Focus of the PhD-Project (Dr. Ute Becherer and Dr. Barbara Niemeyer)

## How do pain sensing neurons respond to increased oxidative stress during inflammation?

We are interested in several aspects concerning release of neuropeptides from dorsal root ganglia (DRG) neurons and regulation of this release by altered ion channel properties and function. Inflammation or injury of tissues will recruit a number of leucocytes to the sites of inflammation. Invading phagocytes (macrophages /microglia) and environmental stressors will locally produce reactive oxygen species (ROS). While ROS are known to amplify pain signalling through the capsaicin receptor TRPV1 and voltage gated sodium channels, little is known how ROS affect the release of neuropeptides from DRG neurons. In chromaffin cells it has been shown that ROS increase the production of the neuropeptide (i.e. NPY) and it's also been shown that nerve injury increases neuropeptide production in DRG neurons.

Our goal is to elucidate ROS dependent signalling pathways within these neurons and also to study possible effects of ROS on the cold- and menthol sensitive TRPM8 channels which are expressed in a subset of DRG neurons and may modulate pain responses. Initially, we will investigate the effects of exogenous ROS on the production of NPY and other neuropeptides (e.g. Substance P, CGRP) and on their release characteristics. Methods will include culturing of DRG neurons, Western Blots, Immuncytochemistry and high resolution fluorescent microscopy (TIRFM, SIM). Because a number of mechanisms also exist for the cell to produce endogenous ROS, we will use genetically modified proteins to serve as endogenous ROS sensors. Transfection of DRG neurons with these sensors will allow for precise detection of local ROS production after stimulation (electric) and exposure of neurons to proinflammatory mediators (bradykinin, ATP, histamine and others).

As a secondary approach we will investigate whether and how ROS affect the menthol receptor TRPM8. The menthol and temperature induced ionic currents mediated by TRPM8 can readily be measured in HEK cells using the patch-clamp technique. Mutagenesis of potential reactive cysteins within TRPM8 will help us to identify the molecular target of ROS regulation of TRPM8.

## Methods

- Western Blots.
- Immunhistochemistry.
- Recombinant DNA technology: constructtion of mutant and wild type proteins
- Cell culture DRG neurons, HEK cells.
- High end microscopic techniques (confocal imaging, total internal reflection
- Fluorescent imaging, cell observer, SIM)
- ROS measurements using fluorescent dyes and recombinant proteins.
- Track and visualize secretion of fluorescently labeled synaptic vesicles and LDCVs.

## Publications


1. Bogeski I, Kappl R, Kummerow C, Gulaboski R, Hoth M, Niemeyer BA (2011) Redox regulation of calcium ion channels: Chemical and physiological aspects. *Cell Calcium*, 50, 407-23.

2. Quintana A, Pasche M, Junker C, Al-Ansary D, Rieger H, Kummerow C, Nunez L, Villalobos C, Meraner P, Becherer U, Rettig J, Niemeyer BA, Hoth M. (2011) Calcium microdomains at the immunological synapse: how ORAI channels, mitochondria and calcium pumps generate local calcium signals for efficient T-cell activation. *EMBO J*, 30, 3895-912.

3. Bogeski I, Kummerow C, Al-Ansary D, Schwarz EC, Koehler R, Kozai D, Takahashi N, Peinelt C, Griesemer D, Bozem M, Mori Y, Hoth M, Niemeyer BA (2010) Differential redox regulation of ORAI ion channels: a mechanism to tune cellular calcium signaling. *Science Signaling*, 3, ra24.

4. Al-Ansary D, Bogeski I, Disteldorf B, Becherer U, Niemeyer BA (2010) ATP modulates Ca2+ uptake by TRPV6 and is counteracted by isoform specific phosphorylation*. Faseb Journal*, 24, 425-435.

5. Zeniou-Meyer, M., Liu, Y., Béglé, A., Olanish, M., Hanauer, A., Becherer, U., Rettig, J., Bader, M-F. and Vitale, N. (2008) The Coffin-Lowry syndrome-associated protein RSK2 is implicated in calcium-regulated exocytosis through the regulation of PLD1. *PNAS*, 105:8434-8439.

6. Becherer U, Pasche M, Nofal S, Hof D, Matti U, Rettig J. (2007) Quantifying exocytosis by combination of membrane capacitance measurements and total internal reflection fluorescence microscopy in chromaffin cells. *PLoS One*, 2(6):e505.

7. Erler I, Al-Ansary D, Wissenbach U, Wagner TFJ, Flockerzi V & Niemeyer BA (2006): Trafficking and Assembly of the cold-sensitive TRPM8 channel. *J Biol Chem* 281: 38396-38404.