

## **The impact of phospholipase C for cardiac excitation contraction coupling and for $\text{Ca}^{2+}$ sparks**

In the  $G_q$ -coupled signalling pathway phospholipase  $C_\beta$  (PLC) is one of the first players transducing stimulation to contraction by activating the production of diacylglycerol and inositol-1,4,5-trisphosphat. This pathway is proposed to be initially involved in the generation of cardiac arrhythmias.

In preliminary experiments we used the PLC pan-activator *m*-3M3FBS on freshly isolated rat ventricular myocytes to investigate its effect on the excitation-contraction coupling. *m*-3M3FBS showed a high arrhythmogenic potential with a total reversibility after washout of the substance. The biological inactive stereoisomer of the PLC-activator (*o*-3M3FBS) was without effect on the ventricular myocytes.

The aim of the project is to provide evidence for an involvement of the  $G_q$ -coupled signalling pathway in the generation of “cellular arrhythmias” by PLC activity stimulation of isolated cardiac myocytes and simultaneous measurements of:

- (i) the kinetics of calcium transients in electrically stimulated ventricular myocytes using the technique of video-imaging and the fluophor fura-2,
- (ii) the change in spontaneous activity ( $\text{Ca}^{2+}$  sparks) of ventricular myocytes by ultra-fast confocal microscopy using fluo-4 as a calcium indicator and
- (iii) the modulatory impact on L-type calcium channels using the patch-clamp technique.

### Methods:

Real-time confocal microscopy; Video-Imaging; Fluorescence Lifetime IMaging, 2-Photon applications; Photometry; Patch clamp technology; quantitative real-time PCR; cell-isolation and primary cell culture; molecular biology methods; etc.

### Selected publications:

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

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Lipp P., Laine M, Tovey S.C., Burrell K.M., Berridge M.J., Li W. & Bootman M.D. (2000) Functional  $\text{InsP}_3$  receptors that may modulate excitation-contraction coupling in the heart. *Current Biology* **10**, 939-942.

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  $\text{Ca}^{2+}$  signalling and the generation of arrhythmias in rat atrial myocytes. *Journal of Physiology*, **541**, 395-409.

Kaestner, L., Juzeniene, A., Moan, J. (2004) Erythrocytes – the “house elves” of Photodynamic Therapy. *Photochem. Photobiol. Sci.*, **3**, 981 – 989.

Kirchhefer, U., Hanske, G., Jones, L.R., Justus, I., Kaestner, L., Lipp, P., Schmitz, W., Neumann, J. (2006) Overexpression of junctin causes adaptive changes in cardiac myocyte  $\text{Ca}^{2+}$  signaling. *Cell Calcium*, in press