Convergence of interleukin-6 and epidermal growth factor (EGF) signaling in microglial cells supports maintenance of hyperexcitability of spinal nociceptive neurons

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Spinal hyperexcitability is a hallmark of many chronic pain states including joint inflammation. In the present study we addressed the role of spinal interleukin-6 (IL-6) in the generation and maintenance of spinal hyperexcitability. We performed extracellular recordings from spinal cord neurons of normal rats *in vivo*, applied IL-6 plus the soluble IL-6 receptor (sIL-6R) to the spinal cord surface and monitored the responses of neurons to mechanical stimuli applied to knee, ankle and paw. IL-6 forms a complex with sIL-R6, and this so-called IL-6-transsignaling mediates most IL-6 effects. Spinal application of IL-6/sIL-6R enhanced the responses of spinal neurons to mechanical stimulation of the leg. This induction of hyperexcitability could be blocked by sgp130 which binds IL-6/sIL-6R complexes, but sgp130 did not reverse established spinal hyperexcitability, when applied 4 hours after IL-6/sIL-6R.

By contrast, a potential inhibitor of EGF, gefitinib, attenuated the established spinal hyperexcitability evoked by IL-6 *trans-signalling* when applied 4 hours after application of IL-6/sIL-6R (n = 6). This indicates an involvement of EGF in the maintenance of IL-6/sIL-6R-induced hyperexcitability. Since microglial cells contribute to IL-6-transsignaling by releasing sIL-6R we asked whether BV2 cells, a microglial cell line, show convergence of IL-6 and EGF signaling. Application of hyper-IL-6 (a combination of IL-6/sIL-6R) activated ERK1/2. This response was inhibited by gefitinib, suggesting convergence of IL-6 and EGF pathways on cellular signaling. The result was confirmed in primary microglia from P2 mice pups. Furthermore, we observed that gefitinib is also capable to inhibit the release of sIL-6R in control condition without any stimulation until 2 hour. These data suggest that, once spinal hyperexcitability is established by IL-6 *trans-signalling*, EGF may play a role in the maintenance of hyperexcitability.