(97)88048-1.

Guimaraes, E.L., Dias, D.O., Hau, W.F., Julien, A., Holl, D., García-Collado, M., Savant, S., Vågesjö, E., Phillipson, M., Jakobsson, L., Göritz, C., 2024. Corpora cavernosa fibroblasts mediate penile erection. Science 383, eade8064. https://doi.org/10.1126/science.ade8064.

Hallen, K., Gustafsson, L.E., Wiklund, N.P., 2005. Nerve-induced release of nitric oxide from the rabbit corpus cavernosum is modulated by cyclic guanosine 3',5'-monophosphate. Neuroscience 133, 169–174. https://doi.org/10.1016/j.neuroscience.2005.02.015.

Hedlund, P., 2005. Nitric oxide/cGMP-mediated effects in the outflow region of the lower urinary tract–is there a basis for pharmacological targeting of cGMP? World J. Urol. 23, 362–367. https://doi.org/10.1007/s00345-005-0019-1.

Holmquist, F., Stief, C.G., Jonas, U., Andersson, K.E., 1991. Effects of the nitric oxide synthase inhibitor N^G-nitro-L-arginine on the erectile response to cavernous nerve stimulation in the rabbit. Acta Physiol. Scand. 143, 299–304. https://doi.org/10.1111/j.1748-1716.1991.tb09236.x.

Ignarro, L.J., Bush, P.A., Buga, G.M., Wood, K.S., Fukuto, J.M., Rajfer, J., 1990. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem. Biophys. Res. Commun. 170, 843–850. https://doi.org/10.1016/0006-291x(90)92168-y.

Irwin, D.E., Milsom, I., Reilly, K., Hunskaar, S., Kopp, Z., Herschorn, S., Coyne, K.S., Kelleher, C.J., Artibani, W., Abrams, P., 2008. Overactive bladder is associated with erectile dysfunction and reduced sexual quality of life in men. J. Sex. Med. 5, 2904–2910. https://doi.org/10.1111/j.1743-6109.2008.01000.x.

Johnstone, M.T., Creager, S.J., Scales, K.M., Cusco, J.A., Lee, B.K., Creager, M.A., 1993. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. Circulation 88, 2510–2516. https://doi.org/10.1161/01.cir.88.6.2510.

Jung, H.C., Mun, K.H., Park, T.C., Lee, Y.C., Park, J.M., Huh, K., Seong, D.H., Suh, J.K., 1997. Role of nitric oxide in penile erection. Yonsei Med. J. 38, 261–269. https://doi.org/10.3349/ymj.1997.38.5.261.

Kendig, D.M., Hurst, N.R., Bradley, Z.L., Mahavadi, S., Kuemmerle, J.F., Lyall, V., DeSimone, J., Murthy, K.S., Grider, J.R., 2014. Activation of the umami taste receptor (T1R1/T1R3) initiates the peristaltic reflex and pellet propulsion in the distal colon. Am. J. Physiol. Gastrointest. Liver Physiol. 307, G1100–G1107. https://doi.org/10.1152/ajpgi.00251.2014.

Kohler, T.S., McVary, K.T., 2009. The relationship between erectile dysfunction and lower urinary tract symptoms and the role of phosphodiesterase type 5 inhibitors. Eur. Urol. 55, 38–48. https://doi.org/10.1016/j.eururo.2008.08.062.

Li, L., Bhatia, M., Zhu, Y.Z., Zhu, Y.C., Ramnath, R.D., Wang, Z.J., Anuar, F.B., Whiteman, M., Salto-Tellez, M., Moore, P.K., 2005. Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. FASEB J. 19, 1196–1198. https://doi.org/10.1096/fj.04-3583fje.

Lima, A.T., Britto-Júnior, J., Moraes, M.O., Moraes, M.E.A., Fregonesi, A., Monica, F.Z., Antunes, E., De Nucci, G., 2024. 6-Nitrodopamine is an endogenous mediator of the rabbit corpus cavernosum relaxation. Andrology 12, 1419–1428. https://doi.org/10.1111/andr.135855.

Lu, K., McDaniel, A.H., Tordoff, M.G., Li, X., Beauchamp, G.K., Bachmanov, A.A., VanderWeele, D.A., Chapman, C.D., Dess, N.K., Huang, L., Wang, H., Reed, D.R., 2005. No relationship between sequence variation in protein coding regions of the Tas1r3 gene and saccharin preference in rats. Chem. Senses 30, 231–240. https://doi.org/10.1093/chemse/bji019Chem.

Martín-Morales, A., Sánchez-Cruz, J.J., Sáenz de Tejada, I., Rodríguez-Vela, L., Jiménez-Cruz, J.F., Burgos-Rodríguez, R., 2001. Prevalence and independent risk factors for erectile dysfunction in Spain: results of the Epidemiologia de la Disfuncion Erectil Masculina Study. J. Urol. 166, 569–575. https://doi.org/10.1016/s0022-5347(05)65986-1.

McVary, K.T., Rademaker, A., Lloyd, G.L., Gann, P., 2005. Autonomic nervous system overactivity in men with lower urinary tract symptoms secondary to benign prostatic hyperplasia. J. Urol. 174, 1327–1433. https://doi.org/10.1097/01.ju.0000173072.73702.64.

McVeigh, G.E., Brennan, G.M., Johnston, G.D., McDermott, B.J., McGrath, L.T., Henry, W.R., Andrews, J.W., Hayes, J.R., 1992. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 35, 771–776. https://doi.org/10.1007/BF00429099.

Meng, J., Ganesan Adaikan, P., Srilatha, B., 2013. Hydrogen sulfide promotes nitric oxide production in corpus cavernosum by enhancing expression of endothelial nitric oxide synthase. Int. J. Impot. Res. 25, 86–90. https://doi.org/10.1038/ijir.2012.39.

Mostafa, T., Rashed, L., Nabil, N., Abo-Sief, A.F., Mohamed, M.M., Omar, M.S., 2019. Cavernosal hydrogen sulfide levels are associated with nitric oxide and hemeoxygenase levels in diabetic rats. Int. J. Impot. Res. 31, 105–110. https://doi.org/10.1038/s41443-018-0084-9.

Muroi, Y., Ishii, T., 2012. Umami taste receptor functions as an aminoacid sensor via Gαos subunit in N1E-115 neuroblastoma cells. J. Cell. Biochem. 113, 1654–1662. https://doi.org/10.1002/jcb.24034.

Navarro-Dorado, J., Climent, B., López-Oliva, M.E., Martínez, M.P., Hernández-Martín, M., Agis-Torres, A., Recio, P., Barahona, M.V., Benedito, S., Fernandes, V.S., Hernández, M., 2023. The bitter taste receptor (TAS2R) agonist denatonium promotes a strong relaxation of rat corpus cavernosum. Biochem. Pharmacol. 215, 115754. https://doi.org/10.1016/j.bcp.2023.115754.

Oya, M., Suzuki, H., Watanabe, Y., Sato, M., Tsuboi, T., 2011. Amino acid taste receptor regulates insulin secretion in pancreatic β-cell line MIN6 cells. Genes Cells 16, 608–616. https://doi.org/10.1111/j.1365-2443.2011.01509.x.

Qiu, Y., Kraft, P., Lombardi, E., Clancy, J., 2000. Rabbit corpus cavernosum smooth muscle shows a different phosphodiesterase profile than human corpus cavernosum. J. Urol. 164, 882–886. https://doi.org/10.1097/00005392-200009010-00066.

Reed, D.R., Li, S., Li, X., Huang, L., Tordoff, M.G., Starling-Roney, R., Taniguchi, K., West, D.B., Ohmen, J.D., Beauchamp, G.K., Bachmanov, A.A., 2004. Polymorphisms in the taste receptor gene (*Tas1r3*) region are associated with saccharin preference in 30 mouse strains. J. Neurosci. 24, 938–946. https://doi.org/10.1523/JNEUROSCI.1374-03.2004.

Reffelmann, T., Kloner, R.A., 2005. Pharmacotherapy of erectile dysfunction: focus on cardiovascular safety. Expet Opin. Drug Saf. 4, 531–540. https://doi.org/10.1517/14740338.4.3.531.

Rendell, M.S., Rajfer, J., Wicker, P.A., Smith, M.D., 1999. Sildenafil for treatment of erectile dysfunction in men with diabetes: a randomized controlled trial. Sildenafil diabetes study group. JAMA 281, 421–426. https://doi.org/10.1001/jama.281.5.421.

Sáenz de Tejada, I., Anglin, G., Knight, J.R., Emmick, J.T., 2002. Effects of tadalafil on erectile dysfunction in men with diabetes. Diabetes Care 25, 2159–2164. https://doi.org/10.2337/diacare.25.12.2159.

Shirazi-Beechey, S.P., Daly, K., Al-Rammahi, M., Moran, A.W., Bravo, D., 2014. Role of nutrient-sensing taste 1 receptor (T1R) family members in gastrointestinal chemosensing. Br. J. Nutr. 111, S8–S15. https://doi.org/10.1017/S0007114513002286.

Simonsen, U., García-Sacristán, A., Prieto, D., 2002. Penile arteries and erection. J. Vasc. Res. 39, 283–303. https://doi.org/10.1159/000065541.

Srilatha, B., Adaikan, P.G., Li, L., Moore, P.K., 2007. Hydrogen sulphide: a novel endogenous gasotransmitter facilitates erectile function. J. Sex. Med. 4, 1304–1311. https://doi.org/10.1111/j.1743-6109.2007.00561.x.

Uckert, S., Hedlund, P., Waldkirch, E., Sohn, M., Jonas, U., Andersson, K.E., Stief, C.G., 2004. Interactions between cGMP- and cAMP-pathways are involved in the regulation of penile smooth muscle tone. World J. Urol. 22, 261–266. https://doi.org/10.1007/s00345-003-0394-4.

Vancleef, L., Thijs, T., Baert, F., Ceulemans, L.J., Canovai, E., Wang, Q., Steensels, S., Segers, A., Farre, R., Pirenne, J., Lannoo, M., Tack, J., Depoortere, I., 2018. Obesity impairs oligopeptide/amino acid-induced ghrelin release and smooth muscle contractions in the human proximal stomach. Mol. Nutr. Food Res. 62. https://doi.org/10.1002/mnfr.201700804.

Waldkirch, E.S., Uckert, S., Yildirim, H., Sohn, M., Jonas, U., Stief, C.G., Andersson, K.E., Hedlund, P., 2005. Cyclic AMP-specific and cyclic GMP-specific phosphodiesterase isoenzymes in human cavernous arteries–immunohistochemical distribution and functional significance. World J. Urol. 23, 405–410. https://doi.org/10.1007/s00345-005-0026-2.

Waldkirch, E.S., Uckert, S., Sigl, K., Satzger, I., Geismar, U., Langnase, K., Richter, K., Sohn, M., Kuczyk, M.A., Hedlund, P., 2010. Expression of cyclic AMP-dependent protein kinase isoforms in human cavernous arteries: functional significance and relation to phosphodiesterase type 4. J. Sex. Med. 7, 2104–2111. https://doi.org/10.1111/j.1743-6109.2010.01808.x.

Xu, H., Staszewski, L., Tang, H., Adler, E., Zoller, M., Li, X., 2004. Different functional roles of T1R subunits in the heteromeric taste receptors. Proc. Natl. Acad. Sci. U. S. A. 101, 14258–14263. https://doi.org/10.1073/pnas.0404384101.

Yilmaz-Oral, D., Kaya-Sezginer, E., Oztekin, C.V., Bayatli, N., Lokman, U., Gur, S., 2020. Evaluation of combined therapeutic effects of hydrogen sulfide donor sodium hydrogen sulfide and phosphodiesterase type-5 inhibitor tadalafil on erectile dysfunction in a partially bladder outlet obstructed rat model. Neurourol. Urodyn. 39, 1087–1097. https://doi.org/10.1002/nau.24333.

Zhang, Y., Yang, J., Wang, T., Wang, S.G., Liu, J.H., Yin, C.P., Ye, Z.Q., 2016. Decreased endogenous hydrogen sulfide generation in penile tissues of diabetic rats with erectile dysfunction. J. Sex. Med. 13, 350–360. https://doi.org/10.1016/j.jsxm.2016.01.002.

Zietman, A.L., Coen, J.J., Shipley, W.U., Willett, C.G., Efird, J.T., 1994. Radical radiation therapy in the management of prostatic adenocarcinoma: the initial prostate specific antigen value as a predictor of treatment outcome. J. Urol. 151, 640–645. https://doi.org/10.1016/s0022-5347(17)35036-x.