Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol12Issue3abst095P.pdf

Analgesic Effect of Polysulfide Compound Dimethyl Trisulfide Is Mediated via TRPA1 Receptors

G Pozsgai, E Saghy, M Payrits, E Szoke, E Steen, Z Helyes, E Pinter. University of Pecs, Pecs, Hungary

Polysulfides were shown to initiate S-sulfhydration of proteins (1). They were reported to activate TRPA1 receptors on astrocytes (2). TRPA1 receptors are mostly expressed in unmyelinated and thinly myelinated nociceptive primary sensory neurons and contribute to mechanical hyperalgesia. Activation of these neurons might lead to neuropeptide release, including somatostatin (SOM). Systemic antinociceptive and anti-inflammatory effects of SOM are mediated by sst_4 receptors (3). The working hypothesis of this study was that polysulfide dimethyl trisulfide (DMTS) might activate primary sensory neurons and induces SOM release. SOM might elicit analgesic effect.

Ca^{2+} influx in TRG neurons of TRPA1 receptor wild-type (WT) and knockout (KO) mice was detected by ratiometric Ca^{2+} imaging with fura-2-AM. SOM release from isolated tracheae of Wistar rats was measured by radioimmunoassay. Heat injury was induced in one hind paw of TRPA1 or sst_4 WT and KO mice by submerging the paw into 51°C water for 15 s under diethyl ether anaesthesia. Mechanical pain threshold of the hind limbs was then determined by dynamic plantar aesthesiometry every 10 min for 60 min. Baseline measurments were taken before heat injury. DMTS in saline containing maximum 5% dimethyl sulfoxide and 1% Tween 80 or vehicle was administered i.p. 30 min before heat challenge. All experimental procedures were carried out according to the European Communities Council Directive of 2010/63/EU and were approved by the Ethics Committee on Animal Research, University of Pécs. Data are expressed as mean±SEM. Analysis was performed by one-way ANOVA followed by Dunnett's and Bonferroni's test or two-way ANOVA followed by Bonferroni's test for SOM release data and mechanical pain threshold data, respectively. Values of $p<0.05$ were regarded as significant.

DMTS induced reproducible elevations of $[Ca^{2+}]_i$ in TRG neurons of TRPA1 WT animals. The response was inhibited by TRPA1 receptor antagonist HC-030031 (10 μmol/L) and was absent in neurons from TRPA1 KO mice. DMTS induced concentration-dependent SOM release from isolated rat tracheae with (EC_{50}=80±0.1 μmol/L, 1.5±0.1 fmol/mg vs. 2.1±0.2 fmol/mg basal and stimulated SOM release at 100 μmol/L DMTS, n=7). SOM release was not affected by HC-030031. DMTS in 100 and 500 μmol/kg doses had analgesic activity in mild heat injury-evoked mechanical hyperalgesia in TRPA1 WT mice (-59.9±4.4% vs. -37±4.2% and -19.1±5.1% changes in pain threshold at 50 min in challenged hind paws for 100 and 500 μmol/kg DMTS, respectively, n=7-9). DMTS lacked analgesic effect in TRPA1 KO animals. DMTS had similar effect in sst_4 receptor WT mice (-59.9±3.7% vs. -33.4±4.6% and -22.5±4.6% at 50 min for 100 and 500 μmol/kg DMTS, respectively, n=7-9). The influence of 100 μmol/kg DMTS on mechanical pain threshold was

abolished in sst_4 KO mice, whereas that of 500 μmol/kg DMTS was unaffected (-67.6±3.6% vs. -29.6±5.4% at 30 min, n=5-6).

The present study provides the first data on the effect of polysulfides on neuropeptide release, as well as on their *in vivo* analgesic effect and *in vivo* activation of TRPA1 by polysulfides. Analgesic effect of DMTS is mediated via TRPA1 receptors. SOM released from sensory neurons might mediate this effect at 100 μmol/kg dose, but other mechanisms are involved at 500 μmol/kg.

This study was funded by grants OTKA PD-112171 and SROP-4.2.2.A-11/1/KONV-2012-0024.

(1) Greiner R *et al.* (2013). *Antioxid Redox Signal* **19**: 1749-1765.
(2) Kimura Y *et al.* (2013). *FASEB J* **27**: 2451-2457.
(3) Helyes Z *et al.* (2006). *Br J Pharmacol* **149**: 405-415.