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**Glial cells as targets for virus-induced neuroinflammation and persistent pain**

Nociceptive and neuropathic pain signals have long been known to result from noxious stimuli, which are converted into electrical impulses within tissue nociceptors whose cell bodies are found in dorsal root ganglions. Information regarding intensity, quality, and location of pain is conveyed to the sensory cortex from the somatosensory thalamus. It has been characterized as a complex equilibrium of pain-signalling and pain-relieving pathways connecting PNS and CNS. Pain-relieving drugs have to date been directed against increased neuronal excitability. During the last years it has been shown that there is a low grade neuroinflammation along the pain pathways probably all the way from the spinal cord to thalamus and the parietal cortex. This neuroinflammation is due to activation of glial cells, astrocytes and microglia, with production of cytokines and other inflammatory mediators within the CNS (Wiesler-Frank et al, 2004; Hansson & Zügner, 2005; Hertz & Hansson, 2007). It is most probable that this low grade neuroinflammation forces a pathological neuronal activation, which is responsible for the pain sensation to be long-term. A new concept for treatment of persistent pain states would thus be to attenuate the neuroinflammation. Our studies focus on mechanisms underlying activation of astrocytes and microglia by pain and inflammatory mediated activators. Furthermore, we try to investigate how this activation could be restored by receptor agonists or drugs back to the physiological state of the cells.

Human neurotropic viruses such as herpes simplex virus (HSV) 1 and 2 and varicella-zoster virus (VZV) all target sensory neurons for persistent infection, and pain is a prominent clinical feature of viral reactivation. In general, astrocytes are more permissive to infection by these viruses as compared to sensory neurons (Bergström et al., 1994). In addition, astrocytes were recently found to react with innate inflammatory responses to viral infection. Interestingly, TLR-3 stimulated signalling was evoked by double-stranded RNA simulating virus infection of astrocytes (Scumpia et al., 2005). In turn, cytokine responses such as by IL-1beta are evoked by this pathway, as well as by experimental infection with Japanese Encephalitis Virus (Das et al., 2008). We hypothesize that, during HSV and VZV infection of sensory neurons, inflammatory responses in adjacent glial cells are evoked which may contribute to sensation of pain.
Astrocytes express receptors for many neurotransmitters and neuroactive substances (Hansson and Rönnbäck, 2004), and the cells act as sensors for ions and neuroactive substances in the extracellular milieu, as well as sensors for pH and osmotic balance. In pain states the permeability of the blood-brain barrier (BBB) is increased due to effects on capillary endothelial cells, important for invasion of peripheral monocytes/macrophages (Abbott et al., 2006). Astrocytes form a syncytial network that allows passage of a multitude of substances, including Ca\(^{2+}\) (Hansson & Rönnbäck, 2003). Intracellular Ca\(^{2+}\) transients are induced, which spread within the astrocyte networks as Ca\(^{2+}\) oscillations and waves (Blomstrand et al., 1999). The microglia release cytokines. It has been shown that the Ca\(^{2+}\)-oscillations stimulate the induction and release of cytokines and growth factors. Thereby new neuronal contacts could be established for the maintenance and spreading of the pain sensation.

In order to imitate the *in vivo* situation with constituents from the BBB, we use astrocytes from newborn rat cerebral cortex in primary culture and co-culture them with adult rat brain microvascular endothelial cells, also in primary culture. The co-cultured astrocytes exhibit a morphologically differentiated appearance with long processes (Hansson et al, 2008). For GFP-labelled HSV-1, we have access to a fully infectious construct (Desai & Person, 1998) in which the nucleocapsid gene vp26 is tagged, allowing an intracellular tracing of the virus.

As astrocytes are intimate co-players with neurons in CNS functionality in physiology and pathology, more knowledge on astrocyte responses to inflammatory activators may give new
insight in our understanding of the involvement of astrocytes, and mechanisms, underlying effects of different drugs in long-term pain states.

The aims of the present project are to investigate:

1) if virus infection (as exemplified by HSV) interacts with and activates TLR-3 on astrocytes
2) if such activation may induce an inflammatory response in form of cytokine release

REFERENCES

FÖRSÖKSprotokoll

Modellsystem:
Astrocyter i primärkultur
Astrocyter i primärkultur samodlade med endotelceller i primärkultur

Metoder:
Bildbehandling för att mäta kalciumförrändringar i cellerna
Protein bestämning
ELISA - IL-1beta, IFN _/ß and _ IP-10.
Western blotting – Cx43
Immunofluorescence för att visualisera aktinfilament
Immunofluorescence för att visualisera TLR-3 receptorn
Infektera astrocyter med GFP (green fluorescent protein)-märkt HSV-1
Infektera astrocyter med UV-besträlda HSV-1
Infektera astrocyter med double-stranded RNA (polyIC)

Försök:

1. Astrocyter infekteras med GFP-märkt HSV-1 på virologiska laboratoriet och cellerna fixeras vid 24h och 36h. Dubbelfärgas med antikroppar mot TLR-3 för att undersöka eventuell co-lokalisation mellan denna receptor på endosomala strukturer och virala nukleokapsider. Bildbehandling hos gliagruppen.

2. Astrocyter infekteras med GFP-märkt HSV-1 på virologiska laboratoriet och supernatant samlas vid 12, 24, och 36 timmar. Analys och kvantifiering av IL-1beta, IFN-ß and , samt IP-10 med ELISA.