Nitric Oxide 65 (2017) 10–21

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Analgesic effect of dimethyl trisulfide in mice is mediated by TRPA1 and sst_4 receptors

Gábor Pozsgai [a, *], Maja Payrits [a], Éva Sághy [a], Réka Sebestyén-Bátai [a], Elise Steen [a], Éva Szőke [a], Zoltán Sándor [a], Margit Solymár [b], András Garami [b], Péter Orvos [c, d], László Tálosi [c], Zsuzsanna Helyes [a], Erika Pintér [a]

[a] Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Szigeti utca 12, Pécs, H-7624, Hungary
[b] Institute for Translational Medicine, Medical School, University of Pécs, Szigeti utca 12, Pécs, H-7624, Hungary
[c] Rytmion Ltd., Ősz utca 27, H-6724, Szeged, Hungary
[d] Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Dóm tér 12, H-6720, Szeged, Hungary

ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form
25 January 2017
Accepted 25 January 2017
Available online 28 January 2017

Keywords:
TRPA1
Dimethyl trisulfide
Somatostatin
sst_4
Mechanical hyperalgesia
Spontaneous locomotor activity

ABSTRACT

TRPA1 receptors are calcium-permeable ligand-gated channels expressed in primary sensory neurons and involved in inflammation and pain. Activation of these neurons might have analgesic effect. Suggested mechanism of analgesic effect mediated by TRPA1 activation is the release of somatostatin (SOM) and its action on sst_4 receptors. In the present study analgesic effect of TRPA1 activation on primary sensory neurons by organic trisulfide compound dimethyl trisulfide (DMTS) presumably leading to SOM release was investigated. Opening of TRPA1 by DMTS in CHO cells was examined by patch-clamp and fluorescent Ca^{2+} detection. Ca^{2+} influx upon DMTS administration in trigeminal ganglion (TRG) neurons of TRPA1 receptor wild-type (WT) and knockout (KO) mice was detected by ratiometric Ca^{2+} imaging. SOM release from sensory nerves of murine skin was assessed by radioimmunoassay. Analgesic effect of DMTS in mild heat injury-induced mechanical hyperalgesia was examined by dynamic plantar aesthesiometry. Regulatory role of DMTS on deep body temperature (T_b) was measured by thermocouple thermometry with respirometry and by telemetric thermometry. DMTS produced TRPA1-mediated currents and elevated $[Ca^{2+}]_i$ in CHO cells. Similar data were obtained in TRG neurons. DMTS released SOM from murine sensory neurons TRPA1-dependently. DMTS exerted analgesic effect mediated by TRPA1 and sst_4 receptors. DMTS-evoked hypothermia and hypokinesis were attenuated in freely-moving TRPA1 KO animals. Our study has presented original evidence regarding analgesic action of DMTS which might be due to TRPA1-mediated SOM release from sensory neurons and activation of sst_4 receptors. DMTS could be a novel analgesic drug candidate.

1. Introduction

Biological effects of the gasotransmitter hydrogen sulfide (H_2S) are well characterised [63]. It has been noted recently that solutions of H_2S uniformly contain polysulfide species. These compounds might be responsible for the biological effects earlier attributed to the gasotransmitter by S-sulfhydration of proteins [18]. Inorganic polysulfides were reported to activate transient receptor potential ankyrin-1 (TRPA1) receptors of astrocytes, murine dorsal root ganglion neurons and being protective against oxidative damage [20,30,31,33]. Organic trisulfides are structurally related to polysulfides, but they participate in different reactions with cysteine sulfhydryl groups [37]. Some organic trisulfides have been detected to release H_2S [5,66]. H_2S solutions uniformly contain inorganic

Abbreviations: TRPA1, transient receptor potential ankyrin 1; DMTS, dimethyl trisulfide; SOM, somatostatin; T_b, deep body temperature; sst_4, somatostatin receptor type 4; TRG, trigeminal ganglion; WT, wild-type; KO, knockout; CHO, Chinese hamster ovary; H_2S, hydrogen sulfide; VO_2, oxygen consumption; DMEM, Dulbecco's-Modified Eagle Medium; PBS, phosphate-buffered saline; NGF, nerve growth factor; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; ECS, extracellular solution; AITC, allylisothiocyanate; CMV, cytomegalovirus; EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid; NMDG, *N*-methyl-D-glucamine; DMSO, dimethyl sulfoxide; CFA, complete Freund's adjuvant; TRPV1, transient receptor potential vanilloid 1; Ca_V 3.2, T-type voltage-gated calcium channel type 3.2; DRG, dorsal root ganglion; NaHS, sodium hydrosulfide; K_V 4.3, voltage-gated potassium channel type 4.3.

* Corresponding author.
E-mail address: gabor.pozsgai@aok.pte.hu (G. Pozsgai).

http://dx.doi.org/10.1016/j.niox.2017.01.012
1089-8603/

polysulfides. TRPA1 activation by inorganic polysulfides or organic trisulfides has not yet been characterised electrophysiologically. It has been shown previously that H_2S induces suspended animation in mice, but thermoregulatory response to organic trisulfides has not been investigated yet [6]. An organic trisulfide compound was shown to exhibit anti-inflammatory properties in human ulcerative colitis and in an animal model of the disease [2,35]. These findings are surprising since H_2S is dominantly associated with pro-algesic effects. Dimethyl trisulfide (DMTS), an organic trisulfide has been patented in the USA for i.m. administration in cyanide intoxication, increasing translational potential of the drug significantly [34].

TRPA1 channel is the only mammalian member of transient receptor potential ankyrin receptor subfamily. It is mainly expressed in unmyelinated and thinly myelinated (C and Aδ) nociceptive primary sensory neurons. More than 25% of TRPA1 positive neurons are peptidergic – they contain calcitonin gene-related peptide, substance P and somatostatin (SOM) [67]. TRPA1 receptors can be activated by various stimuli ranging from temperature to chemicals. Electrophilic agents excite TRPA1 by forming covalent bonds with cysteine residues of the receptor. Activation of TRPA1 induces release of sensory neuropeptides, such as SOM [60]. Participation of TRPA1 receptors in hyperalgesia is well documented [9,41,44].

SOM is a cyclic peptide that appears in the central nervous system and peripheral tissues [13,58]. SOM might be released into the circulation from peptidergic sensory neurons upon their activation [23,55]. A subpopulation of these neurons expresses TRPA1 receptor. Neuron-derived SOM might exert systemic anti-nociceptive and anti-inflammatory effects [48]. These actions of SOM might be mediated by sst_4 receptors [21,22,38,47,51]. Mild heat injury-induced thermal hyperalgesia was also diminished by SOM treatment [38].

The working hypothesis of the present study is that alkylated organic trisulfide compound DMTS might activate TRPA1 receptors of primary sensory neurons inducing release of the neuropeptide SOM that might elicit analgesic effect in animal models of nociception via sst_4 receptors. Actions of DMTS on human TRPA1 receptors expressed in Chinese Hamster Ovary (CHO) cells were characterised by patch-clamp and fluorescent Ca^{2+} detection. Effects of DMTS on TRPA1 receptor-mediated Ca^{2+} signals of murine trigeminal (TRG) neurons were investigated by ratiometric measurement of intracellular free Ca^{2+} concentration. SOM release from sensory neurons of isolated murine skin in response to DMTS was assessed by radioimmunoassay. Analgesic effect in mild heat-injury-induced mechanical hyperalgesia was examined in mice. Changes of deep body temperature (T_b), oxygen consumption (VO_2) and spontaneous locomotor activity in response to DMTS were also studied. Involvement of TRPA1 and sst_4 receptors was tested with gene knockout mice.

2. Materials and methods

2.1. Animals

Primary cultures of trigeminal ganglion neurons were taken from 1 to 3 days old TRPA1 wild-type (WT) and knockout (KO) mice [4]. Mechanical hyperalgesia studies involved female TRPA1 and sst_4 receptor WT and KO mice 2–4 months, 20–25 g; [22]. SOM release studies used TRPA1 WT and KO mice (2–4 months, 20–25 g). Thermophysiological experiments were performed in TRPA1 WT and KO adult (2–4 months) female mice weighing 20–25 g. Age-matched mice were used in the study. Animals were bred and kept in the Laboratory Animal Centre of University of Pécs under standard pathogen free conditions at 24–25 °C, 12 h light/dark cycles. Mice were housed in groups of 5–10 in polycarbonate cages (330 cm^2 floor space, 12 cm height) on wood shavings bedding. Animals were provided standard diet and water ad libitum. All experimental procedures were carried out according to the European Communities Council Directive of 2010/63/EU. The studies were approved by the Ethics Committee on Animal Research, University of Pécs.

2.2. Construction of a cell lines expressing the human TRPA1 and TRPV1 receptor

Human TRPA1 cDNA clone was obtained from the Life Technologies CloneRanger collection (clone ID: 100016279). Human TRPV1 cDNA clone was obtained from the OriGene Inc (TrueClone ID:TC109883). The cDNA was inserted between the CMV promoter and the bovine growth hormone polyA region in pT3CMV vector (unpublished). pT3CMV is a derivate of the pT2/BH Sleeping Beauty transposon vector (Addgene, ID: 26556) which also contains a neomycin expression cassette between the inverted repeats. CHO cells were co-transfected with vectors containing human TRPA1 or TRPV1 cDNA and the pCMVSB100x vector expressing the transposase. Clones with the integrated transposon were selected in medium containing G418 (500 μg/mL). The presence of the functional human TRPA1 or TRPV1 receptors was checked by Fura-2 microfluorimetry and Fluo-4 flowcytometry detecting mustard oil- or capsaicin-induced calcium influx into the CHO cells.

2.3. Measurement of Ca^{2+} influx in TRPA1-expressing CHO cells in response to dimethyl trisulfide by flow cytometry

Culture medium (500 mL Dulbecco's-Modified Eagle Medium (DMEM), 50 mL fetal bovine serum albumin, 10 mL L-glutamine (200 mmol/L), 10 mL MEM non-essential amino acid solution, 500 μL penicillin and streptomycin) was gently removed from cells and trypsin solution (250 μL, 0.1% in PBS) was applied for 5 min. For each sample approximately 10^4 TRPA1-expressing CHO cells were resuspended in 100 μL cell culture medium. Fluo-4 AM (Invitrogen, 0.4 μL, 1 μg/μL in DMSO) was added for 30 min at 37 °C. Extracellular solution (ECS) was added (400 μL, containing (in mmol/L): NaCl, 160; KCl, 2.5; $CaCl_2$, 1; $MgCl_2$, 2; HEPES, 10; glucose, 10; pH 7.3). Appropriate amount of DMTS was added to the cell suspensions in 500 μL ECS. Cell suspensions were analysed by flow cytometry. Fluo-4 AM was excited by 488 nm laser. Fluorescence was detected at 504 nm. Mean green fluorescence of the samples was compared to base fluorescence of dye-loaded control cells. Some cell groups were pre-incubated with selective TRPA1 receptor antagonist HC-030031 (1, 10, 50 and 100 μmol/L in ECS) for 5 min.

2.4. Detection of dimethyl trisulfide-induced TRPA1 receptor activation in CHO cells by automated patch-clamp

Automated patch-clamp experiments were carried out on CHO cells stably transfected with TRPA1. Cell suspension for measurements was derived from running cell culture. Cells were maintained at 37 °C, 5% CO_2 in DMEM (Gibco) supplemented with 10% fetal calf serum, non-essential amino acids, and antibiotics. Medium was supplemented 500 μg/ml G418. Cells were no more than 40–50% confluent during culture procedure. Cells were washed twice with PBS (Life Technologies) before experiments and then TrypLE (ThermoFisher) was added. TrypLE was removed immediately and the cells were kept on room temperature for 2 min in the remaining film-layer. Cells were then resuspended in 1–2 mL external solution and kept in the cell hotel of the Patchliner. Resuspension protocol of the cell hotel was switched off. Under these conditions cells remained suitable for automated patch-clamp recordings for 1–2 h. Patch-clamp experiments were