Is TRPA1 *S*-nitrosylation involved in the inflammatory pain?

L.A. Giancotti[1], F. Lauro[1], S. Ilari[1], C. Montagna[2], M. Gliozzi[1], C. Morabito[3,4], E. Palma[1], G. Di Giacomo[5], V. Mollace[1], G. Filomeni[3,5], C. Muscoli[1]

[1]IRC_FSH, Dept. of Health Sciences, University of Catanzaro Magna Graecia, Catanzaro, Italy
[2]Cell Stress and Survival Unit, Danish Cancer Society Research Center, Copenhagen, Denmark
[3]Research Center, IRCCS San Raffaele Pisana, Rome, Italy
[4]Dept. of Experimental Medicine, University of Rome 'Sapienza', Italy
[5]Dept. of Biology, University of Tor Vergata, Rome, Italy

Recent studies demonstrated the critical role of nitroxidative stress in the development of inflammatory pain and thermal hyperalgesia. Peroxynitrite ($ONOO^-$), generated by the interaction between superoxide (SO) and nitric oxide (NO), is a potent proinflammatory and cytotoxic nitroxidative species leading to the development and maintenance of pain of several etiologies through post-translational nitration of proteins as glutamine synthase (GS), glutamate transporter 1 (GLT1) and glutamate receptor NMDA, key regulators of pain-relevant pathways.
Transient receptor potential ankyrin (TRPA1), a non-selective calcium permeable cation channel, expressed by a subpopulation of primary afferent nociceptive nerve fibres, is also involved in thermosensation and nociception. It is activated not only by exogenous irritans including cisplatin or acrolein but also by a structurally diverse series of oxidative stress byproducts formed during inflammatory reactions such as hydrogen peroxide or 4-hydroxy-trans-2-nonenal (4-HNE).
Since TRPA1 can be activated by covalent modification such as addition of an NO moiety to a reactive sulfhydryl (*S*-nitrosylation) of specific cysteine residues, here we tested the hypothesis that TRPA1 *S*-nitrosylation is involved in hyperalgesia and acute inflammatory pain.
Rats received a subplantar injection of carrageenan (0.1 ml of a 1% suspension in 0.85% saline), a common inflammatory agent, into the hindpaw while antioxidants or vehicle were administered intraperitoneally.
Changes in paw volume were measured by a plethysmometer immediately before the injection of carrageenan and thereafter at hourly intervals for 5 h.
Measurements of thermal hyperalgesia were determined by Plantar Test apparatus according to the Hargreaves' method.
After testing, all the animals were sacrificed and lumbar spinal cord (L4-L6) was removed and used for further analysis as western blotting and *S*-nitrosylated proteins (PSNOs) assay.
Our results demonstrate that intraplantar injection of carrageenan in rats led to time-dependent
development of thermal hyperalgesia and peripheral inflammation as evidenced by paw edema. Pretreatment of rats with antioxidant blocks measured parameters of inflammation and hyperalgesia. Our results also show that during hyperalgesia peak, spinal TRPA1 was nitrosylated and subsequently activated. Furthermore, the antihyperalgesic effect of tested antioxidant is linked to inhibition of TRPA1 nitroxidative modification. To better dissect the contribution of S-nitrosylation to TRPA1 modulation, we employed cells over expressing TRPA1 and then proceed with the silencing of GSNOR (*S*-nitrosoglutathione reductase). The cells maintain the capability to produce NO, but are unable to reduce *S*-nitrosothiols.
These results provide, for the first time, a crucial evidence of the role of *S*-nitrosylation in the inflammatory pain and thermal hyperalgesia. These findings could contribute with novel insight into the involvement of TRPA1 *S*-nitrosylation in the nociceptive signaling identifying a new target for the development of pain therapies.

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