AG Name Stein/Machelska

AG Anesthesiology – Pain Research

Arbeitsgruppenleiter:
Prof. Dr. Christoph Stein (christoph.stein@charite.de)
Prof. Dr. Halina Machelska (halina.machelska@charite.de)

Standort:
Klinik für Anäesthesiologie und operative Intensivmedizin
Charité-Universitätsmedizin Berlin
Campus Benjamin Franklin
Hindenburgdamm 30, D-12203 Berlin

Mitarbeiter:
Dr. Melanie Busch-Dienstfertig, Özgür Celik (Ph.D. student), Dr. Giovanna Del-Vecchio, Dr. Sara González-Rodriguez, Dr. Dominika Labuz, Santhosh Chandar Maddila (Ph.D. student), Dr. Nicolas Massaly, Dr. Antonio Rodriguez-Gaztelumendi, Dr. Yvonne Schmidt, Dr. Viola Spahn, Barbara Trampenau (technician), Nicole Vogel (technician).

Homepage:
http://anaesthesie.charite.de/en/research/pain/

Forschungsgebiet:

Pain and inflammation are almost always associated with each other. Indeed, inflammation accompanies the majority of acute and chronic pain conditions. Examples are postoperative pain, trauma, arthritis, vasculitis, meningitis, invading cancer, visceral pain, and neuropathic pain (resulting from nerve damage). Our group is interested in mechanisms of inflammatory and neuropathic pain and its inhibition by opioids outside the central nervous system (CNS). This can be achieved by activation of opioid receptors on peripheral sensory neurons within injured tissue by exogenous or endogenous opioids. Endogenous opioid peptides (endorphin, enkephalins, dynorphin) are produced by immune cells infiltrating inflamed tissue. Activation of such cells by environmental stressful stimuli or releasing agents (corticotropin-releasing factor, catecholamines, cytokines, chemokines, bacterial components) releases the opioids from immune cells and ameliorates pain.

Projekte:

Macrophages in the regulation of neuropathic pain
In a mouse model of neuropathic pain (chronic ligation of the sciatic nerve), we found that opioid peptide-containing immune cells ($CD45^+$ cells) accumulate at the site of nerve damage. Following stimulation (by corticotropin-releasing factor), immune cells secrete opioids and locally decrease pain. Interestingly, the majority of these cells are macrophages (60-70% of $CD45^+$ cells). In this project we aim to *in vitro* and *in vivo* characterize macrophages and examine their contribution to the inhibition of pain.

Modulation of pain and inflammation in chronic arthritis by opioids
Together with a national consortium, the Division of Rheumatiology CBF (J. Sieper) and the MDC (M. Lipp), this project aims at modulating pain and inflammation by stimulating the production and secretion, or by inhibiting the extracellular enzymatic degradation of endogenous opioids derived from immune cells. In animal models of inflammation and in patients with inflamed joints we will examine pain, inflammatory parameters, release and extracellular concentrations of opioid peptides, modulated by cytokines, chemokines and peptidase inhibitors. In patients we also compare effects of exogenous and endogenous opioids.

AG Name Stein/Machelska

Contribution of opioid peptides and receptors in immune cells to exogenous opioid control of neuropathic pain

In this project we aim at elucidating the contribution of leukocytic opioid peptides and receptors to peripheral opioid analgesia in painful neuropathy. We will examine peripheral endogenous and exogenous opioid analgesia in pharmacologically and genetically immunosuppressed mice with injured sciatic nerves, employing adoptive transfer of leukocytes. The *in vivo* investigations will be accompanied by *in vitro* protocols examining the release of opioid peptides by activation of leukocytic opioid receptors. These studies will provide new insights into mechanisms of analgesic actions based on the interplay between endogenous and exogenous opioids acting at their receptors in immune cells.

The nociceptor pain model

Together with a national consortium, this project applies systems-medicine-based mathematical models of signalling switches involved in pain sensitisation, optimises and expands them by reflection on molecular, cellular as well as animal experiments, to finally translate this knowledge and test the predictive potential in humans. We hypothesize that opioids will inhibit cAMP-dependent and other pathways identified by computational simulation. Ultimately we aim at testing novel analgesic compounds in human patients identified as most suitable, aiming at mechanism-based therapy of inflammatory pain.

Computationally aided design of opioids

In collaboration with the Zuse Institute Berlin (M. Weber), this project uses modeling of opioid receptor conformations and docking of ligands to generate hypotheses to be tested in cell lines and animals expressing wild-type and mutated opioid receptors. We examine ligand binding, G-protein coupling, modulation of action potentials, transient receptor potential vanilloid receptor currents and cAMP formation. Ultimately, we test optimally designed synthetic agonists in animal models and patients with inflammatory pain *in vivo*.

Spezialtechniken:

Animal (rat and mouse) models of inflammatory and neuropathic pain, *in vivo* pain testing in animals (von Frey-, paw pressure-, Hargreaves-tests), pain assessment in patients (pain scales, patient-controlled analgesia), neuronal and immune cell cultures, electrophysiology (*in vitro* skin-nerve preparation, patch-clamp), calcium imaging, FRET, *in vivo* microdialysis, radioimmunoassay, ELISA, radioligand binding, western blot, histological assays (immunohistochemistry, immunofluorescence), DNA/RNA isolation, RT-PCR, transfections, gene and mRNA targeting.

Ausgewählte Publikationen:

Zöllner C, Mousa SA, Fischer O, Rittner HL, Shaqura M, Brack A, Shakibaei M, Binder W, Urban F, **Stein C**, Schäfer M. Chronic morphine use does not induce peripheral tolerance in a rat model of inflammatory pain. J Clin Invest 2008, 118:1065-1073.

Labuz D, Schmidt Y, Schreiter A, Rittner HL, Mousa SA, **Machelska H**. Immune cell-derived opioids protect against neuropathic pain. J Clin Invest 2009, 119:278-286.

Stein C, **Machelska H**. Modulation of peripheral sensory neurons by the immune system: implications for pain therapy. Pharmacol Rev 2011, 63:860-881.

Schmidt Y, Labuz D, Heppenstall PA, **Machelska H**. Cutaneous nociceptors lack sensitisation, but reveal μ-opioid receptor-mediated reduction in excitability to mechanical stimulation in neuropathy. Mol Pain 2012, 8:81.

Schreiter A, Gore C, Labuz D, Fournie-Zaluski MC, Roques BP, **Stein C**, **Machelska H**. Pain inhibition by blocking leukocytic and neuronal opioid peptidases in peripheral inflamed tissue.

FASEB J 2012, 26:5161-5171.

Moshourab R, **Stein C**. Fentanyl decreases discharges of C and A nociceptors to suprathreshold mechanical stimulation in chronic inflammation. J Neurophysiol 2012, 108:2827-2836.

Busch-Dienstfertig M, Labuz D, Wolfram T, Vogel NN, **Stein C**. JAK-STAT1/3-induced expression of signal sequence-encoding proopiomelanocortin mRNA in lymphocytes reduces inflammatory pain in rats. Mol Pain 2012, 13;8:83.

Nockemann D, Rouault M, Labuz D, Hublitz P, McKnelly K, Reis FC, **Stein C***, Heppenstall PA*. The K+ channel GIRK2 is both necessary and sufficient for peripheral opioid-mediated analgesia. EMBO Mol Med 2013, 5:1263-1267.

Schmidt Y, Gavériaux-Ruff C, **Machelska H**. μ-Opioid Receptor Antibody Reveals Tissue-Dependent Specific Staining and Increased Neuronal μ-Receptor Immunoreactivity at the Injured Nerve Trunk in Mice. PLoS One 2013, 8(11):e79099.

Labuz D, **Machelska H**. Stronger antinociceptive efficacy of opioids at the injured nerve trunk than at its peripheral terminals in neuropathic pain. J Pharmacol Exp Ther 2013, 346:535-544.

Spahn V, Fischer O, Endres-Becker J, Schäfer M, **Stein C**, Zöllner C. Opioid withdrawal increases transient receptor potential vanilloid 1 activity in a protein kinase A-dependent manner. Pain 2013, 154:598-608.

Spahn V, **Stein C**, Zöllner C. Modulation of transient receptor vanilloid 1 activity by transient receptor potential ankyrin 1. Mol Pharmacol 2014, 85:335-344.

Laufende Drittmittelprojekte:

Granting agency	Title	Duration
European Society of Anesthesiology	Bypassing Brain: Nanotechnology-enabled delivery of morphine for analgesia without central side effects	2010 – 2014
DFG MA 2437/4-1	Contribution of opioid peptides and receptors in immune cells to exogenous opioid control of neuropathic pain	2010 – 2014
BMBF, VIP0272/03V0364	Nebenwirkungsfreie Analgetika durch Modellierung pathologischer Rezeptorkonformationen (NAMPAR)	2012 – 2015
Virtuelles Helmholtz Institut Multifunktionale Biomaterialien für die Medizin	Multifunctional Nanosystems for Selective Targeting of Inflammation and Pain	2012 –2016
Freie Universität Berlin/ Charité, Berlin Focus Area DynAge	Nanoparticulate opioid conjugates for the treatment of chronic pain	2013 –2014
BMBF e:Bio 0316177B	Schmerzregulation durch Modulation von cAMP/PKA	2013 – 2015
EU FP7-HEALTH-2013-INNOVATION-1; No. 602891-2	Neuropathic pain: biomarkers and druggable targets within the endogenous analgesia system (NeuroPain)	2013 – 2018