Department of Biochemistry of Membrane Receptors (Institute of Physiology, Academy of Sciences of the Czech Republic, Prague) – State of the Art

Department was established in 1989 (J. Vanecek, J. Teisinger, A. Pavlik, E. Amler and P. Svoboda) and since then, the main focus, when expressed in general terms, was oriented to analysis of membrane receptors coupled with trimeric G-proteins (GPCRs). After diversification of specific research interests of founders of original department and in collaboration with Glasgow University supported by the three consecutive The Wellcome Trust grants (with G. Milligan), the research was focused on analysis of cellular and molecular mechanism of *desensitization* of hormone action: agonist-induced solubilisation of G-proteins, changes in subcellular localization of GPCR and G-proteins after prolonged hormone stimulation. Structure-function correlation of Na,K-ATPase was also studied. In this context, special attention was devoted to plasma membrane compartments denominated as membrane-domains (MD). Biochemical methods for isolation of functional MD preparations were developed together with analysis of effect of various detergents and cholesterol-depletion on plasma (cell) membrane structure.

Methodological experience and knowledge collected over the years in studies of desensitization of β1-AR and β3-AR (in brown-adipose tissue, Wenner-Grens Institute, Stockholm, B. Cannon), of β1-AR and β2-R (in S49 lymphoma cells and myocardium; UCSD and Goteborg University, P. A. Insel, L. Ransnas) and of m1-ACh-, IP-prostanoid- and TRH-receptors (in CHO and HEK293 cells; Glasgow University, G. Milligan) was used for analysis of desensitization of opioid receptor (μ-, δ-, κ-OR)-initiated signalling cascades in brain cortex. OR-initiated signalling was studied in the context of drug addiction and effect of long-term exposure of rat brain to high doses of morphine. $GABA_B$-R-signalling pathway and Na,K-ATPase were determined in parallel PM samples. As before, data collected by analysis of natural tissue were compared with characteristics of OR in model cell lines expressing δ-OR and δ-OR-Gi1α (C351-I351).

At present, modern bio-physical and confocal microscopy techniques (fluorescence life time imaging, fluorescence resonance energy transfer, raster image correlation spectroscopy) are being introduced for analysis of agonist-induced change of receptor mobility in HEK293 cell lines transiently expressing δ-OR-CFP, δ-OR-YFP and δ-OR-CFP plus δ-OR-YFP. Stably transfected HEK293 cells expressing TRH-R-eGFP and fluorescence recovery after photo-bleaching are used as reference standard when testing agonist-specific alteration of receptor mobility in living cells. Proteomic analysis and 2D-ELFO is used for detection of PM proteins specifically altered in brain cortex by long-term exposure to morphine. This program includes external collaboration with J. Heyrovsky Institute of Physical Chemistry (M. Hof).

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Personal background: In 2011, the group was strengthened by arrival of J. Wilhelm (supported by Grant Agency of Czech Republic), who has long term experience in the field of the free oxygen radical damage of membrane structures, covalent modification of membrane proteins and detection of endogenous lipofuscin-like pigments (LFP). At present, the group consists of three established investigators, 5 full-time and 3 part-time PhD students and 4 MS students. Besides collaborations mentioned above, the lab has close international exchange of people and materials with M. Parenti (University of Milano, Italy). Tae-Weon Lee at Stanford (Amgene) is preparing methodological background for the future isolation of large amounts of pure GPCR for incorporation /reconstitution in vesicular and planar lipid bilayers of defined composition.

Members of the lab are also active in teaching at Charles University, Prague: Departments of Physiology and Biochemistry, Faculty of Natural Sciences Czech (practical course and lectures in “Molecular Pharmacology”, 1 semester, 3/2).