Focus of the PhD-Project (Prof. Dr. Marc Freichel, Prof. Dr. Peter Lipp)

TRP-like 2 proteins in the heart – subcellular trafficking and functional relevance for cation entry and contractility

TRP (transient receptor potential) proteins form ion channels that mediate transcellular influx of cations such as Ca^{2+} und Na^{+} into the cytoplasm of mammalian cells. Mammalian TRP's are grouped into six subfamilies based on their sequence homology: TRPC, TRPV, TRPM, TRPA, TRPP and TRPML. So far 28 and 27 TRP proteins were identified in mice and men, respectively. TRP-like 2 is one of three members of a new class of membrane proteins that share significant homology to TRP proteins. Hydropathy analysis suggests that TRP-like 2 has between six and ten transmembrane domaines. TRP-like 2 is expressed in the heart, in brain, in endothelial cells, in the lung, in the colon, in the kidney, in the placenta and in mast cells. Following heterologous expression it could not been shown until now that TRP-like 2 proteins form functional ion channels, maybe due to the lack of essential subunits that are not known yet but are expressed in their native environment of primary cells and tissues. The aim of this study is to investigate the function of TRP-like 2 in the heart. Atrial and ventricular cardiomyocytes will be isolated from wild type mice and mouse lines in which we have inactivated the TRP-like 2 gene using gene targeting in embryonic stem cells. The functional relevance of TRP-like 2 proteins for cation entry in cardiomyocytes will be studied using microfluorimetric techniques. Cardiac contractility will be compared in wild type and TRP-like2-deficient mice using organ bath measurements in isolated cardiac tissue preparations and intraventricular pressure measurements in vivo. To study the subcellular trafficking of TRP-like 2 proteins in native cardiomyocytes a targeting construct will be cloned to generate a mouse line in which TRP-like 2 proteins are labeled by fusion of the cDNA of the fluorescent protein mCherry to the exon encoding the carboxyterminus of TRP-like 2. In cardiomyocytes of these mice TRP-like 2 proteins will be traced using laser confocal microscopy before and following receptor stimulation.

Methods:

Isolation and transfection of cardiomyocytes from embryos, neonates and adult mice, gene targeting, confocal microscopy, microfluorimetric Ca^{2+} and Na^{+} imaging, cardiac contraction measurements in vitro and in vivo, telemetric electrocardiogram recording, hormonal induction of cardiac hypertrophy in vivo.

Homepages:

http://wwwalt.med-rz.uniklinik-saarland.de/med_fak/pharma-toxi/Seiten/ExpPharm_frame.html

http://www.lipplab.de

Selected publications:

1. "De novo expression of TRPM4 initiates secondary hemorrhage in spinal cord injury"
V. Gerzanich, K. Woo, R. Vennekens, S. O. Tsymbalyuk, S. Ivanova, A. Ivanov, Z. Geng, Z. Chen, V. Flockerzi, B. Nilius, **M. Freichel** and J. M. Simard.
Nature Medicine, in press.

2. „TRPC3 channels are required for synaptic transmission and motor coordination"
J. Hartmann, E. Dragicevic, H. Adelsberger, H. Henning, M. Sumser, J. Abramowitz, R., Blum, A. Dietrich, **M. Freichel**, V. Flockerzi, L. Birnbaumer and A. Konnerth.
Neuron 59: 392-8 (2008)

3. „Increased IgE-dependent mast cell activation and anaphylactic responses in mice lacking the calcium-activated nonselective cation channel TRPM4"
Vennekens, R., Olausson, J., Meissner, M., Bloch, W., Mathar, I., Philipp, S.E., Schmitz, F., Weissgerber, P., Nilius, B., Flockerzi, V., **Freichel, M**.
Nature Immunology 8: 312-320 (2007)

4. „ Ca^{2+} channel currents and contraction in $Ca_V\beta_3$-deficient ileum smooth muscle from mouse."
Held, B., Tsvilovskyy, V., Meissner, M., Kästner, L., Ludwig, A., Mossmang, S., Lipp, P., **Freichel, M.**, Flockerzi, V.
Cell Calcium, 42: 477-87 (2007)

5. "Critical role for the β regulatory subunits of Cav channels in T lymphocyte function"
Badou, A., Jha, M.K., Matza, D., Mehal, W.Z., **Freichel, M.**, Flockerzi, V., Flavell, R.A.
Proc Natl Acad Sci U S A 103: 15529-34 (2006)

6. "Reduced cardiac L-type Ca^{2+} current in $Ca_v\beta_2^{-/-}$ embryos impairs cardiac development and contraction with secondary defects in vascular maturation "
Weißgerber, P., Held, B., Bloch, W., Kästner, L., Chien, K., Fleischmann, B., Lipp, P., Flockerzi, V., **Freichel, M.**
Circulation Research 99: 749-57 (2006)

7. „ Modulation of Ca^{2+} signalling by Na^+/Ca^{2+} exchangers in mast cells."
Aneiros, E., Philipp, S., Lis, A., **Freichel, M**. and Cavalié, A.
J. Immunol. 174: 119-130 (2005).

8. „Removal of Ca^{2+} channel β_3 subunit enhances Ca^{2+} oscillations frequency and insulin exocytosis"
Berggren, PO, Yang, S., Murakami, M., Efanov, A., Uhles, S., Köhler, M., Moede, T., Fernström, A., Appelskog, I., Aspinwall, C., Zaitsev, S., Larsson, O., Moitosos de Vargas, L., Fecher-Trost, C., Weißgerber, P., Ludwig, A., Leibiger, B., Juntti-Bergren, L., Barker, CJ, Gromada, J., **Freichel, M.**, Leibiger, I. and Flockerzi, V.
Cell, 119, 273-284 (2004).

9. „Contribution of transient receptor potential channels to serotonin-mediated increase in GABA release from dendrites".
Munsch, T., **Freichel, M.**, Flockerzi, V., Pape, H.
Proc Natl Acad Sci U S A 100: 16065-70 (2003).

10. „Voltage dependence of the Ca^{2+} activated cation channel TRPM4"
Nilius, B., Prenen, J., Droogmanns, G., Voets, T., Vennekens, R., **Freichel, M.**, Wissenbach, U.,

Flockerzi, V.
J Biol Chem. 278: 30813-20 (2003).

11. „ The TRPV6 gene, cDNA and protein“
Hirnet, D.*, Olausson*, J., Fecher-Trost, C., Bödding, M., Nastainczyk, W., Wissenbach, U., Flockerzi, V., **Freichel, M**.
Cell Calcium 33: 509–518 (2003).

12. „Impairment of store-operated Ca^{2+}-entry in TRPC4 -/- mice interferes with thrombin–induced increase in lung microvascular permeability.“
Tiruppathi, C., **Freichel, M.**, Vogel, S. M., Paria, B. C., Metha, D., Flockerzi, V., Malik, A. B.
Circulation Research, 91: 70-76 (2002)

13. “Lack of an endothelial store-operated Ca^{2+}-current impairs agonist-dependent Ca^{2+} entry and vasorelaxation in TRP4 (CCE1) -/- mice”
Freichel, M., Suh, S.H., Pfeifer, A., Schweig, U., Trost, C., Weißgerber, P., Biel, M., Philipp, S., Freise, D., Droogmans, G., Hofmann, F., Flockerzi. V., Nilius, B.
Nature Cell Biology, 3: 121-127 (2001)

Reviews

1. “Functional role of TRPC proteins in native systems: implications from knockout and knock-down studies.”
Freichel, M., Vennekens, R., Olausson, J., Stolz, S., Philipp, S., Weißgerber, P., Flockerzi, V.
J Physiol. 567: 59-66 (2005).

2. “Functional role of TRPC proteins *in vivo*: lessons from TRPC-deficient mouse models.
Freichel, M., Vennekens, R., Olausson, J., Hoffmann, M., Müller, C., Stolz, S., Scheunemann, J., Weißgerber, P., Flockerzi, V.
Biochem. Biophys. Res. Commun. 322: 1352-8, (2004)

3. „Store operated cation channels (SOCs) in the heart and cells of the cardiovascular system“
Freichel, M., Schweig, U., Stauffenberger, S., Freise, D., Schorb, W., Flockerzi, V.
Cell. Physiol. Biochem. 9: 270-283 (1999).

Collins J.C., **Lipp P.,** Berridge MJ. and Bootman MD. (2000). Inositol 1,4,5-trisphosphate-induced Ca^{2+} release is inhibited by mitochondrial depolarization. Biochem. J. 347:593-600.

Thomas D., **Lipp P.**, Tovey SC., Berridge MJ., Li W., Tsien RY. and Bootman MD. (2000). Microscopic properties of elementary Ca^{2+} release sites in non-excitable cells. Current Biology 10: 8-15.

Lipp P., Laine M, Tovey S.C., Burrell K.M., Berridge M.J., Li W. & Bootman M.D. (2000) Functional $InsP_3$ receptors that may modulate excitation-contraction coupling in the heart. Current Biology 10: 939-942.

Thomas D., Tovey S.C., Collins T.J., Bootman M.D., Berridge M.J. & **Lipp P**. (2000). A comparison of fluorescent Ca^{2+} indicator properties and their use in measuring elementary and global Ca^{2+} signals. Cell Calcium 28: 213-223.

Anderson K.E., **Lipp P.**, Bootman M.D., Ridley S.H., Coadwell J., Rönstrad L., Holmes A., Painter G.F., Thuring J., Erdjument-Bromage H., Greival A., Tempst P., Stephens L.R. & Hawkins P.T. (2000) DAPP1 undergoes a novel PI3-kinase dependent cycle of plasma

membrane recruitment and endocytosis upon cell stimulation. Current Biology 10: 1403 - 1412.

Berridge MJ., **Lipp P**. and Bootman MD. (2000).Calcium entry – a membrane pas de deux. Science 287: 1604-1605.

Berridge M.J., **Lipp P** & Bootman M.D. (2000) The versatility and universality of calcium signalling. Nature Reviews *Molecular Cell Biology*: 1: 11-21.

Mackenzie L., Bootman M.D., Berridge M.J. & **Lipp P**. (2001) Pre-determined recruitment of calcium release sites underlies excitation contraction coupling in rat atrial myocytes. Journal of Physiology, 530: 417-429.

Collins T.J., **Lipp P**, Berridge M.J. & Bootman M.D. (2001) Mitochondrial Ca^{2+} uptake depends on the spatial and temporal profile of cytosolic Ca^{2+} signals. Journal of Biological Chemistry 276: 26411-26420.

Krugmann S., Ridley S.H., Ansderson K.E., Risso N., McGregor A., Coadwell J., Davidson K., Eguinoa A., Ellson C.D., **Lipp P**., Manifava M., Ktistakis N., Painter G., Thuring J.W., Cooper M.A., Lim Z-Y., Hilmes A.B., Dove S.K., Michell R.H., Grewal A., Nazarin A., Erdjument-Bromage H., Tempst P., Stephens L.R. & Hawkins P.T. (2002) Identification of phosphoinositite-binding proteins by targeted proteomics using selective affinity matrices. Molecular Cell; 9:21-34.

Collins T., **Lipp P**., Berridge M.J. & Bootman M.D. (2002). Mitochondria are heterogeneous in cells. EMBO Journal; 21: 1616-1627..

Lipp P., Egger M. & Niggli E. (2002). Spatial characteristics of SR Ca^{2+} release events triggered by of $I_{Ca,L}$ and I_{Na} in guinea-pig cardiac myocytes. Journal of Physiology; 542: 383-393.

Mackenzie L., Bootman M.D., Laine M., Berridge M.J., Thuring J., Holmes A., Li W.-H. & **Lipp P**. (2002) The role of inositol 1,4,5-trisphosphate receptors in Ca^{2+} signalling and the generation of arrhythmias in rat atrial myocytes. Journal of Physiology; 541: 395-409.

Reither G, Schaefer M, **Lipp P**. (2006) PKCalpha: a versatile key for decoding the cellular calcium toolkit J Cell Biol.174: 521-33.