The origin of pain

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Hydra vulgaris is an elementary organism, belonging to the phylum of Cnidarian, class Hydrozoa, characterized by a saccular structure, composed by an apical end (head) and a basal one (foot). The head has a hypostome with the mouth opening, from which several tentacles (from 5 to 12) depart for defence and feeding. Hydra has a simple nervous system consisting of a nerve net that extends through the body. The aim of our work is to investigate the presence of canonical molecular elements belonging to painful pathways and their original functions in *Hydra*. In particular, we have analyzed both behavioural (e.g., tentacle movements) and molecular changes in the mRNA expression of several pain marker genes after a heat treatment.

Polyps were cultured at 17°C in a specific medium, maintained in a 16h/8h light/dark cycle and fed once a week. In the heat test, animals were moved from 17°C to 34°C hydra medium for 1 min and then placed at 17°C. Heated specimens were collected at specific time points, ranging from 0 to 24h. Animals were continuously monitored using an optical microscope, in order to analyze behavioural changes. In the meantime, animals were processed for quantitative Real-time PCRs and Western Blotting experiments.

Behavioural observation has showed different responses at specific time points. Starting from 0 up to 15 minutes, animals presented an altered response to manual solicitations with a loss of contractile ability of both body and tentacles and of their capacity to adhere to the substrate. On the contrary, a normal response was gradually observed starting from 30 minutes. Real-time PCRs experiments have revealed an increase in mRNA expression of the Heat Shock Protein 70 (HSP70) and the Nitric Oxide Synthase (NOS), two genes induced by Transient Receptor Potential (TRP)-mediated heat painful stimuli in mammals, at specific time points. Thus, we found that the exposition to a Transient Receptor Potential Melastin-3 (TRPM3) agonist (pregnenolone sulfate) is able to induce the expression of HSP70 and NOS and, as expected, these effects are inhibited by a TRPM3 antagonist (mefenafic acid). Furthermore we confirm TRPM3 expression by western blotting analysis. Interestingly, the TRPM3 agonist and heat shock also induce the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and the Superoxide Dismutase (SOD), known as markers of the oxidative stress, thus suggesting an ancestral common network between nociceptive and oxidative stress systems.

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