Functional cross-talk between TLR4 and NOPr in human glial cells

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Neuropathic pain originates from peripheral tissue or nerve fiber injuries that promote the neuronal release of neuromodulators and neurotransmitters: these latter can be detected as 'endogenous danger signal' by glial cells, which in turn become activated and release a variety of neuroexcitatory and pain-enhancing substances that significantly perturbs the physiological neron-glia cross-talk. These altered cellular dynamics relevantly contribute to the onset and maintenance of chronic pain states, such as neuropathic pain, and to the ineffectiveness of analgesic therapies (including opioids) Therefore, a better understanding of the cellular and molecular processes which are altered within pathological pain states associated to glial activation is necessary to find novel therapeutic targets and approaches to successfully treat such chronic pain states.

Glial cells act as neuronal support cells and immune cells in the nervous system; glia can be activated at multiple sites along the pain pathway to produce pro-inflammatory mediators, thus contributing to the onset and maintenance of neuroinflammatory as well as chronic pain states.

The nociceptin/orphanin FQ peptide receptor (NOPr) is a G protein coupled receptor (GPCR) expressed in neurons, lymphocytes, macrophages and in astrocytes: NOPr is involved in a wide range of physiological responses within the nervous system and the immune system and its endogenous ligand, nociceptin (NC), is released in different models of neuroinflammation or chronic pain, thus pointing to a relevant role of NC/NOPr system in neuro-immune and neuron-glia interactions during glial adaptive response to pro-inflammatory stimuli.

Toll-like receptors are pattern recognition receptors (PRRs) belonging to innate immune system that are expressed in many different cell types, including glia.

Within the central nervous system, Toll-like receptor 4 (TLR4) is activated in response to endogenous danger signals ('alarmins'), such as heat shock proteins and cell membrane components released from stressed/damaged cells. Once activated, glial TLRs drive the release of pro-inflammatory cytokines thus being TLR4 a key glial activator in the initiation and maintenance of neuropathic pain.

Considering their expression in glial cells and their potential role in both neuron-glia cross-talk and neuro-inflammatory responses of activated glia, NOPr and TLR4 emerge as very interesting receptors to be investigated in the context of pathological glial activation.

The aim of this research has been to investigate the functional cross-talk between NOPr and TLR4 in a model of human glial cells, either under basal condition or exposed to proinflammatory stimuli (TLR-4 activation by LPS). We found that NOPr activation by NC opposed the TLR-mediated induction of cytosolic calcium increase and the subsequent NF-kB activation; consinstently, NOPr activation by NC also opposed the TLR4-mediated up-regulation of IL-1beta. After glial cells activation by prolonged stimulation of TLR4 with LPS (72h), NOPr expression was significantly down-regulated through transcriptional processes requiring the activation of p38MAPK. Under these conditions, NOPr-mediated inhibition of TLR4-dependent calcium signalling and NF-kB activation were lost, as well as the ability of NC to prevent IL-1beta up-regulation.

Our findings provide new insights into the complex network between NOPr/TLR4 and glial responses to activating stimuli, highlighting NOPr induction by NC within early gilal activation as a potential strategy to block, or at least reduce, aberrant glial proinflammatory responses.

Furthermore, understanding the molecular and cellular processes triggered upon NOPr activation by nociceptin and of their role in modulating the proinflammatory activation of glia will allow to find novel drug targets and innovative therapeutic approaches to treat those chronic pain states, such as neuropathic pain, for which therapies are thus far ineffective.