A COMBINED APPROACH OF PATCH CLAMP AND AFM FOR STUDYING Ca2+ - DEPENDENT INACTIVATION OF CLASS C-TYPE Ca2+ CHANNELS

Gamsjäger Roland[1], Klaus JF Kepplinger[1], Rong Zhu[1], Yves Maulet[2], Klaus Groschner[3], Nikolai M. Soldatov[4], Peter Hinterdorfer[1], Christoph Romanin[1]; [1]University of Linz, Austria, [2]INSERM U 464, Bd Pierre Dramard, Marseille, 13916 France, [3]Karl-Franzens University Graz, Universitätsplatz 2, Graz, 8010 Austria, [4]National Institute on Aging, NIH, 5600 Nathan Shock Drive, Baltimore, Maryland 21224

Ca^{2+}-dependent binding of CaM to an IQ CaM binding motif in the carboxyl-tail of α_{1C} has been sufficiently established, while the functional involvement of the recently identified site tethering CaM (CBR) at resting cell Ca^{2+} is less understood. Quantitative analysis of the molecular mechanism of calmodulin (CaM) mediating Ca^{2+}-dependent inactivation of class C-type Ca^{2+} channels will be performed using a combined approach of electrophysiology and atomic force microscopy. Using the conventional $\alpha_{1C,77}$ and the mutant $\alpha_{1C,77L}$ where most of CBR has been substituted, Ca^{2+}-dependent inactivation will be studied in the inside-out patch. Then, single channel current parameters recorded from $\alpha_{1C,77}$ as well as mutant $\alpha_{1C,77L}$ and single molecule interaction forces between CaM and the respective carboxyl-tail protein are correlated, both in dependence of increasing Ca^{2+} concentrations. Furthermore, conformational changes of carboxyl tail proteins induced by Ca^{2+}/CaM are measured by force sensing, and studying protein mechanics may allow to resolve additional structural details (supp. by: P15387-MOB; Austrian Academy of Science).