Institutionen för Fysiologi och Farmakologi

WNT SIGNALING IN MICROGLIA

- WNTs as novel regulators of microglia

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ABSTRACT

Microglia, the immunocompetent cells of the central nervous system (CNS) and the brain's own macrophages are the most motile cells in the CNS and those with highest plasticity, as they rapidly move their projections to actively screen their environment for any type of injury. Upon cell damage or infection, microglia respond quickly: they proliferate, change morphology from ramified to amoeboid state to migrate or invade towards the injury, secrete many types of cytokines and chemokines to communicate with other inflammatory cells, and phagocytose cell debris.

WNTs are secreted lipoglycoproteins, which bind to and act through the Frizzled family of receptors. The Frizzled (FZD) surface receptors belong to a family of seven transmembrane receptors listed as G protein-coupled receptors because of their structural similarities. WNT/FZD-signaling was historically divided into two main branches of pathways, depending whether or not they induce β-catenin stabilization. With increasing knowledge the WNT pathways are mainly named after their protein-induced intracellular events. WNT/FZD-signaling is important during embryonic development, neurogenesis, synaptogenesis, and tissue homeostasis.

Even though WNTs are expressed in the brain and definitely in contact with microglia cells, a link between microglia and WNTs has just recently begun to emerge. The aim of this thesis is to study how microglia cells respond to stimulation with recombinant WNTs with regards to WNT-induced intracellular signaling and physiological outcome. We have investigated this by the use of classical biochemical techniques, such as immunoblotting, immunochemistry, RT/QPCR, GDP/GTP exchange assay and proliferation assay.

The results show that primary microglia cells isolated from mice and a microglia-like cell line (N13) express several receptors for WNTs and respond to recombinant WNT stimulation. Stimulation with WNT-3A induced the WNT/β-catenin-dependent pathway, and, in parallel, a classical GPCR pathway leading to phosphorylation of the MAPKs ERK1/2. Interestingly, by the use of the $G\alpha_{i/o}$ protein inhibitor, pertussis toxin (PTX), we pinpoint a central role for heterotrimeric G proteins in both WNT-3A-induced pathways. Further, stimulation of microglia with recombinant WNT-5A induced a classical GPCR MAPK signaling pathway recruiting $G\alpha_{i/o}$-protein, PKC, calcium and MEK1/2 to phosphorylate ERK1/2.

In addition, WNT stimulation of microglia induced a substantial proinflammatory response by increasing the expression of several proinflammatory cytokines, prostaglandin synthase COX2, proliferation and invasion. Notably, some of these WNT-induced inflammatory markers could be inhibited by PTX or by a MEK1/2 inhibitor, pointing towards a WNT-induced G protein-dependent mechanism.

Furthermore, in Alzheimer's disease, a chronic neuroinflammatory condition associated with activated microglia, amoeboid-like microglia cells show high levels of β-catenin, suggesting that WNT/β-catenin signaling in microglia plays an important role in AD-associated microglia activation.

In addition, WNT-3A and WNT-5A induced the expression of COX2 dose-dependently, but if microglia are preactivated by the proinflammatory bacterial wall derivate lipopolysaccharide (LPS), WNTs counteract LPS-induced COX2 expression. This suggests a dual regulatory, i. e. pro-and anti-inflammatory effect of WNTs on microglia.

In conclusion, WNTs are expressed in the brain and have impact on microglia's inflammatory activity; this suggests that WNTs may play important roles as modulators of microglia activity in neuroinflammation and tissue homeostasis.

Key words: Microglia, Frizzled, WNT, β-catenin-dependent and -independent signaling, neuroinflammation, heterotrimeric G protein

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