Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol12Issue3abst203P.pdf

Characterization of TRPA1-receptor-mediated cellular and microvascular changes induced by hydrogen sulphide

E PINTER, Zs HAJNA, G POZSGAI, E SZOKE, E SAGHY, M PAYRITS, Zs HELYES. University of Pecs, Pecs, Hungary

The gaseous mediator hydrogen sulphide (H2S) has been suggested to activate transient receptor potential ankyrin 1 (TRPA1) receptors. The present study aimed to analyse the involvement of TRPA1 receptors in the H2S-induced [Ca2+]i increase in trigeminal ganglia neurons (TRG), as well as the role of capsaicin-sensitive sensory neurons and the released pro-inflammatory sensory neuropeptides, CGRP and SP in the H2S-evoked vasodilatation, in vivo.

In vitro [Ca2+]i was investigated with fluorescent indicator fura-2-AM on primary cultures of trigeminal ganglia (TRG) neurons of TRPA+/+ and TRPA1-/- mouse pups. Cells were treated with NaHS and Na2S, allylisothiocyanate (AITC) or KCl. Data are represented as mean ± standard error of mean (SEM). Cutaneous microcirculation was detected in ketamine-xylazine anaesthesia (100 mg/kg and 5 mg/kg, s.c.) by in vivo laser Doppler imaging. Ears of Balb/c, C57BL/6, and NK1-/- mice were treated with NaHS (5%), AITC (2%), NaOH (5%), NaSO3 (5%) or NaCl (5%). C57Bl/6 mice received pretreatment with TRPV1 receptor agonist resiniferatoxin (RTX 10, 20, 30, 70 and 100 μg/kg s.c.). Balb/c mice got CGRP1 receptor antagonist BIBN4096 (0.1-10 mg/kg i.p.) or NK1 receptor antagonist CP-99994 (10-50 mg/kg i.p.), or both, or K+ATP channel blocker glibenclamide (50 mg/kg i.p.) (1). Results of groups were compared by one way ANOVA followed by Bonferroni's post hoc test.

H2S increased [Ca2+]i in TRG neurons of wildtype mice. Most of the NaHS responsive cells also reacted to AITC. NaHS and Na2S had no effect on trigeminal neurons of TRPA1-/- mice.

AITC treatment of mouse ears led to 29.8±2.8% increase in blood flow. NaHS elevated cutaneous blood flow of the mouse ears by 61±4.5%. Treatment with NaOH, NaSO3 and NaCl did not cause any changes in the blood flow. Effect of NaHS on microcirculation was ameliorated by HC-030031, BIBN4096 as well as CP-99994 (n= 5-6/group). Blood flow of TRPA1 KO and NK1 KO mice showed significantly smaller increase in response to NaHS compared to the wild-type counterparts (n= 5-6/group). Microcirculatory responses to NaHS were reduced to 19.14% in RTX-pretreated animals (n= 5-6/group).Our in vivo experiments revealed first that cutaneous vasodilatation induced by topically applied H2S is partly mediated by capsaicin-sensitive sensory neurons. Increase of [Ca2+]i through the activation of TRPA1 receptors is a potential mechanism of these fibres. In the mediation of H2S-induced vasodilatation, pro-inflammatory neuropeptides of sensory neural origin such as CGRP and SP play a crucial role through the activation of CGRP1 and the NK1 receptors. Beside the neurogenic inflammatory component of exogenous H2S-induced vasodilation, this response is partly mediated by K+ATP-channels.

(1) Pozsgai G et al. (2012). Eur J Pharmacol. **689**:56-64.

Supported by the Hungarian Brain Research Program KTIA_NAP_13-1-2013-0001.