Spinal TRPM3 is involved in mediating the hypersensitivity effect induced by sphingolipids in the rat

Hong Wei[1], Zuyue Chen[1], Hugh Chapman[2], Niina Jalava[2], Ari Koivisto[2], Antti Pertovaara[1]

[1] Department of Physiology, Faculty of Medicine, University of Helsinki, Finland, [2] OrionPharma, Orion Corporation, Finland

UNIVERSITY OF HELSINKI
FACULTY OF MEDICINE

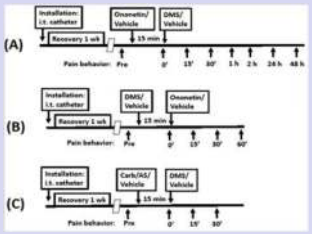
Background and aims:

Earlier results have shown that endogenous sphingolipids in the spinal dorsal horn may contribute to pain hypersensitivity induced by peripheral nerve injury [1]. Also, it has been shown in patch clamp recordings performed in cell cultures that sphingolipids can activate transient receptor potential melastatin-3 (TRPM3) [2]. TRPM3 is a Ca^{2+}-permeable nonselective cation channel that in the primary afferent terminals is involved in transduction of heat [3], while the behavioral effect of spinal TRPM3 is still not clear. Here we studied whether spinal TRPM3 is involved in mediating the pain hypersensitivity effect of sphingolipids. Moreover, since sphingolipids have been shown to activate spinal astrocytes [1], we also assessed effects of drugs acting on spinal astrocyte function on hypersensitivity induced by sphingolipids.

Methods:

- healthy male Han-Wistar rats with a chronic intrathecal (i.t.) catheter for spinal drug administrations
- *N,N*-dimethylsphingosine (DMS; 0.05-0.5 µg) [1]
- ononetin, a TRPM3 antagonist (100 µg) [4]
- carbenoxolone, a gap junction decoupler (inhibitor of astrocyte activation; 10 µg)
- AS-057278, an inhibitor of D-amino acid oxidase (an enzyme in astrocytes; 10 µg)
- monofilament-induced limb withdrawal for assessment of mechanical sensitivity
- heat-induced limb withdrawal for assessment of thermal nociception

1. Time line for experimental procedures



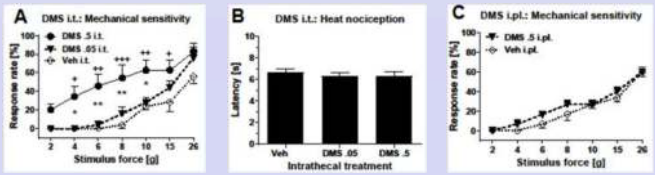
(A)Attempt to prevent development of the *N,N*-dimethylsphingosine (DMS; 0.5 µg i.t.)-induced hypersensitivity by pretreatment with ononetin (TRPM3 antagonist; 100 µg i.t.), see Fig. 3A.

(B) Attempt to attenuate maintenance of the DMS-induced hypersensitivity with ononetin (100 µg i.t.), see Fig. 3B.

(C) Attempt to prevent development of the DMS-induced hypersensitivity by pretreatment with carbonexelone (carb, a gap junction decoupler; 10 µg i.t.) or AS-057278 (AS, a D-amino acid oxidase inhibitor; 10 µg i.t.), see Fig. 4A & 4B.

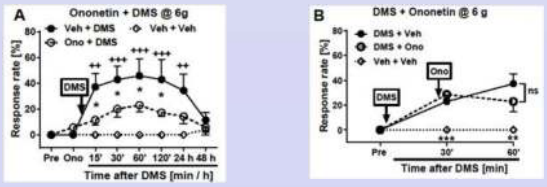
Results:

2. DMS i.t. induced a dose-related mechanical (but not thermal) hypersensitivity, which was not due to spread of DMS to periphery



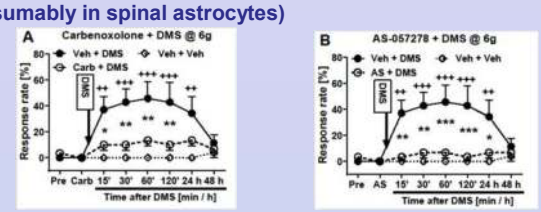
(A) Dose-related effect on mechanical sensitivity by i.t. administration of DMS (at 60 min, which was the time point for the maximal effect). (B) No effect on heat nociception following i.t. administration of DMS. (C) Mechanical sensitivity not changed following intraplantar administration of DMS at a dose that induced hypersensitivity after spinal administration. In A and C, increases in response rate represent facilitation of mechanical sensitivity. Error bars represent S.E.M. (in A and B, $n_{DMS\ .5} = 7$ and $n_{Veh/DMS.05} = 5$, in C, n = 6). $^{*}/^{*}P < 0.05$, $^{++}/^{**}P < 0.01$, $^{+++}P < 0.005$ (t-test with a Bonferroni correction).

3. Development but not maintenance of DMS-induced hypersensitivity was attenuated by a TRPM3 antagonist



(A)Pretreatment with ononetin (a TRPM3 antagonist) prevented development of the DMS-induced hypersensitivity.
(B) Maintenance of the established DMS-induced hypersensitivity was not attenuated by ononetin. Pre, baseline before drug treatments. Veh, vehicle; ns, not significant. In A, ononetin was administered 15 min before DMS and in in B, 30 min after DMS. Error bars represent S.E.M. (n = 7, except for $n_{Veh+Veh} = 5$). $^{*}P < 0.05$, $^{++}/^{**}P < 0.01$, $^{+++}/^{***}P < 0.005$ (t-test with Bonferroni correction).

4. Development of DMS-induced hypersensitivity was attenuated by drugs acting on gap junctions or D-amino acid oxidase (presumably in spinal astrocytes)



Pretreatment with carbenoxolone (Carb; graph A) or with AS-057278 (AS; graph B) attenuated the development of mechanical hypersensitivity induced by DMS. Pre, baseline before drug treatments. Veh, vehicle. Carbenoxolone and AS-057278 were administered 15 min before DMS that was administered at time point 0. Error bars represent S.E.M. ($n_{Veh+DMS} = 7$, $n_{Carb+DMS} = 6$, $n_{AS+DMS} = 6$, $n_{Veh+veh} = 5$). $^{*}P < 0.05$, $^{++}/^{**}P < 0.01$, $^{+++}/^{***}P < 0.005$ (t-test with Bonferroni correction)

Conclusion:

The results are in line with the proposal that spinal TRPM3 and spinal astrocytes contribute to the development of mechanical pain hypersensitivity induced by sphingolipids.

References:

[1] Patti *et al.*, *Nat. Chem. Biol.* 2012;8:232-4. [2] Grimm *et al.*, *Mol. Pharmacol.* 2005;67:798-805.
[3] Vriens *et al.*, *Neuron* 2011;70:482-94. [4] Straub *et al.*, *Br. J. Pharmacol.* 2013;168:1835-50.

Conflicts of interest: Three of the authors (HC, NJ, AK) are employees of OrionPharma Corp. Other authors declare no conflicts of interest.

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