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\(^{2+}\) and 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.
Selected publications:

1. “De novo expression of TRPM4 initiates secondary hemorrhage in spinal cord injury”

2. „TRPC3 channels are required for synaptic transmission and motor coordination“

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., Tsivilovskyy, V., Meissner, M., Kästner, L., Ludwig, A., Mossman, S., Lipp, P., **Freichel, M.**, Flockerzi, V.

5. “Critical role for the \(\beta\) 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.

6. “Reduced cardiac L-type Ca^{2+} current in Ca_{v}\beta_{2} \rightarrow \leftarrow 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.**

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.

8. „Removal of Ca^{2+} channel \(\beta_{3}\) subunit enhances Ca^{2+} oscillations frequency and insulin exocytosis“

9. „Contribution of transient receptor potential channels to serotonin-mediated increase in GABA release from dendrites“.
   Munsch, T., **Freichel, M.**, Flockerzi, V., Pape, H.

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.


11. „The TRPV6 gene, cDNA and protein”

12. „Impairment of store-operated Ca²⁺-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 Ca²⁺-current impairs agonist-dependent Ca²⁺ entry and vasorelaxation in TRP4 (CCE1) -/- mice”
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., Vanheken, R., Olausson, J., Stolz, S., Philipp, S., Weißgerber, P., Flockerzi, V.

Freichel, M., Vanheken, R., Olausson, J., Hoffmann, M., Müller, C., Stolz, S., Scheunemann, J., Weißgerber, P., Flockerzi, V.

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.


